Differential associations between blood biomarkers of inflammation, oxidation, and lipid metabolism with varying forms of coronary atherosclerotic plaque as quantified by coronary CT angiography

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Abstract

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Although epidemiologic data link biomarkers of cardiovascular risk with incident and prevalent coronary artery disease, exact anatomic relationships between biomarkers and coronary atherosclerosis as measured by coronary CT angiography remain unclear. Patients with acute chest pain who ultimately had no evidence of acute coronary syndrome underwent contrast-enhanced 64-slice coronary CT angiography to determine presence, extent and composition of coronary atherosclerotic plaque. We determined the differences in levels of blood biomarkers measured at the time of the CT scan between different CT-based atherosclerotic plaque groups. Among 313 patients (mean age: 51.6 ± 11 years, 62% male) high-sensitivity C-reactive protein (hs-CRP) and matrix metalloproteinase-2 were associated with the extent of calcified plaque ($P = 0.03$ and $P<0.001$), while hs-CRP and apolipoprotein A1 were associated with the extent of non-calcified plaque ($P = 0.03$ and $P = 0.004$; respectively). Despite a generally lower risk profile, subjects with exclusively non-calcified plaque had significantly higher levels of hs-CRP and oxidized low-density lipoprotein ($P = 0.01$ and $P = 0.03$; respectively) and lower levels of adiponectin ($P = 0.03$) when compared to subjects with calcified plaque (n = 130, 42%). Biomarkers reflecting inflammation, vascular remodeling, oxidation, and lipoprotein metabolism maybe associated with different patterns of coronary atherosclerosis as quantified by coronary CT angiography.

Keywords

Biomarkers; Atherosclerosis; Cardiac CT; Imaging; Coronary artery disease

Introduction

A large number of blood biomarkers involved in inflammation, oxidation, and lipid metabolism have been demonstrated to play an important role in atherogenesis [1]. Several of those biomarkers such as high-sensitivity C-reactive protein (hsCRP), tumor necrosis factor alpha (TNF), and oxidized low density lipoprotein (ox-LDL) have also been shown to be independent predictors of cardiovascular events [2].

With advances in imaging of coronary atherosclerosis comes the opportunity to better understand the link(s) between biomarkers predictive of disease presence and complication with the anatomic diagnosis of coronary atherosclerosis. Similarly, as various forms of coronary atherosclerosis, including the presence and extent of coronary artery calcification (CAC) variably predicts cardiovascular events independent of traditional risk profile [3], an opportunity to better understand plaque biology (as detected with computed tomography imaging) using biomarkers is also present. Moreover, with advances in contrast-enhanced computed tomography angiography (CTA), an opportunity to not only evaluate CAC but also detect and quantify non-calcified plaque components is now possible.

As it remained unclear which systemic biomarkers covering different areas of atherogenesis are most closely associated with the extent of atherosclerosis and composition of coronary atherosclerotic plaque, we decided to examine this question in detail, measuring a wide array of biomarkers in patients with detailed CTA examination. Our hypothesis was that there is a correlation between systemic and local markers of atherosclerosis.

Methods

Study sample

This analysis was performed in patients from the "Rule Out Myocardial Infarction Using Computer Assisted Tomography" (ROMICAT) study, designed to assess the clinical utility of coronary CTA to triage subjects with acute chest pain in the emergency department [4]. We included consecutive adult subjects presenting to the ED with acute chest pain but

without diagnostic electrocardiographic (ECG) changes or positive initial cardiac biomarkers (troponin and CK-MB) but excluded subjects who developed an acute coronary syndrome (ACS, as defined by ST-elevation myocardial infarction [MI], non-ST-elevation MI, or unstable angina) and subjects with inflammatory diseases for this study. All patients underwent coronary CTA and blood sampling before hospital admission. The institutional review board of the Massachusetts General Hospital approved the study. All participants provided written informed consent.

