Clinical Investigations

Association of Cystatin C with Ischemia in Patients with Coronary Heart Disease

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ABSTRACT

Background: Elevated concentrations of cystatin C are associated with greater cardiovascular morbidity and mortality. We sought to determine whether elevated concentrations of cystatin C were associated with inducible ischemia in patients with coronary heart disease (CHD).

Methods: We measured serum cystatin C and performed exercise treadmill testing with stress echocardiography in a cross-sectional study of 899 outpatients with CHD.

Results: Among the 241 participants in the highest quartile of cystatin C (>1.30 mg/L), 38% had inducible ischemia, compared with 13% of those in the lowest quartile of cystatin C <0.92 mg/L; adjusted odds ratio [OR]: 2.1; 95% confidence interval [CI]: 1.2 to 3.8; p = 0.01). However, this association differed in participants with and without a history of coronary artery bypass graft (CABG), as well as in users and nonusers of beta-blockers and statins (p values for interaction <0.1). Among participants without a history of CABG, 35% of those in the highest quartile and 9% of those in the lowest quartile of cystatin C had inducible ischemia (adjusted OR: 3.05; 95% CI: 1.3–6.9; p = 0.008). Among participants who were not using beta-blockers, 44% of those in the highest quartile and 7% in the lowest quartile of cystatin C had inducible ischemia (adjusted OR: 5.3; 95% CI: 1.8–15.5; p = 0.002). Among participants who were not using statins, 39% of participants in the highest quartile and 4% of those in the lowest quartile had inducible ischemia (adjusted OR: 10.3; 95% CI: 2.5–43.3; p = 0.001).

Conclusions: Elevated levels of cystatin C are independently associated with inducible ischemia among outpatients with stable coronary disease.

Key words: cystatin C, inducible ischemia, coronary heart disease

Introduction

Decreased kidney function is associated with adverse cardiovascular outcomes.[1–7] Cystatin C is a novel measure of kidney function that appears to have even stronger associations with mortality and cardiovascular risk than creatinine or estimated glomerular filtration rate (GFR) in the ambulatory elderly[8–10] and in hospitalized acute coronary syndrome patients.[11] Whether the increased cardiovascular risk associated with cystatin C elevation is due to increased ischemic burden, higher risk of plaque rupture, or alternative mechanisms is unknown.

Exercise treadmill testing and stress echocardiography can be used to determine ischemic burden\ as a measure of coronary artery disease severity.[12,13] In addition exercise-induced ischemia correlates with an increased likelihood of future unstable angina or myocardial infarction.[14] To determine whether cystatin C is associated with inducible ischemia and to explore the potential role of cardioprotective medications in modifying this association, we examined the association between cystatin C, exercise-induced ischemia, and use of preventive medications in 899 patients with CHD.

Methods

Participants

The Heart and Soul study is a prospective cohort study designed to investigate the influence of psychosocial factors on the progression of coronary artery disease.[15] As described previously, patients were eligible to participate if they had a history of coronary heart disease.[15] Between September 2000 and December 2002, 1,024 participants were recruited from the University of California, San Francisco Medical Center. Our study participants represent a heterogeneous group of patients with stable coronary heart disease. Of these 1,024 participants, 524 (54%) had a history of myocardial infarction, 237 (23%) had a history of revascularization, but not myocardial infarction, and 238 (23%) had a diagnosis of coronary disease that was documented by their physician based on a positive

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angiogram or treadmill test in >98% of cases. In addition, 125 were unable to provide a serum sample or to complete the exercise treadmill test leaving 899 for this cross-sectional analysis. The institutional review board at each of the sites approved this protocol, and all participants provided written informed consent. This study complies with the Declaration of Helsinki.

Measurements

Predictor Variable: Cystatin C: All assays for cystatin C were performed on fasting serum that was drawn prior to the stress echocardiogram and stored at −70°C. Cystatin C was measured by using a BN II nephelometer (Dade Behring, Inc., Deerfield, IL, USA) with a particle-enhanced immunonephelometric assay (N Latex Cystatin C, Dade-Behring, Inc., Deerfield, IL, USA).

