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Impact of gender on the density of intraplaque neovascularization: A quantitative contrast-enhanced ultrasound study

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ABSTRACT

Objective: Atherosclerosis is the main underlying cause of the majority of cardiovascular events. Although cardiovascular diseases (CVD) are a major challenge in both males and females, gender specific differences in the prevalence of CVD have been observed. This may indicate that there are differences in the development of atherosclerosis between males and females. The presence of intraplaque neovessels (IPN) is an imaging marker for plaque vulnerability. The aim of this study was to investigate the impact of gender on IPN.

Methods: A total of 159 patients with $\geq$1 cardiovascular risk factor were included in this prospective study (mean age 56.9 $\pm$ 8.7 years, 47% females). Patients had no symptoms of carotid atherosclerotic disease. All patients underwent a standard carotid ultrasound examination in conjunction with contrast-enhanced ultrasound (CEUS). The presence of atherosclerotic plaques was assessed according to the Mannheim consensus. IPN was assessed using a visual grading scale and semi-automated quantification software.

Results: Subclinical atherosclerosis was detected using standard carotid ultrasound and CEUS in 64 females (86%) and in 79 males (93%) ($p = 0.177$). The mean atherosclerotic plaque sizes were not significantly different ($p = 0.068$). Semi-automated quantification of IPN demonstrated that females had significant more IPN compared to males ($p < 0.05$). After adjustment for clinical variables this association remained significant ($p < 0.05$).

Conclusion: In this population at increased risk for CVD, females had significantly more IPN compared to males. This suggests that the females had a more vulnerable atherosclerotic plaque type.

1. Introduction

Each year, more than 10 million people die due to stroke or coronary heart disease and atherosclerosis is the main underlying cause of these cardiovascular events (CVE) [1,2]. Although cardiovascular diseases (CVD) are a major challenge in both males and females, gender specific differences in the prevalence of CVD have been observed [3]. This may indicate that there are differences in the development of atherosclerosis between males and females. Several studies have reported on gender specific differences in atherosclerotic plaque composition. Females tend to have a more stable atherosclerotic plaque type than males [4–6]. Vulnerable atherosclerotic plaques have an increased risk of rupture, and may cause cardiovascular events. These vulnerable atherosclerotic plaques are characterized by a lipid rich necrotic core and a thin fibrous cap, intraplaque hemorrhage and the presence of intraplaque neovessels (IPN) [7–9]. Contrast enhanced ultrasound (CEUS) is an advanced form of ultrasound that allows dynamic assessment of IPN in carotid atherosclerotic plaques [10,11]. So far, no studies have investigated whether there are gender-specific differences in the density of IPN. Therefore the aim of this study was to investigate the impact of gender on IPN assessed using

Abbreviations: CEUS, contrast-enhanced ultrasound; CVD, cardiovascular diseases; CVE, cardiovascular events; IPN, intraplaque-neovessels.

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carotid CEUS in asymptomatic patients at increased risk of atherosclerosis.

2. Methods

2.1. Patient population and study protocol

This study was set-up as a prospective observational study. Between April 2010 and May 2013 patients from the outpatient clinics of Cardiology and Vascular Medicine in the Erasmus Medical Center were asked to participate in this study. Inclusion criteria were age ≥18 years, and ≥1 cardiovascular risk factor (i.e. hypertension, diabetes mellitus, dyslipidemia, smoking, and/or a family history of cardiovascular disease). Dyslipidemia was defined as total serum cholesterol ≥200 mg/dl. Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg or treatment with antihypertensive medication. Diabetes mellitus was defined as the use of anti-diabetic medication or a diagnosis of diabetes mellitus according to current guidelines [12]. None of the patients had symptoms of carotid atherosclerotic disease. Exclusion criteria were contra-indications for the use of ultrasound contrast agent, such as unstable angina, acute cardiac failure, acute endocarditis, known right-to-left shunts and known allergy for microbubble contrast agents. Clinical characteristics were documented and a physical examination was performed prior to the ultrasound examination, including weight, height, waist-to-hip ratio (WHR) and blood pressure measurements. The study protocol was approved by the local ethical committee and all patients provided written informed consent. All patients underwent a standard ultrasound examination in conjunction with CEUS of the carotid arteries.

2.2. Carotid ultrasound acquisition

Standard carotid ultrasound and CEUS were performed with a Philips iU-22 ultrasound system (Philips Medical Systems, Bothell, USA), equipped with an L9-3 transducer. Standard carotid ultrasound included acquisition of B-mode clips and color Doppler clips. Image acquisition was performed using a standard scanning protocol according to the American Society of Echocardiography consensus statement [13]. In short, both left and right carotid arteries were examined with the patient in a supine position with the head supported at a 45° angle turned to the contralateral side. The left and the right common carotid artery (CCA), carotid bifurcation (BIF), internal carotid artery (ICA), external carotid artery (ECA), and vertebral arteries were imaged by B-mode ultrasound, color Doppler and pulse-wave Doppler. All anatomical sites were examined from different angles of view and a short axis sweep was executed to detect atherosclerotic plaques. Each side was extensively evaluated for the presence of carotid plaques. Gain and imaging depth were adjusted per patient to obtain optimal ultrasound images.