Blood sampling

Blood samples were collected within 1 h prior to the coronary CTA scan and stored at $-70°C$ until analysis. We selected a representative set of serum biomarkers covering aspects of atherogenesis: inflammation, oxidation, and lipid metabolism (Table 2). All measurements were performed in an independent laboratory (Biomarker Laboratory at the Department of Cardiology, University of Ulm, Germany) blinded to the patients clinical and CT findings. Concentrations of hs-CRP, lipoprotein (Lp)(a), and Apo A1 were measured nephelometrically on a BN II analyzer (Dade-Behring, Marburg, Germany). Enzyme-linked immunosorbent assays (ELISA) from R&D Systems (Wiesbaden; Germany) were used to measure TNF-$α$, MMP-2, and adiponectin. An ELISA was also used to determine levels of MPO (Immundiagnostik, Bensheim, Germany), Lp-PLA$_2$ (PLAC, Diadexus, Inc. South San Francisco, CA), and ox-LDL (Mercodia, Uppsala, Sweden). Inter-assay coefficients of variation were<12% for ELISAs, and<5% for nephelometry and for chemoluminescence.

Coronary CT angiography

All subjects underwent ECG-gated contrast enhanced 64-slice coronary CTA (Sensation 64, Siemens Medical Solutions, Forchheim, Germany) with image acquisition during an inspiratory breath-hold. Pre-scan sublingual nitroglycerin and intravenous beta-blockers were given for all subjects with a heart rate greater than 60 beats per minute and no contraindications present.

CT scan parameters included: 32×0.6 mm slice collimation, gantry rotation time of 330 ms, tube voltage of 120 kV, effective tube current of 850–950 mAs, and ECG correlated tube current modulation (when appropriate). The average radiation dose of 14.8 ± 3.8 mSv was estimated based on the measured dose length product (DLP) multiplied by the conversion factor 0.017 mSv mGy^{-1} cm^{-1}. A total of 64 overlapping 0.6 slices per rotation were acquired with the use of a focal spot periodically moving in the longitudinal direction. Contrast agent (Iodhexodol 320 g/cm^3, Visipaque, General Electrics Healthcare, Princeton, NJ) was injected intravenously at a rate of 5 mL per second (mean volume 78 ± 11 mL) with use of a test bolus.

Overlapping transaxial images were reconstructed using a medium sharp convolution kernel (B25f) with an image matrix of 512×512 pixels, slice thickness and increment of 0.75/0.4 mm using an ECG gated half-scan algorithm with a resulting temporal resolution 165 ms in the center of rotation. Image reconstruction was retrospectively gated to the ECG. The position of the reconstruction window within the cardiac cycle was individually optimized to minimize motion artifacts.

Coronary CTA analysis

On average, three data sets per subject were reconstructed. Reconstructed CTA data sets of all subjects were transferred to an offline workstation (Leonardo, Siemens Medical Solutions, Forchheim, Germany) and the reconstruction with the highest image quality was used for further analysis. CTA data sets were interpreted for the presence, extent, and

composition of coronary atherosclerotic plaque on a segmental level using the modified American Heart Association classification (with 17-coronary segments) [5].

Non-calcified plaque (NCAP) was defined as any clearly discernible structure that could be assigned to the coronary artery wall in at least two independent image planes and had a CT density less than 130 HU but greater than the surrounding connective tissue. Calcified atherosclerotic plaque was defined as any structure with a density of 130 HU or more that could be visualized separately from the contrast-enhanced coronary lumen (because its density was above the contrast-enhanced lumen), that could be assigned to the coronary artery wall, and that could be identified in at least two independent planes. Mixed coronary atherosclerotic plaque was defined as the presence of non-calcified and calcified plaque, either because it was "embedded" within non-calcified plaque or adjacent to each other within a coronary segment. All plaque components were assessed on a per segment basis. This approach has previously been validated [6] and shown to have excellent inter-observer agreement on a per subject and per segment level (Cohen's κ = 0.92 and 0.81, respectively) [7].

