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Imaging of plaque perfusion using contrast-enhanced ultrasound — Clinical significance

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Summary The identification of vulnerable and unstable carotid atherosclerotic lesions is up-to-date an important topic of research, in order to adopt the adequate strategy for preventing cerebrovascular events. Plaque inflammation, presence of adventitial vasa vasorum, intimal angiogenesis and plaque neovascularization have been identified in histological studies as indicators of the instability of the atheroma of carotid arteries in cerebrovascular patients and of coronary arteries in cardiovascular patents. Consequently, the identification ''in vivo'' of these pathophysiological aspects has been objective for the development of new imaging techniques. Ultrasound of carotid arteries, with ultrasound contrast agents, is not only able to provide an enhanced visualization of the arterial lumen and plaque morphology, but also allows to directly visualize adventitial vasa-vasorum and carotid plaque neovascularization. This technique and its clinical implications in the unstable plaque identification are discussed in the present paper.

Introduction

The degree of internal carotid stenosis is nowadays no more considered the only parameter to be evaluated when identifying the ''plaque at risk'' to be addressed to carotid endarterectomy [1—3]. Since the 1980s the characterization of the morphology of the carotid plaque has become standard for stroke risk definition and, hence, the efforts for the definition of the ''unstable plaque'' [1,4]. In these regards, carotid ultrasound imaging has represented the cornerstone to describe the plaque characteristics that reflect a higher risk of vulnerability [4—7]. Plaques of moderate echogenicity and with hyperechoic spots are composed of ''hard'' fibrous tissue and calcifications; these plaques are less harmful than heterogeneous plaques with hypoechoic areas that correspond to ''soft'' atheromatous material consisting of cholesterol, lipid deposits, cell debris and necrotic residuals. Intraplaque hemorrhage, another cause of the sudden increase of plaque volume and rupture, is also of low echogenicity. Summarizing, the lower the degree of the echogenicity of a plaque, the higher the risk of the cap thinning and the surface endothelium rupture with subsequent ulceration, distal embolization and stroke. To reduce the biases of the subjective image interpretation, the computerized analysis of ultrasound images has also proved a reliable objective tool for identifying plaques

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with low Gray Scale Median scores, at a ''major risk'' of developing future cerebrovascular events [8,9].

A turning point in the history of atherosclerosis pathophysiological mechanisms comprehension has been the concept that ''inflammation'' may be linked with the disease development and progression. From histology, indeed, it was already known that while stable atheromatic lesions are characterized by a chronic inflammatory infiltrate, in vulnerable and ruptured plaques an active and acute inflammatory process regarding the surface and the plaque core takes place [10]. Consequently, adventitial vasa vasorum, intimal angiogenesis and plaque neovascularization have been considered, and confirmed by histological studies, as important predictors of unstability in atheromasic lesions of cerebro and cardiovascular patients [11—19]. Angiogenesis occurs indeed regularly within atherosclerotic plaques and atheroma vulnerability and symptomatic carotid disease have been associated with an increased number of microvessels [16] that may also be responsible of the intra-plaque hemorrhage, when the rupture of these small newly generated and vulnerable vessels within the plaque occurs.

The possibility that inflammation could represent an index of plaque vulnerability has brought the scientific interest to concentrate on imaging ''in vivo'' the pathophysiological ''functional'' status of the atheroma with the goal to identify, as early as possible, the more vulnerable ones, to adopt the adequate preventive strategy. For this reason, several conventional radiological imaging, such as Computerized Tomography Angiography, Magnetic Resonance Angiography and also 18-FDG Positron Emission Tomography have focused on the evaluation of the ''plaque metabolic activity'', but — up to date — this is an evolving methodology requiring further consensus [20]. Contrast carotid ultrasound (CCU) is nowadays a well-established tool for angiogenesis detection in several fields with the principal advantage of being a simple, low cost and minimally invasive technique. Since the first data of 2006, several papers have now described the possibility to identify adventitial vasa vasorum and neovascularization also in carotid plaques [22—40], with a specific pattern of vascularization in acute symptomatic lesions [41].

Aim of this paper is to describe the methodology and the efficacy of contrast carotid ultrasound to identify plaque vascularization and to discuss the related clinical implications.

Contrast ultrasound investigation methodology and findings

Our experience is based on patients with carotid stenosis electively referred to our ultrasound laboratory for contrast ultrasound investigation [23,27,28,41] and from still ongoing data. The population consists of both asymptomatic patients, referred for vascular screening, as well as by symptomatic stroke patients. Plaques of different morphologies and various degree of stenosis have been investigated. According to the specific indications and guidelines for carotid endarterectomy, symptomatic and asymptomatic patients with a severe degree of stenosis were operated and histological/samples confronted with the ultrasonographic findings.

Apparatus, plaque morphology and technique of ultrasound contrast investigation

Ultrasound carotid duplex scanning were performed with Acuson/Siemens Sequoia 512 and Siemens S2000 systems, with standard vascular presets, and equipped with contrast multi-pulse non-harmonic imaging software ''Cadence contrast Pulse Sequencing'' (CPS) technology. Linear phased array probes (6, 8 and 15 MHz for the Sequoia, 9L4 for S2000) with standard presettings were used. The same machine presets were maintained constant. The technique of investigation is also reported in other published papers on this topic from our group [23,27,28,41].

Plaque echographic morphology was categorized according to criteria already well established in literature [4,7]. Plaque structure according to the echogenicity, and considered as hyperechoic with acoustic shadow, hyperechoic, isoechoic, hypoechoic, and consequently as calcific, fibrous, fibro-calcific, fibro-fatty and hemorrhagic. Plaque surface was defined as regular, irregular and ulcerated, when an excavation $\geq$2 mm was observed. Echogenicity was also quantified with the Gray Scale Median (GSM) computerized analysis [8], in order to better define the plaque risk. The degree of stenosis was evaluated according to European Carotid Surgery Trial (ECST) criteria [42], as percentage of the difference between the original vessel lumen diameter/area and the residual lumen diameter/area at the maximum site of stenosis, and according to blood flow velocities [4,43].

After the standard basal investigation of the plaque, contrast ultrasound investigations were performed with repeated short (0.5—1 ml) bolus injections in an antecubital vein (20 Gauge Venflon) of Sonovue (Bracco Altana Pharma, Konstanz, Germany), for a total contrast administration of up to 2.5 ml, each bolus being promptly followed by a saline flush. The 15 MHz linear array probe for the Sequoia (MI 0.4—1.1) and the 9L4 MHz for the S2000 (MI 0.10) were used for the CPS continuous real-time imaging. The ''Contrast Agent only'' software feature, in which the image is derived only from the signals of the microbubbles, has been used. All the investigations were digitally stored and DICOM files transferred to an external PC equipped with Showcase (v 5.1, Trillium Technology) for the off-line analysis.

Angiogenesis and neovascularization detection

After the bolus injection, few seconds are required for the contrast to be carried through the venous system to the pulmonary filter, heart and to the carotid arterial lumen. After the contrast is detected in the carotid axis, few seconds later, mainly during the diastolic cardiac phase, probably because of the reduced local pressure on the atherosclerotic lesion, the dynamic distribution of the contrast agent inside the plaque allows the visualization of the plaque vascularization. As previously already reported elsewhere [23,27,28], vascularization was detected at the shoulder of the plaque at the adventitial layers, and in the iso-hyperechoic fibrous and fibro-fatty tissue. It is represented by little echogenic spots rapidly moving within the texture of the atheromasic lesion, easily identifiable in the real time motion, and depicting the small microvessels (Fig. 1, Clip 1). In