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Increased expression of endothelial lipase in symptomatic and unstable carotid plaques

Matias Trbušić[1,2,#], Monika Riederer[3,#], Majda Vučić[4], Ivo Lovričević[1,5], Božo Krušlin[1,6], Martin Gauster[7], Sonja Mohrenz[3,7], Andrea Berghold[8], Beate Tiran[9], Vesna Degoricija[1,2§*] and Saša Frank[3§*]

[1]University of Zagreb School of Medicine, [2]Department of Medicine, Sisters of Mercy University Hospital, Zagreb, Croatia; [3]Institute of Molecular Biology and Biochemistry, Center of Molecular Medicine, Medical University of Graz, Graz, Austria; [4]University of Zagreb School of Dental Medicine, and Ljudevit Jurak University Department of Pathology, Zagreb, Croatia; [5]Department of Surgery and [6]Ljudevit Jurak University Department of Pathology, Sisters of Mercy University Hospital, Zagreb, Croatia; [7]Institute of Cell Biology, Histology and Embryology, Center of Molecular Medicine, Medical University of Graz, Graz, Austria; [8]Institute for Medical Informatics, Statistics and Documentation, Medical University of Graz, Graz, Austria; [9]Cinical Institute for Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria.

[#] equally contributed first authors
[§] equally contributed senior authors

Short title: Endothelial lipase in the atherosclerotic carotid disease

*Correspondence:
Dr. Vesna Degoricija, Department of Medicine, Sisters of Mercy University Hospital, Vinogradska 29, 10 000 Zagreb, Croatia. Tel.:+385 91 5033747; Fax:+385 1 3768286; Email: vdegoric@mef.hr
Dr. Sasa Frank, Institute of Molecular Biology and Biochemistry, Center of Molecular Medicine, Medical University of Graz, Harrachgasse 21/III, A-8010 Graz, Austria
Tel.: +43 316 380 4193; Fax: +43 316 380 9615; Email: sasa.frank@medunigraz.at

Abbreviations used: Human carotid artery plaques (HCAPs), endothelial lipase (EL), high density lipoprotein (HDL), fibrous plaque (F), ulcerated non-complicated (UNC), ulcerated complicated (UC), carotid endarterectomy (CEA), Color Doppler Flow Imaging (CDFI), computed tomography angiography (CTA), C-reactive protein (CRP),

ABSTRACT

Aims: To evaluate endothelial lipase (EL) protein expression in advanced human carotid artery plaques (HCAP) with regard to plaque (in)stability and the incidence of symptoms.

Methods: HCAP were collected from 66 patients undergoing carotid endarterectomy (CEA). The degree of plaque (in)stability was estimated by ultrasound and histology. In HCAP sections, EL-expression was determined by immunostaining and the intensity was assessed on a semi-quantitative scale (low: <25%, high: >25% positive cells). Monocytes and macrophages in adjacent HCAP sections were stained with a CD163 specific antibody.

Results: High EL staining was more prevalent in histologically unstable plaques (in 33.3% of fibrous plaques, 50% of ulcerated non-complicated plaques and 79.2% of ulcerated complicated plaques; χ^2 test, p=0.004) and in the symptomatic group (70.8% vs. 42.9% in the asymptomatic group; χ^2 test, p=0.028). The majority of EL immunostainig was found in those HCAP regions exhibiting a strong CD163 immunostaining.

Conclusion: EL in HCAP might be a marker and/or promoter of plaque instability and HCAP-related symptomatology.

Key words: endothelial lipase, carotid artery plaque, high-density lipoprotein, inflammation, atherosclerosis, macrophages

Introduction

Atherosclerosis is a chronic fibro-proliferative and immuno-inflammatory disease affecting large and medium size arteries. It is characterized by endothelial dysfunction, local oxidation of circulating lipoproteins (particularly low density lipoprotein (LDL)), their accumulation in the vascular wall [1,2] and the activation of proinflammatory cytokines [3,4]. Inflammation plays a crucial role in plaque destabilization, as it converts stable atherosclerotic plaques, characterized by a fibrous cap covering the necrotic core, into unstable ones, characterized by a thinned fibrous cap, intra-plaque hemorrhage, surface ulceration, rupture and thrombosis [5].