Covariates and risk factor assessment

Presence of cardiovascular risk factors was established from measurements obtained during the index hospitalization. Hypertension was defined as systolic blood pressure of at least 140 mmHg or diastolic blood pressure of at least 90 mmHg or current antihypertensive treatment. Diabetes mellitus was defined as a fasting plasma glucose ≥126 mg/dL or treatment with a hypoglycemic agent. Hyperlipidemia was defined as total cholesterol of ≥200 mg/dL or treatment with a lipid lowering agent. Subjects were classified as smokers if they had smoked at least one cigarette per day in the year prior to the study. Body-mass index (BMI) was defined as weight (kilograms) divided by the height (meters) squared. Family history (Fx) of coronary artery disease (CAD) was defined as having a first-degree female (<65 years) or male (<55 years) relative with a documented history of MI or sudden cardiac death. Subjects were classified as high-risk (>20%), intermediate (10–20%), or low-risk (<10%) according to their Framingham Risk Score (FRS) [8].

Statistical analysis

Descriptive characteristics for all variables (demographics/risk factors, blood biomarkers, and plaque findings) are expressed as means ± SD for normally distributed continuous variables, medians with interquartile range (IQR) for non-normally distributed continuous variables, and percentages for categorical variables. Differences regarding demographics, cardiovascular risk factors and drug medication between subjects with no plaque, with exclusively non-calcified plaque, and with calcified plaque were tested using Kruskal–Wallis test for continuous variables and Fisher's Exact test for categorical variables. We performed two non-parametric exploratory analyses. First we compared median concentrations of each biomarker in subjects with exclusively non-calcified to subjects with no plaque or with calcified plaque using Wilcoxon Rank Sum test. Biomarkers with significant differences were included in multivariate analysis using quantile regression. A simplex algorithm as described by Barrodale and Roberts [9] has been used to estimate adjusted median, upper and lower quantiles for a 52 year old male subject with none of the included cardiovascular risk factors or drug treatments. The 95% confidence intervals (95% CI) for the differences in biomarkers concentrations between the above described subgroups (no plaque, exclusively non-calcified plaque, calcified plaque) were computed by inverting the rank score test. If the 95% CI excluded zero, the difference was considered as significant with a $P<0.05$. Two different models were fitted. One adjusted for age, gender, and number of cardiovascular risk factors. The second model included additionally all potential confounders, which were different with $P<0.10$ between patients in univariate analysis.

Second, we estimated the association between levels of biomarkers and the extent of plaque (overall plaque burden, burden of non-calcified plaque, and burden calcified plaque) using Spearman's rank correlation. In multivariate analysis, these correlations were adjusted using partial Spearman's rank correlation similar to the approach described above.

All analyses were performed using SAS (Version 9.1, SAS Institute Inc., Cary, NC, USA) and a two sided p-value of <0.05 was considered statistically significant.

Results

From 412 subjects initially enrolled in the study, we excluded 99 subjects due to the occurrence of an ACS during index hospitalization (n = 38), other inflammatory processes (n = 30), prior history of CAD (n = 17), incomplete CTA scanning (n = 10), and four subjects who did not consent to a blood draw (n = 4). Thus, our study cohort consisted of 313 patients (mean age: 51.6 ± 11 years, 62% male; Table 1).

Atherosclerotic plaque burden

Based on coronary CTA, 145 (46%) subjects had any coronary atherosclerotic plaque and 168 (54%) subjects had no plaque; 5.1% had a significant coronary stenosis (n = 16). Among subjects with CAD, 15 (10%) had exclusively non-calcified plaque and 130 (90%) had any calcified plaque. Baseline demographics and cardiovascular risk factors and levels of blood biomarkers stratified by atherosclerotic plaque group are detailed in Table 1 and 2; respectively. In patients with CAD, 4.25 ± 3.49 (range 1–16) segments contained any plaque. On average, non-calcified and calcified plaque were detected in 1.76 ± 2.15 (range 1–14) and 3.71 ± 3.49 (range 1–15) coronary segments; respectively.

