International Journal of Cardiology 177 (2014) 847–853

Contents lists available at ScienceDirect

International Journal of Cardiology

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

Circulating acute phase proteins in relation to extent and composition of coronary atherosclerosis and cardiovascular outcome: Results from the ATHEROREMO-IVUS study ☆

Linda C. Battes, K. Martijn Akkerhuis, Jin M. Cheng, Hector M. Garcia-Garcia, Rohit M. Oemrawsingh, Sanneke P.M. de Boer, Evelyn Regar, Robert-Jan van Geuns, Patrick W. Serruys, Eric Boersma, Isabella Kardys *

Department of Cardiology, Erasmus MC, Rotterdam, The Netherlands

ARTICLE INFO

Article history:
Received 22 May 2014
Accepted 1 November 2014
Available online 4 November 2014

Keywords:
Acute coronary syndrome
Acute phase proteins
Atherosclerosis
Intravascular ultrasound

ABSTRACT

Introduction: We examined whether the acute phase proteins (APPs): Alpha-1-Antitrypsin, Alpha-2-Macroglobulin, Complement C3, ferritin, haptoglobin, and Plasminogen Activator Inhibitor 1 (PAI-1) are associated with cardiovascular outcome, as well as with the extent and composition of coronary atherosclerosis as determined by intravascular ultrasound (IVUS) virtual histology (VH).
Methods: In 2008–2011, IVUS(-VH) imaging of a non-culprit coronary artery was performed in 581 patients from the ATHEROREMO-IVUS study undergoing coronary angiography for acute coronary syndrome (ACS) (n = 318) or stable angina pectoris (SAP) (n = 263). Coronary atherosclerotic plaque volume, composition (fibrous, fibro-fatty, dense calcium and necrotic core) and vulnerability (VH-derived thin-cap fibroatheroma (TCFA) lesions) were assessed. Major adverse cardiac events (MACE; all-cause mortality, ACS or unplanned coronary revascularization) were assessed during 1-year follow-up. We applied linear, logistic and Cox regression.
Results: Mean age was 61.5 ± 11.4 years and 75.4% were men. Higher ferritin was associated with higher coronary plaque volume (beta [95% CI]: 0.19 [0.07–0.31] percent atheroma volume), for the highest vs the lowest tertile of ferritin; p for linear association = 0.013. Higher PAI-1 was associated with higher rates of all-cause mortality or ACS (hazard ratio [95% CI]: 2.98 [1.10–8.06]), for the highest vs the lowest tertile of PAI-1. No clear-cut associations could be demonstrated between APPs and composition of atherosclerosis or plaque vulnerability.
Conclusions: Higher circulating ferritin was associated with higher coronary plaque volume, and higher PAI-1 was associated with higher incidence of all-cause mortality or ACS. None of the APPs displayed consistent associations with composition of atherosclerosis or plaque vulnerability.

1. Introduction

Chronic inflammation of the arterial wall plays an important role in the development of atherosclerosis, and it regulates aspects of plaque biology that trigger the thrombotic complications of atherosclerosis [1]. Inflammation is commonly characterized by increased plasma concentrations of acute phase proteins (APPs). Several studies have demonstrated the ability of the APP C-reactive protein (CRP) to predict adverse coronary events in patients with stable and unstable coronary artery disease (CAD) [2]. In order to further explore the nature of the association of CRP with coronary atherosclerosis, we have previously examined its relation with intravascular ultrasound (IVUS) virtual histology (VH) derived measures of coronary atherosclerosis [3]. The results showed that higher CRP levels were associated with a higher coronary plaque burden, but they were not associated with plaque vulnerability, which was defined as the presence of IVUS-VH- derived thin-cap fibroatheroma (VH-TCFA) lesions. The relation between other APPs and cardiovascular disease has generally been examined to a much smaller extent, and in particular studies on APPs in relation to an in-vivo assessment of the extent and composition of coronary atherosclerosis are lacking [4,5].

APPs including Alpha-1-Antitrypsin (AAT), Alpha-2-Macroglobulin (α2M), Complement C3 (C3), ferritin, haptoglobin, and Plasminogen Activator Inhibitor 1 (PAI-1), are produced by the liver in response to circulating cytokines [6]. APPs contribute to the restoration of homeostasis by neutralizing inflammatory agents, help to minimize the extent of local tissue damage and participate in tissue repair and regeneration [6]. Circulating levels of APPs may potentially be useful for risk stratification of patients with known CAD, and studies on their relation with

☆ Funded by: The ATHEROREMO-IVUS study was funded by the European Commission, Seventh Framework Programme (FP7), theme FP7-HEALTH-2007-2.4.2-1. In addition, the research described in this paper was funded by BBMRI-NL (a research infrastructure financed by the Dutch government, NWO 184.021.007). Jin M. Cheng and Rohit M. Oemrawsingh are supported by the Netherlands Heart Foundation (grant numbers NHS2009B091 and NHS2007B012 respectively).