Endothelial lipase (EL) is a member of the triglyceride lipase gene family, expressed by vascular endothelial cells (ECs), smooth muscle cells (SMCs) and macrophages [6,7]. It primarily exhibits phospholipase-A1 activity, with high affinity for high density lipoprotein (HDL)–phosphatidylcholine (HDL-PC), resulting in the generation of bioactive molecules such as lysophosphatidylcholines (lyso-PC) and free fatty acids (FFA) [8]. Acting on HDL, EL not only promotes HDL remodeling but also lowering of HDL plasma levels, an independent risk factor for atherosclerosis [9]. EL plasma concentrations are strongly associated with elevated plasma interleukin-6 (IL-6), tumor necrosis factor (TNF)-α and C-reactive protein (CRP) levels; a profile typical of metabolic syndrome [10,11]. EL was also shown to promote the expression of inflammatory cytokines, exemplified by attenuation of inflammatory cytokine expression in EL-deficient macrophages [12] as well as by increased production of IL-8 in human endothelial cells over-expressing EL [13].

Taking into account the role of inflammation in plaque destabilization, together with the reported up-regulation of EL under inflammatory conditions, we assumed a positive relationship between the expression level of EL and the degree of plaque instability.

Patients and methods

Patients

Patients were recruited from the Department of Vascular Surgery Sisters of Mercy University Hospital, Zagreb, Croatia, between November 1, 2007 and March 1, 2009. Written informed consent from each patient was obtained prior to the enrollment in the study, which was performed according to Good Clinical Practice and Helsinki Declaration principles. The study was approved by the local Ethics Committee, in accordance with institutional guidelines of the Sisters of Mercy University Hospital, Zagreb.

Inclusion and exclusion criteria

Overall 88 patients with significant (70-99%) carotid artery stenosis (confirmed by carotid Color Doppler Flow Imaging (CDFI) done by an experienced physician in the University Hospital) were screened for inclusion and exclusion criteria. In cases where CDFI imaging was limited by features such as calcified carotid lesions, tortuous or kinked carotid arteries or patient body constitution, contrast enhanced magnetic resonance angiography (CEMRA) or computed tomography angiography (CTA) were performed. The clinical indication for carotid endarterectomy (CEA) was met after examination by the neurologist and vascular surgeon, based on clinical presentation and CDFI (or CEMRA/CTA) results, following guidelines for treatment of extra-cranial carotid artery disease [14,15]. Exclusion criteria were met in case of acute or chronic infection or inflammatory conditions (other than atherosclerosis), severe renal failure (serum creatinine $\geq$ 200 mmol/L), severe hepatic cirrhosis (Child-Pugh Class B or C), neoplasms, recent trauma or surgery, history of ipsilateral CEA or radiotherapy and anticoagulant therapy. Patients with a possible cardiac source of embolism detected through the patients´ history, electrocardiogram or echocardiography (left ventricular aneurysm, previous large anterior cardiac infarction, dilatative cardiomyopathy, aneurysm of inter-atrial septum with patent foramen ovale) were excluded from the study.

Definition of symptomatic carotid disease

The carotid stenosis was considered symptomatic, if a patient had suffered from a focal neurological symptom (ipsilateral stroke, transient ischemic attack or monocular blindness), sudden in onset and referable to the respective carotid artery within 4 months of CEA.

Patient characteristics, history and medication

Medical history was recorded from all patients, and the presence of vascular risk factors, features of metabolic syndrome, previous antihypertensive-, statin- and antiplatelet- treatments were noted. Clinical history was assessed for diabetes mellitus, smoking, hypertension, hyperlipidemia, prior acute myocardial infarction, symptoms of peripheral vascular disease and family history of CHD or IVC in first-degree relatives under the age of 55. The body mass index (BMI) was calculated according to Quetelet's formula as the ratio of body weight to body height squared (kg/m^2). The waist circumference was measured with a flexible tape, placed on a horizontal

plane at the level of the iliac crest. The metabolic syndrome was defined according to the criteria of the International Diabetes Federation issued in 2005 (http://www.idf.org/): increased waist circumference (for Caucasian European men ≥ 94 cm, for women ≥80 cm) plus any two of the following: triglycerides > 1.7 mmol/L (or treatment for elevated triglycerides), HDL cholesterol < 1.03 mmol/l in men or < 1.29 mmol/L in women (or treatment for low HDL), systolic blood pressure > 130 mmHg, diastolic blood pressure > 85 mmHg (or treatment for hypertension), and fasting plasma glucose > 5.6 mmol/L (or previously diagnosed type 2 diabetes mellitus) .
Blood pressure was measured with mercurial sphygmomanometer (Riester, Jungingen, Germany) and calculated as the average of three measurements taken under standardized conditions in a supine position. Hypertension was diagnosed according to World Health Organization (WHO) criteria (blood pressure ≥ 140/90 mmHg or current anti-hypertensive treatment). Diabetes was diagnosed in patients with dietary treatment, anti-diabetics or current fasting plasma glucose levels higher than 7.0 mmol/L. Hyperlipoproteinaemia was defined if the low-density lipoprotein (LDL) cholesterol level was > 3.5 mmol/L, or if a patient was taking a lipid-lowering drug. According to cigarette smoking, patients were classified as current smokers (smoking more than five cigarettes within the past 3 months), former smokers (> 3 months and < 10 years) or non-smokers.