Blood biomarkers stratified by presence and composition of coronary atherosclerotic plaque

Subjects with exclusively non-calcified plaque had significantly higher levels of hs-CRP (2.68 [1.44, 8.42] vs. 1.05 [0.41, 2.40], $P = 0.0005$; Fig. 1a), and a trend towards higher concentrations of ox-LDL (76.1 [67.4, 91.5] vs. 68.1 [54.5, 86.3], $P = 0.07$; Fig. 1b) as compared to subjects without plaque. Further, such patients had lower levels of Adiponectin (3.36 [2.16, 4.95] vs. 4.62 [2.90, 7.57]; $P = 0.03$; Fig. 1c) as compared to subjects without plaque. However, there was no difference in age, gender, or the number of cardiovascular risk factors between subjects with non-calcified plaque and subjects without plaque (48.7 ± 5 vs. 46.7 ± 9 years, $P = 0.27$; 73 vs. 57% male, $P = 0.28$; and 0.83 vs. 0.9; $P = 0.7$; respectively).

In comparison to subjects with calcified plaque, subjects with exclusively non-calcified plaque had a trend towards higher levels of hs-CRP (2.68 [1.44, 8.42] vs. 2.04 [0.95, 3.58]; $P = 0.08$; Fig. 1a), higher ox-LDL (76.1 [67.4, 91.5] vs. 63.7 [47.1, 83.7]; $P = 0.04$; Fig. 1b) and lower levels of adiponectin (3.36 [2.16, 4.95] vs. 5.55 [3.30, 9.77], $P = 0.02$; Fig. 1c). Notably, subjects with non-calcified plaque were significantly younger and had fewer cardiovascular risk factors than subjects with calcified plaque (48.7 ± 5 vs. 58.2 ± 11 years, $P<0.001$ and 0.9 vs. 1.7 cardiovascular risk factors, $P = 0.03$; respectively) and did not differ with respect to gender (73 vs. 67% males, $P = 0.77$; respectively).

No significant differences were found for levels of hs-CRP, ox-LDL, or Adiponectin between subjects with mixed plaque as compared to subjects with exclusively calcified plaque (all $P>0.30$).

In multivariate analysis subjects with exclusively non-calcified plaque had significant higher levels of hs-CRP and lower levels of Adiponectin, as compared with subjects with no plaque

or calcified plaque after adjustment age, gender, and number of cardiovascular risk factors (all $P<0.05$). This association remained significant after adjustment for all potential confounders (Table 3). In contrast, after adjustment, the association between exclusively non-calcified plaque and higher levels of ox-LDL was attenuated (Table 3).

Association between blood biomarkers and the extent of calcified and non-calcified plaque burden

Levels of hs-CRP and MMP-2 were positively and levels of ApoA1 were negatively correlated with the overall extent of coronary atherosclerotic burden ($P = 0.001$, $P = 0.003$ and $P = 0.02$; respectively; Table 4). While levels of hs-CRP were positively correlated with the extent of both, non-calcified and calcified plaque (all $P = 0.03$; Table 3), lower levels of ApoA1 were only correlated with non-calcified plaque ($r = -0.17$, $P = 0.004$). MMP-2 was strongly correlated to the extent of calcified plaque and demonstrated a trend towards an association to the extent of non-calcified plaque ($P = 0.06$). Adjusting for all potential confounders (Table 4), the correlations of hs-CRP and MMP-2 with overall extent of plaque remained significant.

Discussion

Coronary risk is predicted both by blood biomarkers as well as the local extent of atherosclerosis as measured by CT. However, mechanistically, it remains yet unclear why biomarkers predict risk for incident and prevalent coronary atherosclerosis events; furthermore, the link between many biomarkers and the anatomic diagnosis of coronary atherosclerosis remains tenuous. Moreover, with various forms of coronary atherosclerosis present—calcified, non-calcified, fibrous, lipid rich—the association with risk remains similarly yet explained.

In this study we determined the association between blood biomarkers of athero-and thrombogenesis with the presence, extent and composition of coronary atherosclerotic plaque as detected by coronary CTA. Overall, our results suggest that among these markers, hs-CRP, MMP-2, and ApoA1 may be associated with the extent of calcified and/or non-calcified plaque. However, more importantly, we found that a small group of subjects with exclusively non-calcified plaque may have significantly higher levels of hs-CRP and ox-LDL and lower levels of adiponectin than subjects without plaque or calcified plaque, despite that the latter group was older and had a more unfavorable cardiac risk profile.