* Corresponding author at: Erasmus MC, Thoraxcenter, Room Ba-561, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands.
E-mail address: i.kardys@erasmusmc.nl (I. Kardys).

http://dx.doi.org/10.1016/j.ijcard.2014.11.001
0167-5273/

the extent and composition of coronary atherosclerosis may provide further pathophysiological insights with regard to the mechanisms of progression and destabilization of atherosclerotic plaques.

Therefore, the purpose of this study is to examine the associations of AAT, α2M, C3, ferritin, haptoglobin, and PAI-1 with the extent and composition of coronary atherosclerosis as determined in-vivo by IVUS-VH, in patients undergoing coronary angiography. Furthermore, the prognostic value of the APPs for major adverse cardiac outcome during 1 year follow-up in these patients is investigated.

2. Material and methods

2.1. Study population

The design of the European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis-Intravascular Ultrasound (ATHEROREMO-IVUS) study has been described elsewhere [7]. In brief, 581 patients who underwent diagnostic coronary angiography or percutaneous coronary intervention (PCI) for acute coronary syndrome (ACS) or stable angina pectoris (SAP) have been included from November 2008 to January 2011 in the Erasmus MC, Rotterdam, the Netherlands. Intravascular ultrasound (IVUS) of a non-culprit coronary artery was performed subsequent to angiography. The ATHEROREMO-IVUS study has been approved by the Human Research Ethics Committee of Erasmus MC, Rotterdam, the Netherlands. Written informed consent was obtained from all included patients and the study protocol conforms to the ethical guidelines of the Declaration of Helsinki.

2.2. Biomarkers

Blood samples were drawn from the arterial sheath prior to the diagnostic coronary angiography or PCI procedure, and were available in 570 patients for the current study. The blood samples were transported to the clinical laboratory of the Erasmus MC for further processing and storage at a temperature of −80 °C within 2 h after blood collection. CRP was measured in the clinical laboratory of the Erasmus MC in serum samples using a immunoturbidimetric high sensitivity assay (Roche Diagnostics Ltd., Rotkreuz, Switzerland) on the Cobas 8000 modular analyzer platform (Roche Diagnostics Ltd., Rotkreuz, Switzerland). Frozen EDTA-plasma samples were transported under controlled conditions (at a temperature of −80 °C) to Myriad RBM, Austin, Texas, USA, where the concentrations of AAT, α2M, C3, ferritin, haptoglobin, and PAI-1, were measured using a validated multiplex assay (Custom Human Map, Myriad RBM, Austin, Texas, USA). While ferritin, haptoglobin, and PAI-1 were determined in the full cohort of 570 patients, AAT, α2M, and C3, were determined in a random subset of 473 patients. This difference in numbers resulted from batch-wise handling of the samples in combination with an update of the composition of the multiplex assay by the manufacturer in-between two batches.

2.3. Intravascular ultrasound

Following the standard coronary angiography or PCI procedure, IVUS data were acquired in a non-culprit coronary artery without significant coronary disease requiring balloon angioplasty or stent treatment. The order of preference for the selection of the non-culprit vessel was: 1. left anterior descending (LAD) artery; 2. right coronary artery (RCA); and 3. left circumflex (LCX) artery. All IVUS data were acquired with the Volcano s5/s5i Imaging System (Volcano Corp., San Diego, CA, USA) using a Volcano Eagle Eye Gold IVUS catheter (20 MHz). An automatic pullback system was used with a standard pull back speed of 0.5 mm per second. The IVUS images were analyzed offline by an independent core laboratory (Cardialysis BV, Rotterdam, the Netherlands) that had no knowledge of clinical data. The IVUS radiofrequency analyses, also known as IVUS virtual histology (IVUS-VH), were performed using pcVH 2.1 and qVH (Volcano Corp., San Diego, CA, USA) software. The external elastic membrane and luminal borders were contoured for each frame (median interslice distance, 0.40 mm). Extent and phenotype of the atherosclerotic plaque were assessed.

Plaque volume was defined as the percent of the volume of the external elastic membrane occupied by atheroma, i.e. percent atheroma volume [8]. Plaque volume was normalized for the length of the imaged segment. Plaque burden was defined as the plaque and media cross-sectional area divided by the external elastic membrane cross-sectional area and is presented as a percentage. A coronary lesion was defined as a segment with a plaque burden of more than 40% in at least 3 consecutive frames. Using IVUS-VH, the composition of the atherosclerotic plaque was characterized into 4 different types: fibrous, fibro-fatty, dense calcium and necrotic core [9]. A IVUS-VH-derived thin-cap fibroatheroma (VH-TCFA) lesion was defined as a lesion with the presence of >10% confluent necrotic core in direct contact with the lumen.