Methods

Color Doppler Flow Imaging (CDFI)

CDFI of both carotid arteries was performed in all subjects on admission using commercially available equipment (Aloka 5500 and Alfa 10 Premium with 10–MHz linear array transducer). The grade of plaque stenosis was assessed according to defined criteria [16]. Plaques were divided depending on the presence of the echolucency and calcification into: i) fibrous plaques (without echolucent material and calcification), ii) soft plaques (predominantly echolucent material), iii) calcified plaques, iiii) mixed plaques (both calcification and echolucent material present), and iiiii) non-defined plaques (fibrous plaques with small degree of echolucency and calcification). In this regard, echolucency is considered an important factor associated with symptomatic carotid disease [17] and plaque vulnerability.

Tissue sampling, preservation of carotid plaque specimens and histology

Carotid specimens were excised by the vascular surgeon (complete tubular specimen without damage to the luminal surface) and collected during surgery. They were immediately rinsed in 0.9% saline, fixed for 24 hours in 5 mL buffered formalin, followed by several phosphate buffered saline (PBS) washes and storage in PBS for 2 weeks before embedding in paraffin. Plaque morphology was assessed by standard histological examination of hematoxylin and eosin-stained (HE) sections (5 μm) and screened for features indicative of plaque vulnerability. Taking into account the lack of new, prospectively validated criteria for plaque vulnerability, the Stary classification [5] was applied and plaques were defined as: i) fibrous (F) (covered by a smooth luminal surface indicative of an intact fibrous cap), ii) ulcerated non-complicated (UNC) (thin fissured or ruptured cap), and iii) ulcerated complicated (UC) (thin fissured or ruptured cap with intra plaque hemorrhage and/or thrombus).

Immunohistochemistry

Formalin fixed and paraffin embedded human carotid sections (5µm) were mounted on Superfrost Plus slides (Menzel, Braunschweig, Germany) and dried at 40°C over night. Slides were dewaxed twice in xylene for 10 minutes and rehydrated through a graded series of alcohol. Antigen retrieval was performed by sequentially incubating the slides in antigen retrieval buffer pH 9 (Eubio, Vienna Austria) for 2 minutes at 720 Watt and 3 x 5 minutes at 160 Watt in the microwave. Immunohistochemistry was performed using the Ultravision LP detection system (Thermo Scientific, Fremont, USA) according to the manufacturer's instructions. In brief, endogenous peroxidase was blocked by incubation with Hydrogen Peroxide Block (Thermo Scientific) for 10 minutes. Thereafter, slides were washed with PBS and unspecific background was blocked with Ultra V Block (Thermo scientific) for 8 minutes. Rabbit clonal anti-human CD163 (DB Biotech, clone K20-T, diluted 1:100) and rabbit anti-EL serum (1:1500) [18] were diluted in antibody diluent (DAKO) and incubated on slides for 1 h at RT. After PBS washing steps, detection was achieved by incubation with the UltraVision HRP-labelled polymer system for 15 minutes and 3-amino-9-ethylcarbacole (AEC, Dako, Denmark), according to the manufacturer's instructions. For negative controls, slides were incubated with rabbit IgG Ab-1 (2 µg/ml, Neomarkers) instead of the primary antibodies. Nuclei were stained with hemalaun and slides were mounted with Kaiser's glycerol gelatine. Images of sections were taken with an Axiophot microscope equipped with an AxioCam HRc digital camera (Zeiss, Oberkochen, Germany). Importantly, all samples were processed simultaneously using the same batch of reagents. The expression of EL was evaluated on a semi-quantitative scale by 3 independent, experienced investigators blinded to the clinical and histology data. The scoring was arbitrary, as follows: i) (A) low staining (less than 25% positive cells), and ii) (B) high staining (more than 25% positive cells).

Assays

Blood samples for laboratory assays were obtained before surgery at approximately 8 am, following overnight fasting. Blood sampled in ethylenediaminetetraacetic (EDTA)-K3 containing tubes was used for hematological assays (Coulter-Counter S plus junior, Coulter Electornics Limited, Luton, UK). Coagulation parameters were assessed from blood samples in 3.8% sodium citrate. After separation of plasma by centrifugation (3000 g for 10 min), the Quicktest was performed on a Behring coagulation timer (Dade Behring, Marburg, Germany). Serum creatinine, bilirubin, aspartat transaminase, alanin transaminase, blood glucose level, total plasma cholesterol, triglyceride, LDL and HDL were measured on automated, multi-channel selective analyzers Olympus AU2700 and Olympus Fractoscan junior (Olympus Diagnostica GmbH, Hamburg, Germany). High sensitivity (hs) CRP concentrations were measured on automated, multichannel selective analyzer Modular (Roche Diagnostics, Mannheim, Germany). IL-6 concentrations were measured using a specific chemiluminescent ELISA (QuantiGlo; R&D Systems, Wiesbaden-Nordenstadt, Germany) according to the manufacturers' instructions.