Outcome Variable: Inducible Ischemia: The primary outcome was the presence or absence of exercise-induced ischemia. We performed a symptom-limited graded exercise treadmill test according to the standard Bruce protocol. Doppler echocardiography was used just before exercise to obtain a complete resting 2-dimensional echocardiogram. At peak exercise, apical two-chamber and four-chamber views were obtained to detect the development of right or left ventricular dilation or wall motion abnormalities. We defined exercise-induced ischemia as the presence of new wall motion abnormalities not visualized on the baseline rest echocardiogram.

Other Measurements

Age, sex, race, smoking status, and medical history of diabetes, hypertension, myocardial infarction, and coronary revascularization were determined by self report. Serum samples were obtained for measurement of creatinine, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), albumin, C-reactive protein (CRP), and hematocrit. In addition, 24 h urine collection was performed to determine creatinine clearance.

Analyses

Differences in baseline characteristics between participants with and without inducible ischemia were compared using chi-squared tests for dichotomous variables and *t* tests (or nonparametric equivalent) for continuous variables.

We divided participants into quartiles on the basis of their plasma cystatin C concentrations and compared the proportion of participants with inducible ischemia by cystatin C quartile. In addition, we used multivariate logistic regression analyses with quartiles of cystatin C as the predictor and inducible ischemia as the outcome. We entered all variables, including quartiles of cystatin C, into a backward stepwise elimination model. Variables associated with inducible ischemia at $p<0.1$ were retained in the final model. We further adjusted for cardiac function (left ventricular ejection fraction, left ventricular mass index, and exercise capacity) and C-reactive protein. We also adjusted for creatinine clearance in the final model. We tested for interactions of cystatin C with history of myocardial infarction, diabetes, coronary artery bypass grafting (CABG), C-reactive protein (CRP), and use of cardiac preventive medications (beta-blocker, renin-angiotensin system inhibitor, statin, and aspirin). We performed stratified analyses by any variables with p value for interaction test <0.20. All analyses were performed using Statistical Analysis Software (version 9.0, SAS Institute, Inc, Cary North Carolina).

Results

Of the 899 participants, 217 (24%) had inducible ischemia. Compared with participants who did not have inducible ischemia, those with inducible ischemia were older and more likely to be male, white, have a history of myocardial infarction, heart failure, or coronary artery bypass surgery, and to be taking renin-angiotensin system inhibitors (Table 1). Those with inducible ischemia also had a lower diastolic blood pressure, lower ejection fraction, higher LV mass index, lower exercise capacity, and lower creatinine clearance than those without inducible ischemia.

The median cystatin C level was 1.07 mg/L (interquartile range 0.92–1.30 mg/l). Among the 241 participants in the highest quartile of cystatin C (>1.30 mg/L), 38% had inducible ischemia compared with 13% of those in the lowest quartile of cystatin C (<0.92 mg/L; OR: 4.1; 95% CI: 2.6–6.6; $p<0.0001$). This association remained strong after adjustment for potential confounding variables, cardiac function, creatinine clearance, and CRP (Table 2). A similar association was observed between cystatin C and inducible ischemia after excluding women from the analysis. Among the 744 men, 41% (78/192) of those with a cystatin C concentration in the highest quartile had ischemia compared with 14% (25/178) of those in the lowest quartile (OR: 4.2; 95% CI: 2.5–7.0; $p<0.0001$). This association remained strong after adjustment for age, race, history of MI, history of angioplasty, history of CABG, and history of CHF (OR: 2.4; 95% CI: 1.4–4.3; $p = 0.002$).

We observed no difference in the association of cystatin C with ischemia in users and nonusers of aspirin or renin-angiotensin system inhibitors or among patients with or without a history of myocardial infarction, diabetes, congestive heart failure, or elevated CRP (all p values for interaction >0.2). However, the association of cystatin C with ischemia differed in participants with and without a history of CABG (p for interaction = 0.06) and in users and nonusers of beta-blockers (p for interaction = 0.01) and statins (p for interaction = 0.03).

