Atherosclerosis 234 (2014) 185–192

Contents lists available at ScienceDirect

Atherosclerosis

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

Alterations in plasma lecithin:cholesterol acyltransferase and myeloperoxidase in acute myocardial infarction: Implications for cardiac outcome

Robin P.F. Dullaart [a,*], Uwe J.F. Tietge [b], Arjan J. Kwakernaak [a], Bert D. Dikkeschei [c], Frank Perton [a,d], René A. Tio [e]

[a] Department of Endocrinology, University of Groningen, University Medical Center Groningen, P.O. Box 30.001, Groningen 9700 RB, The Netherlands
[b] Department of Pediatrics, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands
[c] Department of Clinical Chemistry, Isala Klinieken, Zwolle, The Netherlands
[d] Laboratory Center, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands
[e] Department of Cardiology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

ARTICLE INFO

Article history:
Received 13 December 2013
Received in revised form
30 January 2014
Accepted 25 February 2014
Available online 11 March 2014

Keywords:
Acute coronary syndrome
Atypical chest pain
Lecithin:cholesterol acyltransferase
Myeloperoxidase
STEMI
Non-STEMI

ABSTRACT

Background: The cholesterol esterifying enzyme, lecithin:cholesterol acyltransferase (LCAT), plays a key role in HDL maturation and remodeling. Myeloperoxidase (MPO) may compromise LCAT enzymatic activity. We tested the extent to which plasma LCAT activity is altered in acute myocardial infarction (MI) in conjunction with abnormal MPO levels. We also assessed the impact of LCAT and MPO on newly developed major adverse cardiovascular events (MACE).
Methods: Two-hundred one consecutive patients referred for acute chest pain of whom 134 had MI (95 with ST-elevation) participated. Forty-five new MACE were ascertained during 1203 (range 13–1745) days of follow-up among 185 patients. Plasma LCAT activity was measured using an exogenous substrate assay. MPO mass was assayed by chemiluminescent microparticle immunoassay.
Results: Plasma LCAT activity was decreased by 15%, coinciding with 7-fold increased MPO levels in acute MI patients vs. patients with non-cardiac chest pain ($p < 0.001$ for both; correlation: $r = -0.343$, $p < 0.001$). MI at admission was associated independently with both lower plasma LCAT activity and higher MPO (age- and sex-adjusted odds ratio per 1 SD increment: 0.46 (95% CI, 0.31–0.68), $p < 0.001$ and 7.58 (95% CI, 3.34–17.11), $p < 0.001$, respectively). In an analysis with LCAT and MPO together these associations were modestly attenuated. MPO mass (hazard ratio: 1.59 (95% CI, 1.15–2.19), $p = 0.004$), but not LCAT activity (hazard ratio: 0.87 (95% CI, 0.65–1.19), $p = 0.39$), predicted newly manifest MACE.
Conclusion: In acute MI patients, plasma LCAT activity is decreased coinciding with increased MPO levels. Higher MPO but not lower LCAT activity prospectively predicts adverse cardiac outcome.

1. Introduction

The high residual risk of cardiovascular disease (CVD) that remains during currently available lipid lowering treatment provides a major rationale to identify novel risk markers and treatment strategies aimed to improve cardiovascular protection [1]. In view of the consistently observed inverse relationship of high density lipoprotein (HDL) cholesterol with the risk of cardiovascular disease (CVD) [2], it is clinically relevant to more precisely delineate the extent to which the development of atherosclerosis is affected by factors involved in HDL metabolism [1].

The enzyme lecithin:cholesterol acyltransferase (LCAT; EC 2.3.1.43), which catalyzes the formation of cholesteryl esters from free cholesterol, has a key function in HDL maturation and remodeling [3–5]. LCAT has long been considered to play a pivotal role in early steps of the anti-atherogenic reverse cholesterol transport (RCT) pathway by creating a concentration gradient of free cholesterol between the cell membrane and extracellular cholesterol acceptors [3]. More recently, the possible impact of LCAT on atheroprotection has been questioned [5,6]. Macrophage-to-feces cholesterol transport, an overall estimate of *in vivo* RCT, is only in part inhibited in LCAT knockout mice, and is not enhanced

* Corresponding author. Tel.: +31 503613731; fax: +31 503619392.
E-mail address: r.p.f.dullaart@umcg.nl (R.P.F. Dullaart).

http://dx.doi.org/10.1016/j.atherosclerosis.2014.02.026
0021-9150/

in LCAT overexpression models [7]. In addition, genetic *LCAT* deficiency, which causes low HDL cholesterol, does not unequivocally determine increased carotid artery intima media thickness (IMT) [5], an established biomarker of subclinical atherosclerosis. Also, higher plasma LCAT activity or mass levels are not predictive of a lower IMT [8,9], and higher plasma LCAT levels do not confer decreased risk of future cardiovascular disease in population studies [10,11]. Of further interest, both higher and lower plasma LCAT activity has been documented in subjects with chronic ischemic heart disease [12,13]. With few exceptions all these studies were carried out in clinically stable subjects [8–14]. It is, therefore, relevant to document whether plasma LCAT activity is altered in the acute phase of coronary artery disease.