Our results confirm earlier studies that have suggested that selected blood biomarkers of atherogenesis including inflammation, oxidation, and lipid metabolism to moderately correlate with morphologic measures of cardiovascular risk, including CAC and the degree of stenosis in invasive coronary angiography [10–14], intima-media thickness and ankle-brachial index [15], visceral abdominal and pericardial fat [16,17]. This observation emphasizes that levels of blood biomarkers such as hs-CRP may represent systemic but not local atherothrombogenic potential [18]. It is further interesting that biomarkers associated with plaque burden do not point to a single component of atherogenesis linked to CAD burden, rather markers associated with the extent of calcified (hs-CRP and MMP-2) and non-calcified (hs-CRP and ApoA1) represent all contributing pathways to atherothrombosis [19,20]. However, all of these associations were attenuated when adjusted for confounders. To this end, our results are in line with prior studies, which used coronary calcification or coronary angiography as surrogate markers of CAD [10–14,20]. One explanation maybe that as a systemic marker, levels of blood biomarkers may reflect diffuse atherosclerosis involving all vascular beds while coronary atherosclerosis may not represent overall plaque burden due to its local nature [18]. This is supported by data showing stronger correlations

between blood biomarker levels and subclinical extra-coronary atherosclerosis (i.e. carotid intima-media thickness or ankle brachial index) [21,22].

We did not systematically study the association of mixed plaque, a plaque type containing calcified and non-calcified plaque components, which has shown some associations with plaque progression and future events [23,24]. However, it was a predefined aim of the study to assess different stages of atherosclerosis as detected by CT including absence, early stage as non-calcified, and calcified plaque. Moreover, it is known from histology studies that the calcified plaque most often does have a non-calcified component [25].

An interesting and provocative finding of our study is that subjects with exclusively non-calcified plaque had generally higher levels of ox-LDL and hs-CRP and lower levels of adiponectin. This observation, although limited by the relatively small number of patients, is important as non-calcified plaque preceeds calcified plaque in the natural hisory of atherosclerosis [26], has been found highly prevalent in culprit lesions in patients with ACS [27,28] as well as in patients with myocardial infarction but without significant epicardial stenosis. In contrast, calcified coronary plaque is thought to represent more advanced stage of the natural history of disease (and may be less prone to ACS) [25].

Research on mechanisms of atherosclerotic plaque formation has identified more than 240 biomarkers associated with CAD [1]. Both hs-CRP and ox-LDL are among the more established blood biomarkers that have been linked to the initiation and formation of early atherosclerosis [29] and cardiovascular events [30–32]. In animal models, intimal accumulation of ox-LDL occurs prior to the development of coronary plaques [33] as ox-LDL is taken up by macrophages via the scavenger receptors, promoting foam cell formation and fatty plaque development [34]. Further, in humans increased ox-LDL is strongly associated with endothelial-dependent vasomotor function [35] and can be histologically found in lipid-rich plaque [36]. Similarly, CRP appears to serve not only as a marker of the inflammatory process of atherosclerosis but also as an active partaker in all stages of atherogenesis, since it is present in atherosclerotic lesions but not in the normal vessel wall [37]. Latest research suggests that hs-CRP is an independent predictor of cardiovascular event risk [1].

Previously, Hausleiter et al. found marginal differences in levels of CRP in patients with non-calcified plaque when compared to those without plaque (1.7 vs. 1.0 mg/L, $P = 0.045$) [38]. Our results extend these observations and we specifically demonstrate that hs-CRP significantly elevated in patients with exclusively non-calcified plaque when compared to patients with calcified plaque, despite that these patients were older and had a less favorable cardiovascular risk profile. These findings support the central role of inflammation in initiation and formation of early atherosclerosis and generate the hypothesis that detection of non-calcified plaque may improve cardiovascular risk prediction beyond traditional risk assessment and calcified plaque detection. Clearly, long-term follow-up studies in larger populations are needed to establish a role of non-calcified plaque detection by coronary CTA in cardiovascular risk predicition.