After standard carotid ultrasound, CEUS was performed using intravenous administration of SonoVue™ contrast agent (Bracco S.p.A., Milan, Italy). The contrast mode of the ultrasound system was selected using amplitude modulation to enhance the ultrasound contrast and suppress the tissue. A mechanical index of 0.06–0.08 was used to optimize the CEUS examination. CEUS clips were acquired according to a standard acquisition protocol. CEUS clips were recorded with a side-by-side display mode for simultaneous standard B-mode ultrasound and CEUS view. The ultrasound contrast agent was injected in boluses of 0.5 ml followed by a flush with 2.0 ml NaCl 0.9% solution. After administration of contrast agent, high-quality contrast images could be obtained for approximately 1 min. Bolus injections of the contrast agent were repeated when necessary up to a maximum total dose of 9.6 ml. Both carotid arteries were examined, focusing on the presence of atherosclerotic plaques. If plaques were present, the plaque was visualized in the longitudinal axis of the carotid artery. Still frames and cineclips were digitally stored.

2.3. Carotid ultrasound analysis

Standard carotid ultrasound clips and CEUS clips were reviewed offline. First, carotid plaque screening was performed using standard carotid ultrasound clips including color Doppler and CEUS clips. Atherosclerotic plaque was defined as a focal structure encroaching into the lumen of at least 0.5 mm or 50% of the surrounding CIMT, or demonstrating a thickness >1.5 mm as measured from the media–adventitia interface to the intima–lumen interface [14]. The presence of atherosclerotic plaques was recorded for each side and all individual carotid segments (i.e. CCA, BIF, ICA, ECA). Maximum atherosclerotic plaque thickness was measured perpendicular to the carotid longitudinal axis. In addition, for each segment that exhibited atherosclerotic plaques, the presence of wall irregularities and/or ulcerations was recorded. Atherosclerotic plaque ulceration was defined as a disruption in the plaque-lumen border ≥1 × 1 mm. If plaques were present, carotid luminal diameter was measured proximal from the atherosclerotic plaque.

Assessment of IPN was performed using the CEUS clips. Due to pseudo-enhancement artifact only near-wall plaques were eligible for assessment of IPN [15,16]. Clips without atherosclerotic plaques or with atherosclerotic plaques that could have been affected by pseudo-enhancement were not eligible and as a consequence excluded from further analysis. Therefore, all far wall plaques and all near-wall plaques that were behind a contrast-pool (e.g. jugular vein) were excluded from assessment of IPN. Per carotid artery with ≥1 atherosclerotic plaque on the near wall of the carotid artery, the clip with visually the highest amount of contrast enhancement was selected for analysis (Fig. 1). In patients with bilateral plaques, the thickest plaque was selected for patient based analysis. The amount of intraplaque neovascularization (IPN) was visually assessed according using a previously published 3 point ordinal scale as 0 = absent IPN, 1 = mild to moderate IPN and 2 = extensive IPN [17]. Semi-automated quantification of IPN was performed using dedicated software. DICOM files of side-by-side B-Mode and contrast mode ultrasound clips were imported in the software to assess different plaque perfusion parameters after selection of the time-frame. The software calculated several parameters based on region-of-interest (ROI) analysis after motion compensation of the carotid artery. These calculations were based on maximum intensity projections and time intensity curves which resulted in the following parameters: 1. IPN surface area in mm^2 (IPN-SA), 2. IPN surface area ratio in % (IPN-SA ratio), 3. mean percentage of the plaque filled with contrast over time (MPCP), 4. plaque mean intensity (PMI) and 5. plaque area in mm^2 [18]. Table 1 provides a short description of how the parameters were derived. The reproducibility of the parameters of this software was good to excellent. Intra-observer intra-class correlation ranged from 0.835 to 0.984. Inter-observer intra-class correlation ranged from 0.682 to 0.968. Bland–Altman plots showed a low variability of repeated measurements. The reproducibility of the measurements was reported by Akkus et al. [18].

2.4. Statistical analysis

Statistical analysis of the data was performed using SPSS PASW software for Windows (Version 20.0.0, SPSS Inc., Chicago, IL, USA). Continuous and ordinal data were expressed as mean ± standard deviation or median and inter-quartile range. Categorical variables were expressed as counts and percentages and/or median value. Differences in patient characteristics were analyzed. Depending on