Statistical analysis

Data were summarized using mean and standard deviation or median, minimum, maximum as appropriate for continuous variables and counts as well as percentages for categorical variables. Data distribution was analyzed by Kolmogorov-Smirnov test. According to these results, appropriate parametric or non-parametric tests were

used in further analyses. Unpaired Student´s t-test or Mann-Whitney U test was performed to assess differences among quantitative variables between symptomatology and immunohistochemistry groups. Differences between ordinally scaled histological groups were analyzed with Kruskal-Wallis test, whereas Chi square test was applied to analyze differences in qualitative and categorical variables. The association between quantitative data was evaluated using Spearman-rank correlation analysis. Data were analyzed using PASW 17.02 (IBM, Chicago, IL, USA) software and all p values below 0.05 were considered significant.

Results

Sample size and clinical characteristics

Out of 88 patients assessed for eligibility, 22 patients were excluded (see Fig. 1). The final study population included 66 patients (43 males and 23 females, mean age 67.5) stratified into two groups: the asymptomatic group (42 pts) and the symptomatic group (24 pts). The patients´ clinical characteristics and medications are shown in Table 1. The two groups did not differ significantly according to age, gender, individual risk factors, medication, or coexistence of other vascular diseases (peripheral vascular disease, past myocardial infarction, angina pectoris). In the symptomatic group, however, a higher incidence of patients with increased waist circumference was found (62.5% vs. 38%; χ^2test, p= 0.049). The BMI was also higher in the symptomatic group (28.2 ± 3.0 vs. 26.8 ± 3.0) but the difference did not reach statistical significance (p= 0.085). Overall, there was no significant difference in the number of metabolic risk factors. Basic biochemical and hematological parameters as well as inflammatory parameters (WBC, hs-CRP, erythrocyte sedimentation rate, fibrinogen and IL-6) did not differ between the groups (Table 2).

Ultrasound and histological characteristics of plaques

The grade of carotid artery stenosis detected by CDFI (or CEMRA/CTA) was considerably higher in the symptomatic group (χ^2 test, p=0.027) (Table 3). Additionally, plaques with morphologic characteristics suggestive of plaque vulnerability (echolucent zones with or without ulcerated morphology) seen on CDFI were more frequent in the symptomatic group (χ^2 test, p=0.039). Finally, the majority of asymptomatic plaques (50%) were F plaques, whereas the majority of symptomatic plaques (50 %) were UC plaques (not statistically significant) (Table 3).

EL immunostaining in plaques

In F plaques (Fig. 2 a-c), the necrotic core was covered with a fibrotic cap with preserved endothelium and only few inflammatory cells (Fig. 2 a). In UC plaques (Fig. 2 d-e), in contrast, the necrotic core was covered with inflammatory infiltrates, composed of lymphocytes, macrophages, siderophages and importantly of erythrocytes, indicating intra-plaque hemorrhage (Fig 2d). In F plaques, a weak EL immunostaining (type A) was detected in the endothelial layer and in the fibrous cap (Fig. 2b). The fibrous cap also exhibited moderate CD163 immunostaining, indicative of the presence of monocytes and macrophages (Fig. 2c). Compared with F plaques, EL staining was more pronounced (type B) in UC plaques (Fig. 2e). Here, EL staining localized primarily to cells of the inflammatory infiltrate (Fig. 2e), which showed a strong staining for CD163 as well (Fig. 2f).

Importantly, high intensity EL immunostaining (type B) was most frequently observed in UC plaques (79%; 19 of 24 pts), followed by UNC and F with 50% (6 of 12 pts) and 33% (10 of 30 pts), respectively (Fig. 3) (χ^2 test, p=0.004). Furthermore, EL immunostaining type B was significantly more prevalent in plaques of symptomatic patients (Table 3) (χ^2 test, p=0.028). Inflammatory markers (IL-6 and hsCRP) as well as HDL plasma levels were

comparable between patients with EL immunostaining type A and B (Mann-Whitney U test, p=0.17 for IL-6; p=0.44 for hsCRP; p=0.76 for HDL).

