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Associations of inflammatory markers with coronary artery calcification: Results from the Multi-Ethnic Study of Atherosclerosis

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ABSTRACT

Objective: Inflammatory markers predict coronary heart disease (CHD). However, associations with coronary artery calcium (CAC), a marker of subclinical CHD, are not established.
Methods: We examined cross-sectional associations of C-reactive protein (CRP), interleukin-6 (IL-6) and fibrinogen with CAC presence (Agatston score > 0 by computed tomography) in 6783 Multi-Ethnic Study of Atherosclerosis (MESA) participants.
Results: In all participants, those in the highest, compared to lowest, quartile of CRP had a relative risk (RR, 95% confidence interval) of 1.13 (1.06–1.19; $p < 0.01$) for CAC in age, sex and ethnicity adjusted models. For highest versus lowest quartiles, relative risks were 1.22 (1.15–1.30; $p < 0.01$) for IL-6 and 1.18 (1.11–1.24; $p < 0.01$) for fibrinogen. Adjusting for CHD risk factors (smoking, diabetes, blood pressure, obesity and dyslipidemia) attenuated RRs. RRs for CAC were 1.05 (0.99–1.12; $p = 0.63$) for CRP, 1.12 (1.06–1.20; $p < 0.01$) for IL-6 and 1.09 (1.02–1.16; $p = 0.01$) for fibrinogen in multivariable adjusted models. Results were similar for men and women and across ethnic groups.
Conclusion: Inflammatory markers were weakly associated with CAC presence and burden in MESA. Our data support the hypothesis that inflammatory biomarkers and CAC reflect distinct pathophysiology.

1. Introduction

Inflammation plays a central role in atherosclerosis. In multiple epidemiologic studies, inflammatory biomarkers C-reactive protein (CRP), fibrinogen and interleukin-6 (IL-6) have been associated with increased risk of coronary heart disease (CHD) [1,2]. However, associations of biomarkers with coronary artery calcium (CAC), a surrogate marker of subclinical CHD, are not clear. Previous studies range from null to weak associations between CRP and/or fibrinogen and CAC in asymptomatic individuals [3–7]. Associations of IL-6 with CAC have not been extensively examined although IL-6 is reported to be associated with other measures of subclinical atherosclerosis [8,9].

It is likely that there is also a link between inflammation and calcification. In order to elucidate associations between the two pathophysiologic processes, we examined these associations in white, black, Chinese and Hispanic men and women in the Multi-Ethnic Study of Atherosclerosis (MESA). We analyzed associations of three biomarkers, CRP, IL-6 and fibrinogen, with CAC presence and burden in this large population-based cohort.

2. Methods

2.1. Multi-Ethnic Study of Atherosclerosis (MESA)

MESA was initiated to investigate prevalence, correlates and progression of subclinical cardiovascular disease [10]. MESA comprises 6814 men and women, 38.6% white, 27.6% black, 11.8% Chinese and 22.0% Hispanic, who were 45–84 years of age at baseline, July 2000–August 2002 [10]. Exclusion criteria included: (1) clinical cardiovascular disease; (2) active treatment for cancer; (3)

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pregnancy; (4) weight > 300 pounds; (5) cognitive inability; and (6) living in a nursing home. Each clinic recruited an equal number of men and women according to specific age/ethnicity proportions. Participants were recruited by random digit dialing and mail. Baseline exams included anthropometry, medical and lifestyle histories and blood collection. All subjects gave informed consent for participation in the study and all procedures were conducted under institutionally approved protocols for human subjects research.

2.2. Cardiac computed tomography (CT)

CT scanning of the chest was performed by an ECG-triggered (at 80% of the RR interval) electron-beam CT scanner or by prospectively ECG-triggered scan acquisition at 50% of the RR interval with a multidetector CT system [11]. Each participant was scanned twice. Scans were read centrally and calcium scores among field centers and between participants were adjusted with a standard calcium phantom scanned simultaneously with the participant [12]. The average Agatston score for the two scans was used for analyses.

2.3. Definitions

Body mass index (BMI) was weight in kilograms divided by height in meters squared (kg/m^2). Smoking was defined as never, former (no cigarettes within the past 30 days) or current. Diabetes (fasting glucose ≥126 mg/dl) and impaired fasting glucose (fasting glucose 110-125 mg/dl) were classified by 1997 American Diabetes Association guidelines.

2.4. Laboratory methods

Fasting blood was drawn, processed and stored using standardized procedures [13]. Total and HDL cholesterol, triglycerides and glucose were measured. Analytical coefficients of variation (CVs) were ≤4% for all. LDL cholesterol levels were calculated.

