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Review

The association between biomarkers in the blood and carotid plaque composition-focusing on oxidized lipids, oxysterols and plaque status

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ABSTRACT

Human atherosclerotic plaque is composed of a large mixture of elements, predominantly lipids and oxidized lipids, lipid-loaded macrophages and smooth muscle cells, forming foam cells. Plaque contents undergo dynamic changes during the plaque's progression, being in a constant interaction with the circulating blood. During the mutual interaction between blood and plaque and the specific biochemical processes occurring in both, specific molecules can be generated in the serum which might provide information on plaque status. This information, mostly on plaque vulnerability, is highly important for making appropriate treatment decisions before neurological symptoms appear. The present review summarizes plaque contents, mostly lipids, oxidized lipids, oxidized products of cholesterol (oxysterols), and covers the recent literature on their association with biomarkers in the blood and on the possibility of using them for providing information on plaque status.

1. Atherosclerotic disease

Atheroclerosis is a chronic systemic disease characterized by the accumulation of lipids and oxidized lipids that aggregate in the intima, forming lipid-filled macrophages, called foam cells. Foam cells are a hallmark of early atherosclerosis, the major cause of morbidity and mortality in the western world [1–3]. The lipid-filled macrophages are derived mainly from circulating monocytes that adhere to endothelial cells in the arterial wall, cross the endothelial layer and enter the sub-endothelial space. There they differentiate into mature macrophages that are able to engulf various lipids, including oxidized LDL (Ox-LDL), in an unregulated process which further enhances foam-cell formation [4]. This leads to the formation of arterial lesions known as plaques. Atherosclerosis involves progressive luminal narrowing, which may leads to acute cardiac syndromes with ischemia, myocardial infarction or cerebrovascular events.

2. Plaque – formation, composition, stability (vulnerability)

Plaque formation is a progressive process which includes inflammation, accumulation of lipids and oxidized lipids, thrombosis, proteolysis, apoptosis and angiogenesis within the plaque matrix. The progression of the atherosclerotic plaque from a fatty streak to an advanced atherosclerotic plaque is characterized by increasing levels of Ox-LDL, HDL, phospholipids, arachidonic acid derivatives, and the accumulation of fibrinogen, apo-AI, clusterin and paraoxonase, as well as calcification [5–7]. Developing atherosclerotic lesions contain significant areas of extracellular lipid representing the "core", whereas mature lesions exhibit a complex structure with calcified fibrous areas and visible ulceration [1,2]. Using emerging mass spectrometry techniques, comprehensive lipid profiles of human plaque were assessed, identifying 150 lipid species from nine different classes. This lipid profile elucidated mostly polyunsaturated cholesteryl esters with long-chain fatty acids, predominantly the linoleic acid, sphingomyelin species, phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine and their lyso-derivatives [8], and various oxidized fatty acid metabolites such as aldehydes (4-hydroxynonenal and malondialdehyde). Recently, the phagocyte protein myeloperoxidase was found to be associated with HDL in human atherosclerotic intima and to break down urea to form cyanate in vivo. The cyanate carbamylates the HDL, which contributes to foam-cell formation in the lesion [9]. Protein carbamylation occurs at the site of inflammation which facilitates pro-atherosclerotic activities [10]. Such carbamylation promotes loss of HDL's anti-inflammatory and antioxidant activities, including impaired activity of the HDL-associated antioxidant enzyme paraoxonase 1 (PON1), and HDL's ability to activate lecithin–cholesterol acyltransferase [11]. Plaque contents of patients who had never

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suffered from ipsilateral cerebrovascular events were compared with those from patients who suffered from such events prior to revascularization. Patients in group I had higher plaque smooth muscle cell, more calcified plaques and less intra-plaque hemorrhages [12].

Plaques can be categorized as either stable or vulnerable to rupture. Stable plaques tend to be characterized by a smaller lipid core, a thick fibrous cap, and shoulder regions with few inflammatory cells. Vulnerable plaques contain considerable lipid in their core, a thin fibrous cap, a robust population of macrophages and T cells in their shoulder regions, high matrix metalloproteinase 9 (MMP-9) activity and low collagen and smooth muscle cell content. These differences in morphology suggest that vulnerable plaques are structurally weaker and more likely to rupture in response to the physical forces of blood flow. It is assumed that intra-plaque hemorrhaging in the developing necrotic core is a critical factor in atherosclerotic plaque growth and destabilization [13]. Plaque destabilization has been found to be associated with increased hyaluronic acid metabolism and elevated CD44 levels, presumably due to increased MMP-9 activity and stimulation of angiogenesis [14]. The identification of biomarkers which can provide information on the plaque's stability, whether they will become symptomatic and when symptoms will occur is important. Patient plaque status could dictate the optimal type of intervention and treatment. To date, there is no blood biomarker available for regular clinical use to assess the vulnerability status of human carotid plaque. Biochemical processes occurring in the blood and tissues, including atherosclerotic plaques, impact plaque morphology and stability. These may generate biomarkers in the blood that are linked to a specific process occurring in either the tissue or the blood. Such biomarkers could be used as a complementary information to that collected from the various imaging techniques.

Hermus et al. [15] provide a comprehensive review, covering investigations up until 2010 linking serum biomarker identification to plaque status. Parameters of inflammation such as serum Ox-LDL, phospholipase A2, HDL, C-reactive proteins, cytokines such as interleukin (IL)-6, IL-8 and IL-18, serum amyloid A, interferon γ and TNF-α, and proteolytic enzymes such as MMP-9 and MMP-12, have all been found to correlate with plaque instability [15], although none of these have been selected for routine clinical use.