Discussion

The major findings of the present study are: i) higher EL immunostaining in unstable HCAP and ii) higher EL immunostaining in plaques of patients exhibiting HCAP - related symptomatology. Probably the main connector between plaque stability, inflammation and EL are macrophages, which play an important role in plaque destabilization [19]. Using imunohistochemistry, Azumi et al. [20] identified macrophages and smooth muscle cells as main source of EL in HCAP. This was confirmed by a Danish study [21], which detected EL mRNA and protein in areas between the fibrotic cap and the necrotic core, co-localizing primarily with macrophage specific CD68; however, that study exclusively included symptomatic carotid patients (26 pts). In the present study, the intensity of both EL and CD163 immunostaining increased with plaque instability, however not all CD163 positive regions/cells were positive for EL staining. This might be explained by the fact that CD163 (a scavenger receptor for haptoglobin/hemoglobin complexes) is expressed by both monocytes and macrophages [22], whereas EL protein expression is negligible in monocytes but prominent in macrophages and foam cells [21]. Accordingly, the up-regulation of EL (in plaque macrophages) might aggravate inflammation by promoting cytokine production [13,12] or LDL uptake [23,24].

Most studies, addressing EL in the context of inflammation and atherosclerosis, were based on the plasma levels of EL. In healthy subjects with a family history of premature coronary heart disease (CHD), increased plasma EL concentrations were associated with subclinical CHD [10]. Badelino and al. [25] reported increased plasma EL levels under experimental endotoxemia and in patients with metabolic syndrome, a known proinflammatory condition.

In the present study, where all patients exhibited advanced atherosclerotic lesions in at least one (target) artery, inflammatory serum markers, such as IL-6 and hsCRP, were not different between patients with low and high EL expression in HCAP (Mann-Whitney U test, $p=0.17$ for IL-6; $p=0.44$ for hsCRP). This might probably reflect the diffuse nature of atherosclerosis that masks the effect of increased local inflammatory molecule production in unstable HCAP, highlighting the importance and relevance of localized EL expression at the site of pathology. Furthermore, this lack of association between local EL expression in HCAP and IL-6 and hsCRP might be due to the analysis of the total cohort irrespective of gender [26], age [27], or routine exercise [28] - factors with established impact on the plasma levels of inflammatory molecules. Additionally, the expression of inflammatory molecules might have been differently affected by medication - considering pharmacological properties, dosage and interactions of the therapeutics, and the duration of the treatment.

In the present study, there was no significant difference in plasma HDL levels between patients with high (B) and low (A) EL expression in plaques (mean value in both groups 1.3 mmol/L, Mann-Whitney U test, $p=0.76$). Importantly, in the study group, 69% of patients were treated with statins (atorvastatin 19 pts, simvastatin 23 pts, fluvastatin 3 pts) known to increase HDL plasma levels by regulating various molecules that participate in reverse cholesterol transport, such as apo AI [29], scavenger receptor B type I (SR-BI) [30], or ATP binding cassette A1 (ABCA1) [31]. Furthermore, statins were shown to decrease EL expression [32,33]. Considering this and the fact that patients were treated with various doses of different statins, known to vary in their potency to modulate HDL plasma levels, it is tempting to assume that the lack of association between EL in HCAP and HDL plasma levels in the present study is partially due to medication. In addition to statins, angiotensinogen II (AT II) receptor antagonists (10 pts), as well as angiotensin converting enzyme inhibitors (33 pts) might, considering the capacity of AT II to modulate EL expression [34], affect the relationship between EL in HCAP and HDL plasma

levels as well as between EL in HCAP and inflammatory markers. Interestingly, neither statins nor ACE inhibitors impacted significantly the intensity of EL immunostaining in HCAP (not shown). Furthermore, increased EL mass in HCAP does not necessarily have to be accompanied by increased local EL phospholipase activity, considering reduced phospholipase activity of various genetic variants [35]. Finally, the relative contribution of EL in HCAP to the total systemic EL phospholipase activity might be too low to significantly affect HDL plasma levels. Besides, even serum EL levels were found not to correlate significantly with HDL levels [36,11].

The results of our study are in line with previous reports showing no obligatory association between symptomatology of carotid disease and histological features of plaque vulnerability. This might be due to: i) the lack of strong uniform criteria for plaque instability, ii) artificial (laboratory) disruption of the plaque surface, or iii) the fact that some unstable plaques resolve spontaneously without causing symptoms [37-39]. In addition, some of the ischemic events are not caused by the carotid lesion itself, but rather by embolic material derived from heart and aorta. Using echocardiography, we could exclude only some of the patients with suspected cardiac source of embolism. Moreover, it is known that not only the vulnerable plaque makes the patient vulnerable, but also that the vulnerable environment (e.g. blood prone to thrombosis) plays an important role in the overall outcome.

