Atherosclerosis 246 (2016) 293–300

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Determining carotid plaque vulnerability using ultrasound center frequency shifts

Tobias Erlöv [a, *], Magnus Cinthio [a], Andreas Edsfeldt [b], Simon Segstedt [a], Nuno Dias [c], Jan Nilsson [b], Isabel Gonçalves [b, d]

[a] Department of Biomedical Engineering, Faculty of Engineering, Lund University, Sweden
[b] Experimental Cardiovascular Research Group, Department of Clinical Sciences Malmö, Lund University, Sweden
[c] Vascular Center, Skåne University Hospital, Sweden
[d] Department of Cardiology, Skåne University Hospital, Sweden

ARTICLE INFO

Article history:
Received 18 September 2015
Received in revised form
29 December 2015
Accepted 11 January 2016
Available online 15 January 2016

Keywords:
Atherosclerosis
Imaging
Ultrasound
Vulnerable plaque

ABSTRACT

Background: The leading cause of morbidity and mortality worldwide is atherosclerotic cardiovascular disease, most commonly caused by rupture of a high-risk plaque and subsequent thrombosis resulting in stroke, myocardial infarction or sudden death depending on the affected arterial territory. Accurate, non-invasive methods to identify such lesions known as vulnerable or high-risk plaques are currently sub-optimal. Our aim was to validate a new non-invasive ultrasound method to identify high-risk carotid plaques.

Methods: We evaluated a new method based on the center frequency shift (CFS) of the ultrasound radio frequency data obtained from carotid plaques compared to a reference phantom. We evaluated the method both *ex vivo*, on 157 sections from 18 plaques, and *in vivo*, in 39 patients 1-day prior to carotid plaque removal, and correlated the data with histology.

Results: The CFS correlated with a plaque vulnerability index based on histological areas stained for lipids, macrophages, hemorrhage, smooth muscle cells and collagen ($r = -0.726$, $P = 1.7 \times 10^{-8}$). Plaques with CFS below median had larger cores, more macrophages and were less rich in collagen in agreement with the definition of rupture-prone plaques. The accuracy to detect plaques with high vulnerability index was 78% (confidence interval (CI) 61–89%), with sensitivity 77% (CI 61–89%) and specificity 78% (CI 62–89%).

Conclusions: Our method is the first to characterize atherosclerotic plaque components that affect plaque vulnerability using CFS.

1. Introduction

The rupture of atherosclerotic plaques with subsequent thrombosis is the cause of most acute cardiovascular events. Rupture-prone, vulnerable plaques, are characterized by a core of necrotic debris and lipids, accumulation of inflammatory cells and a reduced content of fibrous tissue and smooth muscle cells (SMC) [1]. They are often covered by a thin fibrous cap. Identification and treatment of vulnerable plaques before they give rise to acute events represents a great challenge in cardiovascular medicine today. Over the past decade, many invasive and non-invasive imaging modalities which visualize the structure and composition of atherosclerotic plaques and which may improve detection of high-risk atherosclerotic lesions have been developed. Coronary plaque features can be assessed with high resolution by intravascular ultrasound (IVUS) [2] and carotid plaques by magnetic resonance imaging (MRI) [3]. Computed tomography (CT) readily identifies vascular calcification and discriminates between soft, intermediate and calcified lesions [4]. CT combined with 18-fluorodeoxyglucose positron emission tomography (PET) detects plaque inflammation [5]. Other characteristics such as ulceration, intraplaque hemorrhage, microemboli (assessed by transcranial Doppler), echolucent juxta-luminal areas and other aspects of plaque texture have also

* Corresponding author. Biomedical Engineering, Lund University, Ole Römers väg 3, SE-22363 Lund, Sweden.
E-mail address: tobias.erlov@bme.lth.se (T. Erlöv).

http://dx.doi.org/10.1016/j.atherosclerosis.2016.01.019
0021-9150/

been suggested as tools to identify high-risk plaques [6,7]. Although several of these techniques are well documented with respect to identification of plaque features associated with increased plaque vulnerability their usefulness for clinical risk stratification has not been determined in large study populations. Moreover, a broader clinical use of these techniques in the identification of high-risk plaques is limited by several factors including invasiveness of the procedures, the use of ionizing radiation, as well as cost and time required for the procedures.

Ideally, a clinically applicable plaque imaging modality should be time-efficient, non-invasive, low-cost, easily tolerable, give real-time results and with no use of radiation, contrast or manual post-processing. The techniques that embrace most of these demands are ultrasound-based. In carotid ultrasound the standardised gray-scale median (GSM) has been used as a plaque echogenicity score [8], at times usefully applied in combination with clinical information [9]. These studies demonstrated that GSM, plaque area, discrete white areas, together with degree of stenosis and clinical characteristics in asymptomatic patients, could improve the stratification of the annual risk of stroke from <1% to 10%. However, these features are affected by user-defined settings and are still time-consuming as well as hard to reproduce [10].