Activator protein-1 was found to increase in plaques associated with symptomatic patients and with cholesteryl ester content, and was thus suggested as a marker of plaque vulnerability [16]. An interesting factor related to statin therapy which may contribute to the beneficial effects of statin on plaque stabilization, in addition to their cholesterol lowering effect, was suggested [17]. Those authors showed that statins decrease cholesterol crystal density, forming more dissolving crystals in human arteries in vivo and in vitro and thus providing plaque stabilization. Age also seems to affect atherosclerotic plaque stability. A large number of plaques from patients undergoing carotid endarterectomy (1385) were tested showing that plaque stability decreases gradually with age. In elderly patients, larger atheromas and heavy calcifications were observed with a decreased amount of smooth muscle cells in the plaque, all of which contribute to vulnerability [18]. The level of chondroitin sulfate isomers in the plasma was suggested as diagnostic tool for the presence of atherosclerotic plaque [19]. Investigation of fatty acid binding protein (FABP4) expression in carotid atherosclerotic lesions in relation to plaque composition and future cardiovascular events revealed a correlation between FABP4 level and unstable plaque and symptomatic lesions. Patients with increased FABP4 plaque level were at twofold higher risk for cardiovascular events [20].

Investigation of serum neopterin as a biomarker for inflammatory activity in vulnerable carotid plaques revealed that it might be related to the presence of atherosclerotic disease but not to carotid plaque vulnerability [21]. Serum levels of C-reactive protein, IL-6 and TNF-α were found to be closely comparable with the severity of patients' carotid atherosclerotic plaques (Chen Caixia et al. Shandong Yiyao, 51, 62–3 2011, written in Chinese). An additional predictive marker in the plaque's morphology for the occurrence of future vascular events was reported from a prospective study showing that patients whose excised carotid plaque revealed plaque hemorrhage or marked intra-plaque vessel formation, demonstrated increased risk for a primary outcome. Macrophage infiltration, large lipid core, calcification, collagen and smooth muscle cell infiltration were not associated with clinical outcome [7]. Human carotid plaque was shown to contain a lipid fraction with the capacity to facilitate atherogenesis. This lipid extract enhanced LDL and macrophage oxidation and inhibited HDL-mediated cholesterol efflux from macrophages in a dose-dependent manner [22], induced macrophage foam-cell formation [23] and inhibited the HDL-associated antioxidant enzyme PON1. Structural elucidation of the major atherogenic element in the plaque extract revealed that it is linoleic acid hydroperoxide (LA–OOH), which enters into a specific hydrophobic grove within the enzyme, directing its OOH group to the enzyme's thiol Cys^{284} and oxidizing it, probably to sulfenic acid derivative [24]. Modeling study was carried shows that the interaction between the LA–OOH and PON1 is specific whereas arachidonic hydroperoxides or *t*-butyl hydroperoxide do not feat PON1 groove. LA–OOH is a powerful damaging element in the plaque, a correlation between its concentration in the lipid fraction of the plaque to parameters in the blood was carried out [25]. Human carotid plaques were extracted and the level of LA–OOH in each extract was analyzed and compared with various clinical parameters taken from blood tests of the patient carrying the plaque. LA–OOH in the plaque was inversely correlated with HDL level in the circulation and to HDL-PON1 activity (paraoxonase and lactonase activities, Fig. 1), and

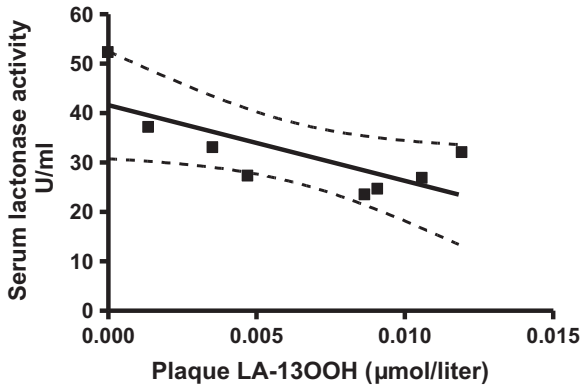

Fig. 1. Interrelation between paraoxonase1 activity (unit/ml) in patients serum versus their plaque linoleic acid hydroperoxide (LA–OOH) content. Human carotid plaques were taken from patients undergoing routine endarterectomy in the Department of Vascular Surgery in Carmel Hospital (Haifa, Israel). Each plaque was ground into a powder by liquid nitrogen and extracted with ethyl acetate. The dried extract was dissolved in DMSO to a final concentration of 50 mg/mL and used for detection of LA–13OOH level by liquid chromatography–mass spectrometry (LC–MS). LA–OOH amount was determined from calibration curves prepared from LA–OOH at position 9 or 13, each synthesized separately from linoleic acid incubated with the specific lipoxigenase. Serum samples of the same individuals were diluted 1:20 and detected for PON1 lactonase activity using 5-(thiobutyl)-butyrolactone (TBBL) as substrate (see Ref. [22]) and correlated to plaque LA–13OOH level. A Pearson's linear regression was calculated using GraphPad Prism 4 software, $r^2 = 0.52$ and p value was $p = 0.04$. The dotted lines present a 95% confidence interval of the regression line. Results of Fig. 1 are representing one set of plaques out of other four separate sets, all showing similar correlation between LA–OOH level in plaque versus serum PON1 lactonase activity.