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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 6,783 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.

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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.

Keywords

Atherosclerosis; Calcium; Inflammation; Population

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.

Methods

Multi-Ethnic Study of Atherosclerosis (MESA)

MESA was initiated to investigate prevalence, correlates and progression of subclinical cardiovascular disease[10]. MESA comprises 6,814 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) 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.

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.

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.

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 $\leq 4\%$ 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 ultra-sensitive 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.

6,762 participants had CRP measurements, 6,767 had fibrinogen and 6,662 had IL-6 measurements. 6,783 participants had at least one marker measured and were included in these analyses.

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

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. 6,599 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.

Results

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).

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, systolic blood pressure, dyslipidemia and BMI) attenuated associations of biomarkers with CAC presence although IL-6 and fibrinogen associations remained significant (Model 2). Results were similar in sex- and ethnicity-stratified analyses. In general, biomarkers were modestly associated with CAC presence in minimally adjusted models (age and ethnicity or age and sex) in all subgroups. Results were attenuated when CHD risk factors were added.

We also examined associations of inflammatory markers with CAC presence comparing those with significant CAC burden (Agatston scores >100) to those with no detectable CAC. In the full cohort, the RR (95% CI) for the presence of significant CAC was 1.00 (0.97-1.03, $p=1.00$) for the highest compared to lowest quartile of CRP, 1.09 (1.05-1.12, $p<0.01$) for IL-6 and 1.06 (1.02-1.08, $p<0.01$) for fibrinogen. Sex- and ethnicity-stratified analyses were likewise similar to the results described for the presence of any detectable CAC.

In addition, we created a composite model of inflammation status using quartiles of the three biomarkers. Compared to those with the lowest levels of all biomarkers, increasing inflammation score was associated with higher RRs for the presence of CAC in multivariable adjusted models (Figure 1). The RR (95% CI) for those with the highest levels of all three markers was 1.34 (1.24-1.45, $p<0.01$) in models adjusted for age, sex and ethnicity and 1.20 (1.11-1.30, $p<0.01$) in multivariable adjusted models.

Inflammatory Marker Association with CAC Burden

In participants with detectable CAC, IL-6, but not CRP or fibrinogen, was associated with Agatston score in age, sex and ethnicity adjusted linear regression models (Table 3, Model 1). Adding CHD risk factors to the models attenuated associations (Model 2). Trends were similar in sex- and ethnicity-stratified analyses.

Discussion

The main findings of this large population-based study of men and women from four ethnic groups were modest associations of inflammatory markers, CRP, IL-6 and fibrinogen, with CAC presence and burden. Combining the three markers, representing inflammatory burden, showed a stronger association than any single marker, but the combined effect was still relatively small. Prevalence and quantity of CAC and levels of inflammatory markers varied by sex and ethnicity; however, associations of biomarkers with CAC were similar in men and women and across ethnic groups.

This is the first study to directly compare associations of inflammatory markers with CAC in a population-based sample of men and women. Elevated levels of CRP, IL-6 and fibrinogen were associated with CAC presence in minimally adjusted models (age, sex and ethnicity). Adding CHD risk factors (smoking, diabetes, systolic blood pressure, dyslipidemia and BMI) attenuated associations. Associations of biomarkers with CAC burden were limited; only IL-6 was weakly associated with CAC burden. Overall, our results parallel those of previous studies in apparently healthy individuals. Comparing those with no detectable CAC to those with high levels, fibrinogen has been reported to be weakly associated with CAC in men and an age-dependent association was reported for women[6]. Unlike MESA, these participants were selected based on results of CAC assessment. While other studies also found no association of CRP with CAC after adjustment for risk factors[3,4], elevated CRP was associated with CAC in men and women in the Framingham Heart Study[5]. However, CRP was measured 4 to 8 years prior to CAC assessment and the association may have been modified by CAC progression[5]. Although IL-6 has been associated with other measures of subclinical atherosclerosis in older and middle-aged men and women[8,9], we found only a weak association of IL-6 with CAC in MESA.

Atherosclerotic calcification is likely an organized, regulated process similar to bone formation [14]. A variety of inflammatory mediators such as interleukins 1 β , -4, -6, -10 and -12, tumor necrosis factor- α and interferon- γ play key roles in bone formation and likely calcification as well. Plaque morphology changes over time and general inflammatory markers like CRP may be more strongly associated with or influenced by plaque progression processes other than calcification; processes such as plaque rupture and thrombosis[15]. Our data and previous studies support the hypothesis that inflammatory markers and CAC provide distinct information and may therefore be complementary in CHD risk prediction.