Importantly, HDL particles may lose their anti-oxidative functionality in acute coronary syndrome, which contributes to increased oxidative stress [15]. Interestingly, both apolipoprotein (apo) A-I, a key activator of LCAT, and LCAT protein itself are modified by oxidative stress [16–19]. One such oxidative pathway involves myeloperoxidase (MPO), a heme-containing enzyme that is abundantly released from activated leukocytes and monocytes during pro-inflammatory conditions such as acute coronary syndrome [17,20,21]. MPO is able to generate hypochlorous acid (HOCl) from hydrogen peroxide at physiological chloride concentrations [16,17]. As a consequence, HOCl modifies critically important apoA-I amino acid residues, which has been shown to result in impaired LCAT enzymatic activity [18]. In addition, HOCl-modified low density lipoproteins may also compromise LCAT enzymatic activity *in vitro* [19]. In this context it is relevant that plasma MPO is elevated in acute coronary syndrome patients, has been shown to predict major adverse cardiovascular events (MACE), and may improve risk stratification for acute coronary syndrome [21,22]. Collectively, these findings raise the possibility that plasma LCAT activity is decreased, whereas MPO is increased in the setting of an acute coronary syndrome.

Our present study was initiated first to simultaneously compare plasma LCAT activity and MPO levels between myocardial infarction (MI) patients and patients with non-cardiac chest pain in the acute setting. Second, we determined the impact of plasma LCAT activity and MPO, as obtained in the acute setting, on newly manifest MACE during follow-up.

2. Patients and methods

2.1. Patient recruitment, end-point validation and risk factor assessment

The patients included in the present study participated in a protocol, the Quick Identification of acute Chest pain patients Study (QICS) [23]. The primary aim of QICS is to determine the diagnostic yield of biomarkers in discriminating between cardiac and non-cardiac causes of acute chest pain. A more detailed description of the study design, inclusion criteria, and routine laboratory measurements has been published [23]. Results with respect to biomarker outcome have been reported elsewhere [24]. The protocol complies with the Declaration of Helsinki, and has been approved by the responsible medical ethics committee of the University Medical Center Groningen. QICS involves the anonymous storage of frozen plasma samples, for which approval from the local ethical committee was requested but waved, because such storage is performed as standard for additional testing in order to evaluate future clinically important diagnostic considerations.

Patients referred with chest pain suspected for having an acute coronary syndrome to either the emergency department or the coronary care unit of the University Medical Center Groningen, The Netherlands, were enrolled if they presented at weekdays between 8 a.m. and 5 p.m. The University Medical Center Groningen serves as regional referral center for STEMI patients [23]. For the present analysis the time frame of enrollment of patients was between 2006 and 2008. Patients with MI were categorized as MI without ST-elevation (non-STEMI) and MI with ST-elevation (STEMI), as judged by attending cardiologists following international guidelines that were used during the inclusion period [25,26]. To be eligible other clues for a cardiac origin, pulmonary embolism or aortic pathology had to be absent. If sequential electrocardiograms and laboratory tests, in particular troponin T (determined again at least 6 h after onset of chest pain) were normal, patients were classified as having non-cardiac chest pain, and were discharged in case of low suspicion or if a bicycle exercise test was negative [27]. Clinical characteristics and laboratory and electrocardiographic data were obtained from medical records and hospital case records [23,24]. Blood pressure was measured at least twice at admission using a sphygmomanometer. The maximal levels of cardiac enzymes were established in each patient during the in-hospital observation period. Previously diagnosed hypertension was established by primary care physicians (defined as systolic blood pressure $>$ 140 mm Hg and/or diastolic blood pressure $>$ 90 mm Hg and/or initiation of antihypertensive medication). Diabetes mellitus had been previously diagnosed using the Dutch College of General Practitioners guidelines (fasting plasma glucose $\geq$ 7.0 mmol/l and/or non-fasting plasma glucose $\geq$ 11.1 mmol/l) [28]. Smoking was defined as current cigarette smoking.

2.2. Follow-up

From all participants follow-up data were obtained with respect to newly manifest MACE, which was chosen as the combined end-point. MACE was defined as cardiac mortality, MI, percutaneous coronary intervention, and/or coronary artery bypass grafting. Cardiac mortality was defined as death due to acute MI, heart failure or sudden cardiac death. Causes of death were obtained by linking the number of the death certificate to the primary cause of death as coded by the Central Bureau of Statistics (Voorburg/Heerlen, The Netherlands). Recurrent MI was diagnosed with international criteria that were used during the follow-up period [25,26,29]. Census date was chosen as the date of newly manifest MACE or as the date of the latest verification of the status of the patient, which was at least 1000 days after initial presentation.

2.3. Laboratory analyses

Venous blood samples were obtained directly after admission for routine measurements, including troponin T, creatinine kinase (CK) and the MB fraction of CK (CK-MB). Additionally, venous blood was collected in EDTA-containing tubes (1.5 mg/ml) for subsequent assay of lipids and lipoproteins, LCAT activity, MPO and other assays. These plasma samples were prepared by centrifugation at 1400 g for 15 min at 4 °C, and were kept frozen at −80 °C until analysis.

Plasma cholesterol was assayed by a routine enzymatic method (Roche/Hitachi cat no 11875540; Roche Diagnostics GmbH, Mannheim, Germany). HDL cholesterol was measured with a homogeneous enzymatic colorimetric test (Roche/Hitachi, cat no 04713214; Roche Diagnostics GmbH, Mannheim, Germany). Non-HDL cholesterol was calculated as the difference between total cholesterol and HDL cholesterol. LDL cholesterol was calculated by the Friedewald formula. Apolipoprotein (apo) A-I and B were assayed by immunoturbidimetry (Roche/Cobas Integra Tina-quant cat no. 03032566 and 033032574, respectively, Roche Diagnostics).

Plasma LCAT activity was determined using excess exogenous substrate containing [^{3}H]-cholesterol as described [8,30]. In brief,