Among participants without a history of CABG, 35% of those in the highest quartile and 9% of those in the lowest quartile of cystatin C had inducible ischemia (adjusted OR:

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Table 1. Characteristics of 899 participants by presence of inducible ischemia

Variable	Ischemia N = 217	No ischemia N = 682	P-value
Demographic			
Age (years)	70±10	66±11	<.0001
Sex (% Male)	190 (88%)	553 (81%)	0.03
Race			
White (%)	150 (69%)	399 (59%)	0.006
Black (%)	24 (11%)	113 (17%)	0.05
Other (%)	43 (20%)	169 (25%)	0.13
Current Smoker	39 (18%)	139 (20%)	0.43
Regular alcohol	58 (27%)	208 (31%)	0.28
Physically active	139 (64%)	446 (66%)	0.68
BMI	28±5	28.5±5	0.14
Medical history			
MI	144 (66%)	333 (49%)	<.0001
Hypertension	153 (71%)	473 (70%)	.79
Diabetes	61 (28%)	166 (24%)	.27
Angioplasty	77 (35%)	278 (41%)	.15
CABG	107 (50%)	221 (33%)	<.0001
CHF	53 (24%)	97 (14%)	.0005
Stroke	35 (16%)	82 (12%)	.12
Weekly Angina	36 (17%)	124 (18%)	.59
Medications			
ACE/ARB	132 (61%)	332 (49%)	.002
Statin	144 (66%)	444 (65%)	.73
Aspirin	173 (80%)	533 (78%)	.62
Beta Blockers	130 (60%)	387 (57%)	.41
Cardiac Function			
Systolic BP	130±18	132±19	.19
Diastolic BP	71±10	75±10	<.0001
LVEF	.59±. 11	.63±.09	<.0001
LV Mass Index	102±28	95±24	.0008
METS	6.4±3.5	7.6±3.4	<.0001
Cholesterol (mg/dl)	176±41	177±42	.87
LDL (mg/dl)	104±33	103±33	.95
HDL (mg/dl)	46±16	46±13	.98
Hemoglobin (g/dl)	13.7±1.4	13.9±1.4	.06
Renal function			
CrCl (ml/min)	74±26	85±28	<.0001
Inflammation			
CRP (mg/L)	4.8±7.0	4.3±8.4	.38

3.05; 95% CI: 1.3–6.9; p = 0.008). Among participants who were not using beta-blockers, 44% of those in the highest quartile and 7% of those in the lowest quartile of cystatin C had inducible ischemia (adjusted OR: 5.3; 95% CI: 1.8–15.5; p = 0.002). Finally, among those who were not using statins, 39% of participants in the highest quartile and 4% of those in the lowest quartile had inducible ischemia (adjusted OR: 10.3; 95% CI: 2.5–43.3; p = 0.001). We did not observe a significant association between cystatin C and inducible ischemia among participants who were treated with CABG, statins, or beta-blockers (Figure 1).

Discussion

We found that elevated concentrations of cystatin C were associated with inducible ischemia among outpatients with CHD particularly in those without a history of CABG and in those who were not being treated with beta-blockers or statins. After adjustment for baseline demographics, comorbidities, medications, creatinine clearance, cardiac function, and CRP, the highest cystatin C quartile predicted inducible ischemia. Although our cross-sectional study cannot determine the causal pathway between cystatin C and inducible ischemia, the observed association suggests that renal specific mechanisms are present that increase the risk of ischemic burden within this population.

Cystatin C is an emerging risk factor for adverse outcomes in patients with and without CHD, but its precise role in the development and progression of cardiovascular disease are far from clear. Our study suggests that cystatin C is capturing an element of risk beyond established risk factors. Furthermore, neither creatinine clearance nor CRP predicted inducible ischemia in multivariate models containing cystatin C. That creatinine clearance no longer predicted ischemia after cystatin C adjustment may imply that cystatin C is a better correlate of underlying GFR; or, that cystatin C has mechanisms of association with ischemia that are independent of kidney function.[16–18]

Certain non-renal mechanisms have been proposed to explain cystatin C's association with cardiovascular risk—either direct pathologic effects or associations with

Table 2. Association between Cystatin C and exercise-induced ischemia in 899 participants with coronary disease

Cystatin quartile	*mg/L*	*Proportion with ischemia*	*Unadjusted OR (95% CI)*	*P value*	*Adjusted OR (95% CI)**	*P value*
I	<0.92	13% (31/258)	1.0		1.0	
II	0.92 – 1.07	24% (53/239)	2.1 (1.3–3.5)	.002	1.7 (1.0–2.9)	.07
III	1.07–1.30	23% (54/252)	2.0 (1.2–3.3)	.004	1.4 (0.8–2.4)	.26
IV	>1.30	38% (79/241)	4.1 (2.6–6.6)	<. 0001	2.1 (1.2–3.8)	.01