Limitations

We acknowledge the cross-sectional nature of our study design, which precludes establishing causality. Our findings, especially in subjects with exclusively non-calcified, plaque are significantly limited by sample size, which resulted in statistical trends in some biomarker analyses. However, this is the largest study on the association of blood biomarkers and coronary plaque to date and it has become evident that subjects with exclusively non-calcified plaque present a minority (between 5 and 10%) in subjects without known CAD. Thus, our finding in subjects with exclusively non-calcified plaque is

hypothesis generating and requires further confirmation in future studies. Our sample was drawn from a population of patients presenting with acute chest pain, which may limit the generalizability of our findings to asymptomatic patients. However, we excluded subjects with history of CAD and subjects with ACS from the analysis and thus all subject had acute chest pain without known origin. Also, we did not quantitatively assess the amount of plaque as prior research suggested high inter-observer variability of these measures.

Acknowledgments

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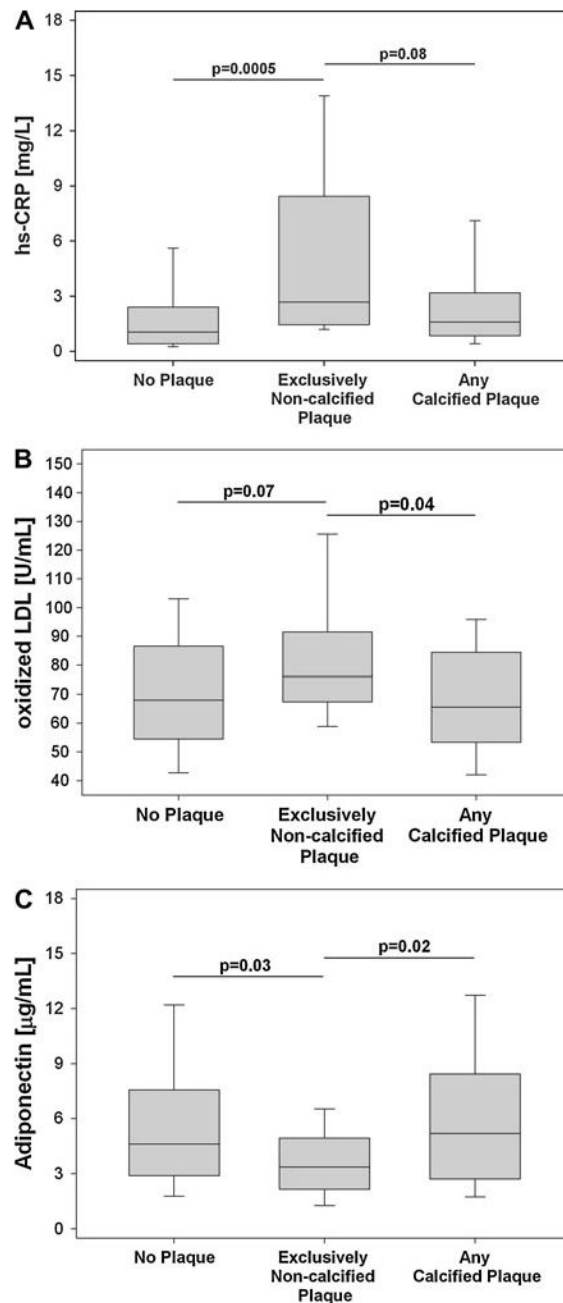
Fig. 1.
Box-and Whisker Plots showing differences in levels of **a** high-sensitivity C-reactive protein (*hs-CRP*), **b** oxidized low density lipoprotein (*oxidized LDL*), and **c** Adiponectin between subjects without plaque, subjects with non-calcified plaque, and subjects with calcified plaque. The Whisker was defined as 1.5-times of the interquartile range, if all outliers were within this range, the Whisker stopped at the minimal/maximal point