To our knowledge this is the first study addressing the relationship between the intensity of EL immunostaining in HCAP and the degree of HCAP vulnerability and HCAP-related symptomatology. The higher EL immunostaining in vulnerable and symptomatic HCAP suggests a strong involvement of EL in the local inflammatory process, a key element of plaque destabilization. Whether EL in HCAP is only a marker or an active causative factor for cerebrovascular events and whether EL in HCAP has a prognostic value for the incidence and velocity of carotid artery re-stenosis or other cerebro- and/or cardio-vascular events remains to be examined.

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Conflict of interest statement: Authors declare that they have no conflict of interest.

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Figure legends

Figure 1 Flow chart of the study

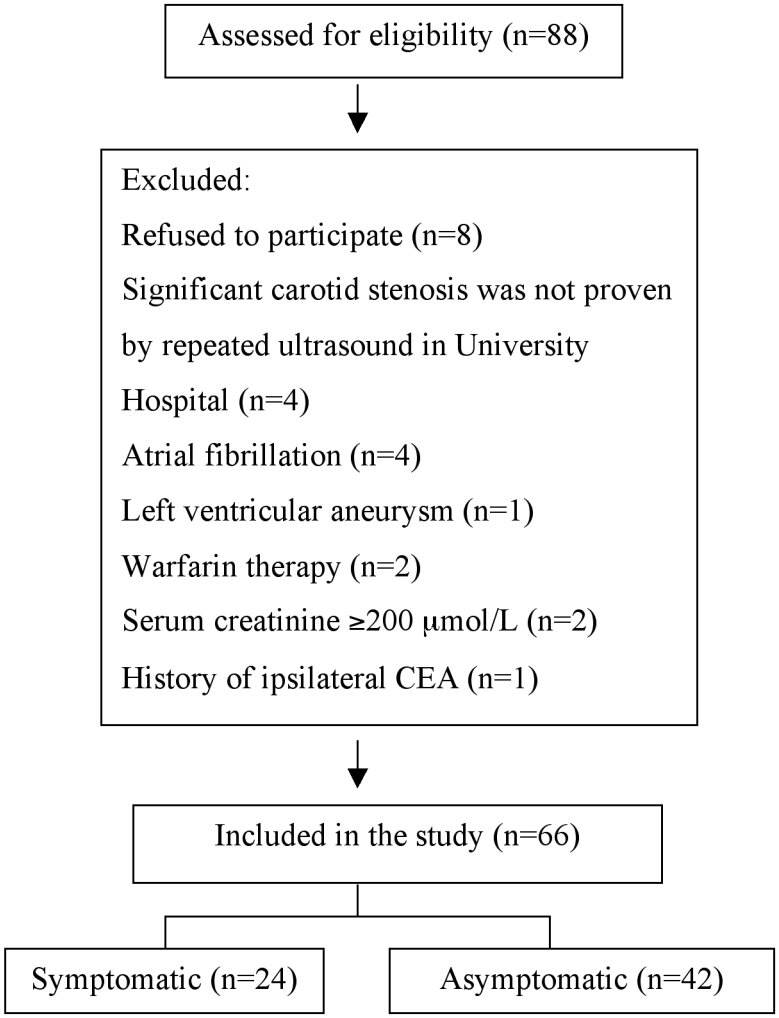

Fig. 1

Figure 2 HCAP histology and immunostaining of EL and CD163

For histology, serial sections of HCAP were stained with HE and for immunohistochemical detection of EL and CD163, with antibodies against EL and the mononocyte/macrophage marker CD163. A fibrous plaque (F) is shown in panels a-c: (a) HE-stained F plaque with fibrous cap (filled arrow heads) and necrotic core (asterisk). (b) EL staining (red) in the endothelium (empty arrows) and in cells within the fibrous cap (filled arrows). (c) CD163 immunostaining (red).

An ulcerated plaque (UC) is shown in panels d-f: (d) HE-stained UC plaque with inflammatory infiltrate (asterisks) and intra-plaque erythrocytes (empty arrow heads). (e) EL immunostaining. (f) CD163 staining. Scale bar represents 50 µm.