* All variables from Table I were entered into a backwards elimination logistic regression model including the four cystatin categories. Other variables associated with associated with exercise-induced ischemia (at p<0.10) were older age, history of MI, no history of angioplasty, history of CABG, increased SBP, lower DBP, lower LVEF, increased cholesterol, lower LDL, lower HDL and lower triglyceride.

other risk factors. Cystatin C may reflect an increased inflammatory state that contributes to atherosclerotic plaque vulnerability and a higher risk of plaque rupture and thrombotic complications.[19] Others have noted the correlation of cystatin C with levels of CRP,[8,20] supporting this hypothesis. Basic investigations demonstrate that thrombus formation is related to the level of collagen in the atheroma's cap.[21] Mice that are doubly deficient in cystatin C and apolipoprotein E have consistently increased collagen contents and better developed fibrous caps. This suggests that cystatin C expression may induce degradation of the collagen forming cathepsins.

We also noted an intriguing interaction between cystatin C and 2 cardio-preventive medications for predicting inducible ischemia. In stratified analyses, we observed the strongest association between cystatin C and ischemia among participants who were not using beta-blockers and statins. Both pharmacologic therapies are known to inhibit atherogenesis and lead to regression in plaque development.[22–25] Plaque stabilization could potentially explain why cystatin C might be a less sensitive marker for inducible ischemia among patients treated with these therapies. We also observed an interaction of cystatin C with participants who had no history of CABG. Elevations in cystatin C concentrations may signify earlier stages of cardiorenal disease and atherogenesis. As a result, it may show greater correlates with inducible ischemia among those participants without a history of CABG.

Several limitations should be considered in interpreting our results. First, our study participants were mostly men with known CHD. Thus we are unable to determine whether our results generalize to women or to patients without known CHD. However, the cystatin C values in our study population are similar to those in other outpatient studies and correlate with the cut points identified for determining risk of future cardiac events.[8–10] Second, stress echocardiography may have misclassified some ischemic participants as nonischemic. However, misclassification usually results in weaker not stronger associations. Third, our study design was cross-sectional. However, whatever the

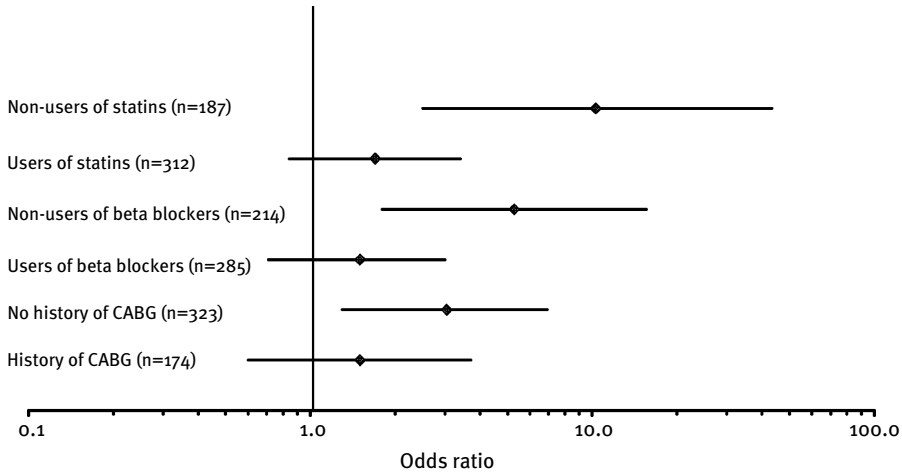

Figure 1. Association between elevated cystatin C and inducible ischemia among participants in the highest versus lowest quartile of cystatin C, stratified by use of beta-blockers, use of statins, and history of CABG.

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causal direction, our data provide evidence that cystatin C is associated with an important measure of ischemic burden, providing insight into a potential biological mechanism linking elevated cystatin C with future events.

In summary, we found that elevated levels of cystatin C are associated with inducible ischemia in 899 outpatients with coronary heart disease. These findings suggest that greater ischemic burden, as represented by inducible ischemia, may contribute to the adverse cardiovascular outcomes associated with kidney dysfunction.

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