CRP (minimum detection level 0.17 mg/l) and fibrinogen (50 mg/dl) were determined by BNII nephelometer (N High Sensitivity CRP and N Antiserum to Human Fibrinogen; Dade Behring Inc., Deerfield, IL). IL-6 (0.16 pg/ml) was measured by ultrasensitive ELISA (Quantikine HS Human IL-6 Immunoassay; R&D Systems, Minneapolis, MN). Analytical CVs for CRP, fibrinogen, and IL-6 were 3.6%, 2.7% and 6.3%, respectively. All measurements were made in duplicate, in random order and in a blinded fashion.

6762 participants had CRP measurements, 6767 had fibrinogen and 6662 had IL-6 measurements. 6783 participants had at least one marker measured and were included in these analyses.

2.5. Statistical analyses

Data were analyzed using STATA (version 8.0). Participants were stratified by CAC status: no detectable CAC (Agatston score = 0) or presence of CAC (Agatston score > 0). Unadjusted means (standard deviations) or proportions (percentages) were calculated for demographic variables, CHD risk factors and inflammatory markers by CAC status. Differences between groups were assessed by analysis of variance or χ^2 statistics.

Relative risk regression models with robust standard errors were used to determine the probability of CAC presence (Agatston score > 0) for marker quartiles 2–4 compared to quartile 1 for all participants and using sex- and ethnic-specific quartiles. Models were adjusted for age, ethnicity and sex. Additional adjustments were smoking, diabetes, systolic blood pressure, dyslipidemia (total cholesterol/HDL cholesterol ratio > 5 or taking cholesterol lowering medication including statins) and BMI. We did not adjust for hormone replacement therapy, aspirin or non-steroidal anti-inflammatory agents as including their use in models adjusted for age, sex and ethnicity did not significantly change effect estimates.

Table 1
Baseline characteristics by CAC status.

Variables	CAC status		*p*-Value
	None	Present	
Demographics	(*n* = 3397)	(*n* = 3386)	
Age, years (mean, SD)	58.0 (9.1)	66.4 (9.5)	<0.001
White	1122 (43)	1494 (57)	–
Black	1061 (57)	813 (43)	0.001
Chinese	399 (50)	404 (50)	<0.001
Hispanic	815 (55)	675 (45)	<0.001
Male	1246 (39)	1959 (61)	<0.001
CHD risk factors			
BMI, kg/m^2 (mean, SD)	28.3 (5.7)	28.4 (5.3)	0.73
Dyslipidemia	873 (26)	1452 (43)	<0.001
Diabetes	362 (11)	606 (18)	<0.001
Hypertension	1555 (41)	1831 (61)	<0.001
Current smoking	449 (13)	433 (13)	0.60
Agatston score (mean, SD)	0	293 (553)	–

Values are *n* (%) unless noted. SD is standard deviation.

Linear regression was used to model associations of continuous Agatston score with inflammatory markers (one standard deviation change in marker level) in those with a positive Agatston score. We used ln-transformed Agatston score; regression coefficients were exponentiated for presentation. Models were adjusted as above. In the full group, we had sufficient power (approximately 100%) to detect a 1.27-fold increase in CAC per 1 unit standard deviation change in biomarker level. In the Chinese sub-cohort, the smallest selection, we had 80% power.

To determine if associations between biomarkers and CAC were stronger in those with significant CAC, we utilized relative risk regression models comparing those with no detectable CAC (Agatston score = 0) to those with an Agatston score >100. Linear regression was used to model associations of continuous Agatston score with inflammatory markers in those with an Agatston score >100. 571 women and 1026 men had Agatston scores >100 (793 whites, 353 blacks, 168 Chinese and 283 Hispanics).

We also created a composite measure of inflammation status by combining CRP, fibrinogen and IL-6 quartiles. 6599 participants with all three biomarker measurements were included in these analyses. Quartiles were assigned scores: quartile 1 = 0, quartile 2 = 1, quartile 3 = 2 and quartile 4 = 3. Possible scores ranged from 0 to 9. Based on trends observed comparing scores of 1–9 to 0, we created three categories: low (inflammation score 0–1; reference category), intermediate (2–7) and high (8–9). 1064 individuals were low and 1094 were high. Relative risk regressions with robust standard errors were used to examine associations of composite scores with CAC presence.

3. Results

3.1. Baseline characteristics

Participants with detectable CAC were older and more likely to be diabetic, hypertensive and have dyslipidemia than those with no detectable CAC (Table 1). Current smoking was similar between the two groups. More men than women had detectable CAC and the prevalence of CAC was lower in blacks, Chinese and Hispanics compared to whites ($p \leq 0.001$ for all comparisons).

3.2. Inflammatory marker association with presence of CAC

In all, adjusting for age, sex and ethnicity, those with the highest CRP had an increased risk of having detectable CAC (Table 2, Model 1). Associations of IL-6 and fibrinogen with CAC presence were analogous (Model 1). Adding CHD risk factors (smoking, diabetes,