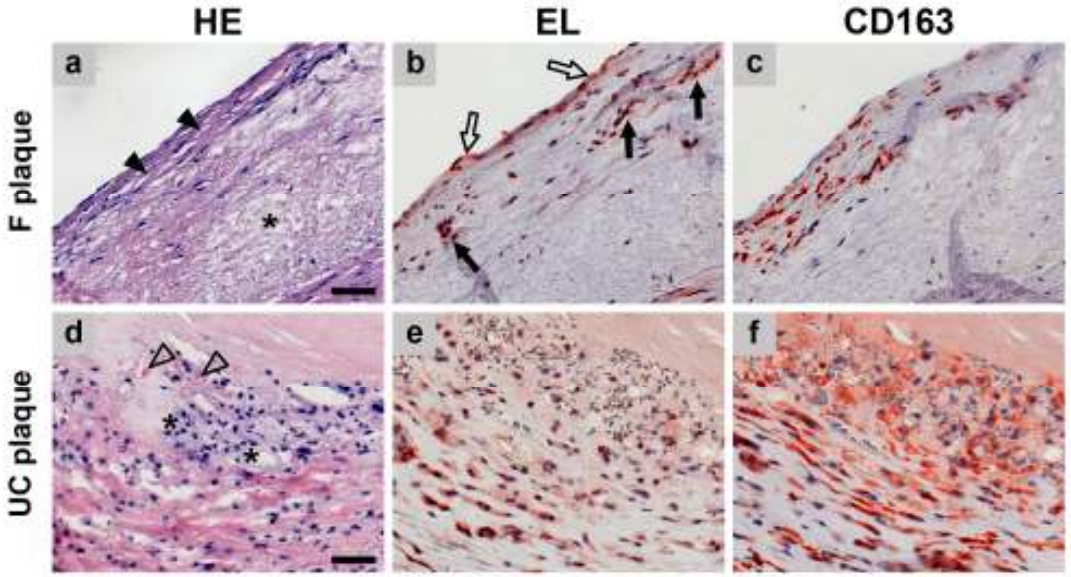

Fig. 2

Figure 3 The intensity of EL immunostaining increases with plaque instability

The intensity of EL immunostaining was determined in different types of plaques, defined by histology as fibrous plaques (F), ulcerated non-complicated plaques (UNC), and ulcerated complicated plaques (UC). Grey columns represent the number of patients exhibiting low intensity of EL immunostaining (A), whereas black columns denote those with high intensity of EL immunostaining (B) in plaques. 79.2% of UC plaques were type B, 50% of UNC plaques were type B, whereas 66.7% of F plaques exhibited type A immunostaining. χ^2 test, p=0.004.

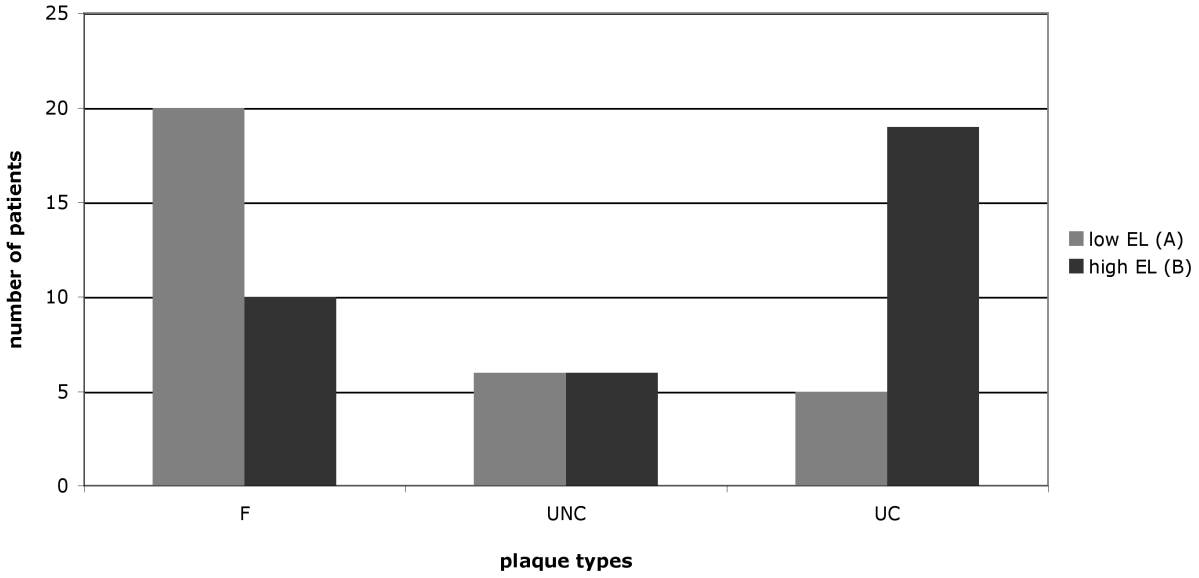

Fig. 3

Table 1. Clinical characteristics and patient medications at the enrollment into the study