Several methods developed to characterize plaques are based on ultrasound radio frequency (RF) data, i.e. the raw data which is processed to provide the ultrasound images seen by the user; these include the use of e.g. integrated backscatter [11] and spectral analysis [12–17]. These methods utilize that the frequency and amplitude of the reflected ultrasound is dependent on characteristics of the scattering tissue. For instance the size, shape and elastic properties of the components in the scattering tissue affects the frequency content of the RF data [18]. Methods have been derived to extract tissue specific parameters from a spectrum of the RF data [18,19]. The spectrum is usually normalized to a reference spectrum in order to remove the frequency dependence caused by the instrumentation [20,21]. Shi et al. [17] showed preliminary results from such a method on *in vivo* measurements of 10 carotid plaques. These were classified as either calcified or soft by an experienced radiologist based on B-Mode and color Doppler images. They found that calcified regions differed from softer regions in both scatter size (120–180 μm vs 280–470 μm) and attenuation (1.4–2.5 dB/cm/MHz vs 0.3–1.3 dB/cm/MHz).

All methods that are based on spectral analysis are subjected to a trade-off in time-frequency resolution, i.e. increased spatial resolution will decrease the spectral resolution. For instance Gerig et al. [22]. showed both theoretically and practically that the variance of scatter size estimates increase with increased spatial resolution (decreased calculation window).

In the present study we describe and validate *ex vivo* and *in vivo* a new non-invasive ultrasound-based method that allows real-time assessment of plaque composition. The method uses single-frame analysis based on the center frequency shift (CFS) of the ultrasound RF data from carotid plaques compared to a reference phantom. The center frequency is, similar to an entire spectrum of frequencies, affected by the size, shape and elasticity of the tissue specific components. However, the CFS can be measured efficiently in the time domain (i.e. without spectral conversion) and is therefore not subjected to the same trade-off as in spectral analysis. We demonstrate that the CFS correlates with the amount of plaque components known to be related with rupture risk. The method could potentially become a useful tool to identify patients at risk for development of acute cardiovascular events as well as to monitor response to interventions.

2. Material and methods

2.1. Clinical samples

All patients were preoperatively assessed by an independent neurologist as having significant stenosis (stenosis >70% for the plaques associated with ipsilateral symptoms (TIA, strokes or amaurosis fugax) or >80% for the asymptomatics). Stenosis grade was measured according to the velocity criteria assessed by ultrasound performed as clinical routine in our university hospital/tertiary care setting [23].

We collected in total 206 fragments of human atherosclerotic plaques obtained by carotid endarterectomy from 57 patients (Table 1).

To test our method under optimal conditions, we first conducted an *ex vivo* study where 157 fragments from atherosclerotic plaques, obtained from 18 symptomatic patients (10 males, 72.2 ± 6.6 years old), were imaged and analyzed. The method was then validated in an *in vivo* study, in which we analyzed 49 fragments from 39 patients: 5 asymptomatic, 7 *amaurosis fugax*, 13 transient ischemic attacks (TIA) and 14 strokes. From the 39 patients studied, 37 (95%) were under statin treatment (Table 1), 35 (90%) had antiplatelet therapy, 6 (15%) anticoagulants, 23 (59%) betablockers, 18 (46%) angiotensin-converting enzyme inhibitors, 8 (20%) angiotensin receptor blocker, 14 (36%) diuretics, 8 (20%) calcium channel blockers and 8 (20%) anti-diabetic medications. In all 39 patients a fragment was chosen from the most stenotic region. In 10 of those patients an extra fragment was chosen close to a clear anatomic landmark, so that the matching between ultrasound and histology could be guaranteed. Each patient was imaged twice to assess intra-operator variability. The coefficient of variation was 4%. The study was approved by the local ethical committee and all subjects gave informed consent, both oral and written consent.

2.2. Ultrasound acquisition

To access ultrasound RF data, the Ultrasound Advanced Open Platform (ULA-OP) was used for the acquisitions [24] with 9 MHz center frequency (LA523 linear array, Esaote, Italy). The transmitted pulse length was 0.3 μsec and the derated mechanical index was below 0.5. The RF data was stored and demodulated to obtain a complex representation. The data was then sent to a computer for offline analysis using Matlab (The Mathworks Inc., Natick, MA) where the method was implemented.

Table 1
Characteristics of the patients whose plaques were assessed with our method *ex vivo* and *in vivo*.

	Ex vivo	*In vivo*
	Patients (n = 18)	Patients (n = 39)
Age (years)	72.2 ± 6.6	72.4 ± 9.4
Sex (males)	10 (56%)	24 (62%)
Symptoms	18 (100%)	34 (87%)
Degree of stenosis (%)	79 ± 12	83 ± 13
Type 2 Diabetes	4 (22%)	8 (21%)
Hypertension	12 (67%)	34 (87%)
Smoking (past or currently)	12 (67%)	35 (90%)
Statin use	16 (89%)	37 (95%)
C-reactive protein (CRP) (mg/L)	3.5 (2–7.8)	3.3 (1.2–9.9)
Fasting lipoproteins (mmol/L):		
Cholesterol	4.0 ± 1.0	4.5 ± 1.5
Low-density lipoprotein (LDL)	2.5 ± 0.9	2.8 ± 1.3
High-density lipoprotein (HDL)	1.0 ± 0.3	1.3 ± 0.4
Triglycerides	1.4 ± 0.8	1.3 ± 0.5

Values are presented as mean ± standard deviation or as median (interquartile range), if not normally distributed.