2.4. Clinical study endpoints

In this study, follow-up lasted up to 1 year after angiography. Post-discharge survival status was obtained from municipal civil registries. Post-discharge rehospitalizations were prospectively assessed. Questionnaires focusing on the occurrence of major adverse cardiac events (MACE) were sent to all living patients. Subsequently, hospital discharge letters were obtained and treating physicians and institutions were contacted for additional information whenever necessary. The primary endpoint was the occurrence of MACE, defined as the composite of all-cause mortality, ACS or unplanned coronary revascularization. The secondary endpoint was the composite of all-cause mortality or ACS. ACS was defined as the clinical diagnosis of ST segment elevation myocardial infarction (STEMI), non-STEMI or unstable angina pectoris in accordance with the guidelines of the European Society of Cardiology [10,11]. Unplanned coronary revascularization was defined as unplanned repeat PCI or coronary artery bypass grafting (CABG). The endpoints were adjudicated by a clinical event committee that had no knowledge of biomarkers and IVUS data.

2.5. Statistical analysis

Categorical variables are presented in percentages. The distributions of continuous variables, including biomarker levels and IVUS parameters, were examined for normality by visual inspection of the histogram and calculation of the skewness coefficient. Normally-distributed continuous variables are presented as mean ± standard deviation (SD), while non-normally distributed continuous variables are presented as median and interquartile range (IQR). For reasons of uniformity, all biomarker levels are presented as medians (with IQR).

In further analyses, biomarker concentrations were examined both as continuous and as categorical variables (the latter by dividing the variables into tertiles). Biomarkers with a non-normal distribution were ln-transformed or were transformed by using the square root.

First, we examined associations of biomarker concentrations with the extent of atherosclerosis according to IVUS. We applied linear regression analyses with biomarker concentrations as the independent variable (transformed or categorized when appropriate) and, consecutively, plaque volume and plaque burden in the imaged coronary segment as the dependent variable. The results are presented as βs (per unit increase in transformed biomarker concentration or per category of biomarker concentration) with 95% confidence intervals (95% CIs).

Subsequently, we examined the associations between biomarker concentrations and 4 types of atherosclerosis composition (fibrous, fibrofatty, necrotic core, and dense calcium), each expressed in percentages. The results are again presented as βs. We also examined the associations between biomarker concentrations and the presence of VH-TCFA lesions by using logistic regression analyses with biomarker concentrations as the independent variable. The results are presented as odds ratios (ORs) per unit increase in transformed biomarker concentration or per category of biomarker concentration, with 95% CIs.

Moreover, we examined associations of biomarker concentrations with MACE and with the composite of all-cause mortality or ACS, during 1 year follow-up. Patients lost to follow-up were considered at risk until the date of last contact, at which time-point they were censored. We used Cox proportional hazard regression analyses with biomarker concentration as the independent variable. The results are presented as hazard ratios (HRs) per unit increase in ln-transformed biomarker concentration or per category of biomarker concentration, with 95% CIs.

All above-described analyses were performed univariably. Subsequently, we adjusted for age, gender, indication for coronary angiography, diabetes, hypertension and CRP. Additionally, to further examine possible effect modification by indication for baseline coronary angiography, we repeated the analyses separately in patients with acute coronary syndrome and in patients with stable angina pectoris.

All data were analyzed with SPSS software (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY, USA). All statistical tests were two-tailed and p-values < 0.05 were considered statistically significant.

3. Results

3.1. Baseline characteristics

Baseline characteristics are summarized in Table 1. Mean age was 61.5 ± 11.4 years and 75% were men. Coronary angiography or PCI was performed for several indications: 159 (28%) patients had an acute myocardial infarction, 150 (26%) patients had unstable angina pectoris and 261 (46%) patients had stable angina pectoris. The median length of the imaged coronary segment was 44.1 [33.7–55.4] mm. C3 was the only biomarker with a normal distribution. AAT, α2M, ferritin, haptoglobin and PAI-1 concentrations were not normally distributed; haptoglobin was square root-transformed for further analyses, and the remaining biomarkers were ln-transformed.

3.2. Biomarkers and extent of atherosclerosis

The results of the analyses for (ln-transformed) normalized plaque volume normalized for the length of the segment are shown in Table 2. Higher ferritin levels were associated with higher coronary plaque volume (β [95% CI]: 0.19 [0.07–0.31], for the highest vs the lowest tertile of ferritin, and β [95% CI]: 0.14 [0.02–0.27], for the middle vs