Clinical characteristic and medications	asymptomatic (n=42)	symptomatic (n=24)
	(mean±SD)	
Age (years; median, range)	69 (43-83)	65.5 (50-84)
BMI (kg/m^2; mean ±SD)	26.8 ± 3.0	28.2 ± 3.0
Waist circumference (cm; mean±SD)	91.6 ±7.51	94.1 ± 8.5
	No. (%) of patients	
Gender:		
men	28 (66.6)	15 (62.5)
women	14 (33.3)	9 (37.5)
Increased waist circumference†	16 (38.1)	15 (62.5)
Cardiovascular risk factors:		
Arterial hypertension	36 (85.7)	22 (91.6)
Hyperlipoproteinaemia	31 (73.8)	21 (87.5)
Diabetes mellitus or glucose intolerance	20 (47.6)	15 (62.5)
Tobacco (current or recent)	16 (38.0)	14 (58.3)
Family history‡	9 (21.4)	4 (16.7)
Peripheral vascular disease	21 (50.0)	9 (37.5)
Coronary artery disease	12 (28.5)	6 (25.0)
Medications:		
Statins	29 (69.0)	16 (66.6)
ACE inhibitors	21 (50.0)	12 (50.0)
Angiotensin receptor blockers	7 (16.6)	3 (12.5)
Calcium channel blockers	19 (45.2)	8 (33.3)
Acetyl salicylic acid	25 (59.2)	19 (79.1)
No of metabolic elements present		
0	3 (7.1)	1 (4.2)
1	4 (9.5)	2 (8.3)
2	15 (35.7)	5 (20.8)
3	8 (19.0)	4, (16.7)
4	9 (21.4)	11 (45.8)
5	3 (7.1)	1 (4.2)

Abbreviations: BMI–body mass index, ACE–angiotensin-converting enzyme

† Fisher exact test, p=0.049

‡familiar history of cardiovascular disease or stroke in first relatives younger than 55 years

Table 2. Patient blood parameters at the enrollment in the study

Parameter	asymptomatic (n=42)	symptomatic (n=24)
	(mean±SD)	
Platelets ($x10^9$/L)	237 ±67	219 ±52
Hemoglobin (g/L)	137.8 ±14.1	140.5 ±9.1
WBC ($x10^9$/L)	8.21 ±2.4	7.33 ±1.65
fibrinogen (g/L)	4.28 ±0.86	4.62 ±1.02
APTT (sec)	25.2 ±4.6	26.4 ±3.4
PT (%)	104.3 ±11.8	103.6 ±8.9
ESR (mm/h)	18.5 ±8.7	19.2 ±11.2
hs-CRP (μg/mL)	1.4 (0.1-23.0) §	3.6 (0.4-31.5) §
serum creatinine (μmol/L)	95.1 ±21.7	96.7 ±20.4
Total cholesterol (mmol/L)	5.2 ±1.5	5.5 ±1.4
LDL (mmol/L)	3.1 ±1.3	3.5 ±1.3
HDL (mmol/L)	1.3 ±0.4	1.3 ±0.3
Triglycerides (mmol/L)	1.8 ±0.7	1.8 ±0.9
Lp(a) (mg/dl)	23.4 ±21.2	19.9 ±18.7
IL-6 (pg/ml)	1.4 (0.0-15.4) §	1.8 (0.0-43.3) §

Abbreviations: WBC White blood cells; APTT–activated partial thromboplastin time; PT–prothrombin time; ESR–erythrocyte sedimentation rate at first hour; hs-CRP–high sensitivity C-reactive protein; LDL–low density lipoprotein; HDL–high density lipoprotein; IL-6–interleukin-6; Lp(a)–Lipoprotein (a); § results are median (range)

Table 3. Ultrasound and histological characteristics of carotid stenosis

	No. (%) of patients (total n=66)	
	asymptomatic (n=42)	symptomatic (n=24)
Grade of stenosis (%) †		
70-85	18 (42.9)	4 (16.7)
85-95	5 (11.9)	1 (4.2)
95-99	19 (45.2)	19 (79.2)
Morphology of plaques by ultrasound ‡		
fibrous	5 (11.9)	0 (0.0)
soft	11 (26.2)	12 (50.0)
calcified	4 (9.5)	3 (12.5)
mixed	14 (33.3)	9 (37.5)
non-defined	8 (19.0)	0 (0.0)
Histology classification		
fibrous	21 (50.0)	9 (37.5)
UNC	9 (21.4)	3 (12.5)
UC	12 (28.6)	12 (50.0)
Immunohistochemistry for EL §		
A (0-25% cells)	24 (57.1)	7 (29.2)
B (>25% cells)	18 (42.9)	17 (70.8)

Abbreviations: UNC–ulcerated non complicated plaques (without intra-plaque hemorrhage and/or thrombus); UC–ulcerated complicated (with intra-plaque hemorrhage and/or thrombus); EL-endothelial lipase. † χ^2, p = 0.027; ‡ χ^2, p = 0.039; § χ^2, p = 0.028