Table 1

Main characteristics of 313 patients who underwent blood sampling for measurement of a selected set of blood biomarkers and 64-slice cardiac computed tomography for the assessment of coronary atherosclerotic plaque burden

Variables	Overall cohort	Subjects with			P-value
		No plaque	Exclusively non-calcified plaque	Any calcified plaque	
N	313	168	15	130	
Age, years	51.6 ± 11.27	46.8 ± 9.0	48.7 ± 5.0	58.2 ± 11.2	< 0.0001
Gender male	194 (62.0%)	96 (57.1%)	11 (73.3%)	87 (66.9%)	0.15
Cardiovascular risk factors					
Number of risk factors	1.4 ± 1.2	1.1 ± 1.0	1.3 ± 1.1	1.9 ± 1.2	< 0.0001
Diabetes mellitus	33 (10.5%)	12 (7.1%)	2 (13.3%)	19 (14.6%)	0.08
Family history of CAD	72 (23.0%)	36 (21.4%)	3 (20.0%)	33 (25.4%)	0.72
Hypertension	114 (36.4%)	41 (24.4%)	4 (26.7%)	69 (53.1%)	< 0.0001
Hyperlipidemia	105 (33.7%)	34 (20.1%)	8 (53.3%)	63 (48.5%)	< 0.0001
Obesity (BMI ≥ 30)	105 (33.7%)	51 (30.5%)	8 (53.3%)	46 (35.4%)	0.16
Smoking	151 (48.2%)	67 (39.9%)	6 (40.4%)	78 (60.0%)	0.002
Medication					
Statin use	80 (25.6%)	26 (15.5%)	4 (26.7%)	50 (38.5%)	< 0.0001
Beta blocker use	61 (19.5%)	24 (14.3%)	2 (13.3%)	35 (26.9%)	0.02
ACE inhibitor use	46 (14.7%)	15 (8.9%)	2 (13.3%)	29 (22.3%)	0.004

Data are provided as mean ± standard deviation or n (%). The *P*-value compared the distribution of the variables across subjects with no plaque, exclusively non-calcified plaque and any calcified plaque

CAD coronary artery disease, *FRS* Framingham risk score

Table 2

Measured concentration of blood biomarkers in 313 patients undergoing coronary CT angiography stratified by atherosclerotic plaque group

Variables	Overall cohort	Subjects with			P value
		No plaque	Exclusively non-calcified plaque	Any calcified plaque	
N	313	168	15	130	
hs-CRP (mg/L)	1.35 [0.59; 2.88]	1.05 [0.4; 2.4]	2.68 [1.4; 8.4]	1.59 [0.8; 3.1]	< 0.0001
TNF-α (pg/mL)	1.05 [0.67; 1.86]	1.0 [0.6; 1.8]	1.56 [0.7; 2.5]	1.05 [0.7; 1.9]	0.37
MMP-2 (ng/mL)	161.0 [139.0; 186.0]	1.57 [135; 176]	156 [138; 172]	171 [147; 199]	0.1
Oxidized LDL (U/mL)	67.7 [54.9; 85.4]	68.05 [54.5; 86.3]	76.1 [67.4; 91.5]	65.6 [53.4; 84.5]	0.01
MPO (U/ML)	19.7 [13.6; 35.1]	18.7 [13.1; 32.4]	36.0 [12.8; 57.2]	21.1 [13.8; 36.1]	0.21
Lp-PLA$_2$ (ng/mL)	171.0 [142.0; 201.0]	174 [144; 205]	166 [124; 205]	166 [140; 196]	0.42
ApoA1 (g/L)	1.52 [1.28; 1.77]	1.57 [1.3; 1.8]	1.36 [1.1; 1.5]	1.48 [1.3; 1.8]	0.07
ApoB (g/L)	0.91 [0.77; 1.09]	0.92 [0.8; 1.1]	1.1 [0.8; 1.2]	0.89 [0.7; 1.1]	0.21
Adiponectin (µg/mL)	4.76 [2.70; 7.52]	3.36 [2.2; 5.0]	4.6 [2.9; 7.5]	5.2 [2.7; 8.3]	0.06
LP(a) (ng/mL)	0.12 [0.04; 0.30]	0.09 [0.03; 0.33]	0.12 [0.04; 0.3]	0.12 [0.04; 0.3]	0.83