There are several limitations to this study. We selected several commonly measured biomarkers of generalized inflammation and found a modest association of inflammation with CAC. It is possible that biomarkers representing other facets of bone formation/calcification, such as osteoprotegerin, or markers of vascular inflammation, such as pentraxin 3, would have shown stronger associations with CAC. The study was cross-sectional and we cannot infer causality. In addition, participants in MESA were volunteers from six geographic areas in the United States and not a truly random population sample. However, MESA participants were free of clinical cardiovascular disease at baseline and not referred for CAC assessment. Men and women from four ethnic groups were also represented.

In summary, in this population-based cohort of men and women from four ethnic groups, CRP, IL-6 and fibrinogen were modestly associated with both CAC presence and burden regardless of sex or ethnicity. Our results support the hypothesis that inflammatory biomarkers and CAC may provide integrative information about CHD risk and supports the use of biomarkers to better estimate risk in individuals with established CHD burden as outlined recently by Hamirani et al[15]. MESA, an ongoing, prospective cohort study, and other current and future studies will determine the utility of combining measurement of CAC and circulating biomarkers in risk stratification and prediction of cardiovascular events.

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Figure 1. Adjusted relative risks for the presence of CAC by inflammation status

Inflammation scores were calculated by combining quartiles of CRP, fibrinogen and IL-6.

Scores were 0 (lowest quartiles of all) to 9 (highest quartiles of all). RRs for CAC presence for intermediate (2-7) and high (8-9) compared to low scores (0-1) were determined using models adjusted for age, sex, ethnicity, smoking status, diabetes, systolic blood pressure, dyslipidemia and BMI.

Table 1
Baseline characteristics by CAC status

Variables	CAC Status		p-value
	None	Present	
<u>Demographics</u>	(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
<u>CHD Risk Factors</u>			
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.	

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

Table 2

Associations of inflammatory markers with CAC presence

Quartiles		Model 1 [*] RR (95% CI)	p-value	Model 2 [*] RR (95% CI)	p-value
CRP	2	1.01 (0.96-1.07)	0.68	1.00 (0.95-1.05)	1.00
	3	1.03 (0.97-1.08)	0.35	0.98 (0.92-1.03)	0.43
	4	1.13 (1.06-1.19)	<0.01	1.05 (0.99-1.12)	0.10
IL-6	2	1.14 (1.07-1.21)	<0.01	1.09 (1.03-1.17)	<0.01
	3	1.17 (1.10-1.24)	<0.01	1.10 (1.03-1.17)	<0.01
	4	1.22 (1.15-1.30)	<0.01	1.12 (1.06-1.20)	<0.01
Fibrinogen	2	1.04 (0.98-1.10)	0.17	1.02 (0.96-1.08)	0.52
	3	1.11 (1.05-1.17)	<0.01	1.06 (1.00-1.12)	0.05
	4	1.18 (1.11-1.24)	<0.01	1.09 (1.03-1.16)	<0.01

Relative risk (RR) and 95% confidence intervals (CI) with robust standard errors.

* Model 1 adjustments: age, ethnicity, sex.

* Model 2: Model 1 and smoking, diabetes, systolic blood pressure, BMI, dyslipidemia. Bolded values significant (p<0.05).

Table 3
Regression coefficients of inflammatory markers in separate linear models of Agatston score in participants with a positive score

Marker	Mean (SD)	Model 1 *		Model 2 *	
		Coefficients	p-value	Coefficients	p-value
CRP	3.78 mg/l (5.89)	1.01 (0.96-1.06)	0.70	0.99 (0.93-1.04)	0.74
IL-6	1.56 pg/ml (1.23)	1.06 (1.00-1.12)	0.05	1.02 (0.96-1.08)	0.52
Fibrinogen	347 mg/dl (74)	1.04 (0.98-1.10)	0.20	0.99 (0.94-1.05)	0.70

Regression coefficients (95% confidence intervals) from ln-transformed Agatston score exponentiated to represent coefficients for one standard deviation (SD) changes in marker levels.

* Model 1 adjustments: age, ethnicity, sex.
* Model 2: Model 1 and smoking status, diabetes, systolic blood pressure, BMI, dyslipidemia. Bolded values significant (p<0.05).