Data are provided as medians with [interquartile range]

hs-CRP high-sensitivity C-reactive protein, *TNF-α* tumor necrosis factor alpha, *MMP-2* matrix metalloproteinase 2, *oxidized LDL* oxidized low-density lipoprotein, *MPO* myeloperoxidase, *Lp-PLA2* lipoprotein-associated phospolipase A2, *ApoA1* apolipoprotein A1, *ApoB* apolipoprotein B, *Lp(a)* lipoprotein (a)

Table 3

Estimated concentrations of biomarkers stratified by atherosclerotic plaque types

	hs-CRP [mg/L]			oxidized LDL [U/mL]			Adiponectin [µg/mL]		
	Median	IQR	P value	Median	IQR	P value	Median	IQR	P value
Adjusted for age, gender, CVRF									
No plaque	0.75	0.25–1.41	<0.05	67.32	53.86–87.92	NS	4.83	3.26–6.41	<0.05
Exclusively non-calcified plaque	1.91	1.36–7.90	-ref-	75.15	62.08–89.93	-ref-	3.54	2.46–3.94	-ref-
Any calcified plaque	0.90	0.61–1.66	<0.05	64.41	51.52–85.03	NS	5.49	3.10–7.66	<0.05
Adjusted for age, gender, diabetes, HTN, HLP, smoking, medication[a]									
No plaque	0.78	0.32–1.76	<0.05	69.76	54.35–84.90	NS	4.70	2.91–6.68	<0.05
Exclusively non-calcified plaque	1.92	1.33–8.52	-ref-	71.55	63.49–85.08	-ref-	3.52	2.45–4.43	-ref-
Any calcified plaque	0.96	0.58–1.76	<0.05	63.40	48.36–77.67	NS	5.30	3.00–8.19	<0.05

Levels were adjusted in two different models, the first included age, gender and number of cardiovascular risk factors (CVRF), the second included all potential confounder. The presented median, upper and lower quartile corresponds to a 52 year old male subject with none of the included cardiovascular risk factors or drug treatments

HTN hypertension, *HLP* hyperlipidemia, *hs-CRP* high-sensitivity C-reactive protein, *oxidized LDL* oxidized low-density lipoprotein

[a]Medication includes Statin, Beta-Blocker, ACE inhibitor use

Table 4

Association between a selected set of blood markers and the extent of any, calcified and non-calcified atherosclerotic plaque burden

	Extent of any plaque		Extent of non-calcified plaque		Extent of calcified plaque	
	r	P	r	P	r	P
Univariate, un-adjusted analysis						
Hs-CRP	0.19	0.001	0.12	0.03	0.12	0.03
MMP-2	0.17	0.003	0.07	0.06	0.20	<0.001
ApoA1	−0.13	0.02	−0.17	0.004	−0.08	0.15
Adjusted for age, gender, CVRF						
Hs-CRP	0.11	0.06	0.07	0.27	0.02	0.75
MMP-2	0.13	0.03	0.05	0.37	0.16	0.007
ApoA1	−0.05	0.39	−0.08	0.17	0.009	0.88
Adjusted for age, gender, diabetes, HTN, HLP, smoking, medication[a]						
Hs-CRP	0.12	0.04	0.08	0.19	0.03	0.65
MMP-2	0.14	0.02	0.05	0.40	0.17	0.005
ApoA1	−0.04	0.49	−0.06	0.29	0.02	0.79

CVRF number of cardiovascular risk factors, *HTN* hypertension, *HLP* hyperlipidemia, *hs-CRP* high-sensitivity C-reactive protein, *MMP-2* matrix metalloproteinase-2, *ApoA1* apolipoprotein A1

[a]Medication includes Statin, Beta-Blocker, ACE Inhibitor use