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The prospective association of Chlamydia pneumoniae and four other pathogens with development of coronary artery calcium: the Multi-Ethnic Study of Atherosclerosis (MESA)

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Abstract

Objective—Previous basic and cross-sectional studies obtained conflicting results regarding the association of pathogens with coronary artery calcium (CAC). The aim of this study is to prospectively evaluate this association in a population-based cohort.

Methods—We examined 5,744 individuals aged 45-84 years at baseline (2000-02) who underwent repeated CAC assessment on average 2.4 years later (a half at visit 2 [2002-04] and the other half at visit 3 [2004-05]). CAC incidence was defined as newly detectable CAC at follow-up (475 cases of 2,942 participants). CAC progression was defined as annualized change in CAC Agatston score 10 units/year if baseline CAC score >0 to <100 or 10%/year if baseline score 100 (1,537 cases of 2,802 participants). Seropositivity was assessed in the entire cohort for Chlamydia pneumoniae and in a random sample (n=873) for Helicobacter pylori, cytomegalovirus, herpes simplex virus, and hepatitis A virus.

Results—Seropositivity to Chlamydia pneumoniae was not significantly associated with CAC incidence (odds ratio [OR] 1.11 [95% CI, 0.88-1.39], $P=0.371$) or progression (1.14 [0.96-1.36], $P=0.135$) even in unadjusted models. When CAC incidence and progression were combined, we

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Disclosures
None.

observed significant association with *Chlamydia pneumoniae* seropositivity before adjustment (OR 1.17 [1.03-1.33], $P=0.016$) but not in a model adjusting for traditional risk factors (1.04 [0.90-1.19], $P=0.611$). The results were consistent across subgroups according to age, sex, and race/ethnicity. None of five pathogens or their accrual was associated with CAC incidence and progression in the subsample.

Conclusion—Our prospective study does not support the pathophysiological involvement of these pathogens in CAC development.

Keywords

Coronary Calcium; Atherosclerosis; Pathogens; Infection

1. Introduction

Coronary artery calcium (CAC) is characteristic of coronary atherosclerosis (1, 2), and its quantity is correlated with atherosclerotic plaque burden (3). A growing body of evidence suggests that CAC is a strong predictor of cardiovascular disease (4, 5). CAC provides predictive information beyond conventional risk factors (6) and predicts cardiovascular events better than carotid intima-media thickness, a representative measure of subclinical atherosclerosis (7). Although CAC and other manifestations of atherosclerosis share risk factors to great extent, some unique properties of CAC have been also reported. For example, statin use has been reported to reduce overall atherosclerotic burden but not CAC in clinical trials (8, 9).

Several studies have suggested that infection may play a role in the development of calcification (10, 11). Pathogens such as *Chlamydia pneumoniae* infect human macrophages, endothelial cells and arterial smooth muscle cells (12), and induce production of cytokines and matrix metalloproteinases, thus potentially contributing to calcification (11, 13, 14). An intense inflammatory process by pathogen infection may result in debris of cellular membranes of the bacteria and host cells, creating a substrate for calcium deposition (15). Indeed, several pathogens have been found in calcified tissues (10, 11). Also, a cross-reaction of antibodies to pathogen and human heat shock protein has been proposed as a link between pathogens and calcification (16).

A few epidemiological studies have investigated the association between pathogens and CAC with conflicting results (1, 16). Zhu et al reported a positive association between *Helicobacter pylori* infection and CAC levels (16). Several other studies also reported a positive association of pathogens with calcification in vasculature other than coronary (17, 18). On the other hand, a larger study from the Multi-Ethnic Study of Atherosclerosis (MESA) reported no significant association between several pathogens (*Chlamydia pneumoniae*, *Helicobacter pylori*, cytomegalovirus, herpes simplex virus and hepatitis A virus) and prevalence of CAC (1). However, these studies are cross-sectional and thus not conclusive.

Examination of the association between pathogens and CAC in a prospective cohort will help better understand the involvement of pathogens in the development of CAC. Therefore, the objective of this study was to evaluate the prospective associations of pathogens with incidence and progression of CAC in a community-based cohort. Our primary exposure of interest was *Chlamydia pneumoniae*, which was measured in the entire MESA cohort. We also studied *Helicobacter pylori*, cytomegalovirus, herpes simplex virus and hepatitis A virus, and pathogen burden (number of positive pathogens) in a random sample of approximately 900 participants.

2. Methods

2.1. Participants

A detailed description of the study has been reported previously (19). Briefly, the MESA is a population-based study of 6,814 men and women aged 45 to 84 years who were free of clinical cardiovascular disease at study entry (July 2000–August 2002). Participants were recruited from six communities in USA (Baltimore, Maryland; Chicago, Illinois; Forsyth County, North Carolina; Los Angeles, California; New York, New York, and St Paul, Minnesota) and consisted of 38.5% whites, 27.8% African-Americans, 11.8% Chinese, and 22.0% Hispanics. MESA has been approved by the Institutional Review Board at each contributing institution, and all participants have given informed consent for their participation at each visit. For the current study, we excluded participants with missing data of follow-up CAC evaluation at visit 2 [2002-04] or 3 [2004-06] (n=1,056) and seropositivity to *Chlamydia pneumoniae* at baseline (n=14), leaving final sample of 5,744 participants (2,737 men and 3,007 women) for this study. As anticipated, those without follow-up CAC evaluation had higher risk profile than those with follow-up scan (e.g., age 63.9 ± 10.5 vs. 61.8 ± 10.2 years, current smokers 16.6% vs. 12.4%, diabetes 16.5% vs. 12.0%, and hypertension 51.0% vs. 43.8%, respectively). Of those 5,744 participants, seropositivity to *Helicobacter pylori*, cytomegalovirus, herpes simplex virus and hepatitis A virus was measured in a random sample of 873 participants.

2.2. Seropositivity to pathogens

Serum samples for determination of pathogens were frozen at -70°C (1). Serum IgG antibodies to *Chlamydia pneumoniae* were detected using a microimmunofluorescent antibody assay with a two-stage sandwich procedure (Focus Technologies, Cypress, California, USA). Indirect enzyme immunoassays were used to determine IgG antibodies to *Helicobacter pylori*, cytomegalovirus, and herpes simplex virus types 1 and 2 antigens (Diamedix Immunosimplicity Test Kits, Diamedix Corporation, Miami, Florida, USA). IgG antibodies are known to reflect previous history of infection (16). Total serum antibodies to hepatitis A virus were detected using the IMx times; HAVAB qualitative microparticle enzyme immunoassay (Abbott Laboratories, Abbott Park, Illinois, USA). Positivity values were as follows: *Chlamydia pneumoniae*, intensity of fluorescence ≥ 0 ; *Helicobacter pylori*, at least 1.1 index value; cytomegalovirus, at least 8.0 EU/ml; herpes simplex virus, at least 16 EU/ml; hepatitis A virus, at least 20 mIU/ml (1).

2.3. Incidence and progression of CAC

CAC was measured by multidetector computer tomographic scanning and electron beam computed tomography, as previously described (1, 20), in every participant at baseline (2000-02) and then at visit 2 (2002-04) in a random half of the study sample (n=2,950) and at visit 3 (2004-05) in the other half (n=2,794). One-fifth of participants in this study (n=1,117) had third CT scan at visit 4 (2005-07), which was used for several sensitivity analyses. Every participant was scanned twice at each visit. The Agatston score was based on the phantom-adjusted sum of calcium measures from the left anterior descending, left circumflex, and right coronary arteries and was recorded as the average of two scans (21). Custom software was developed at the CT reading center (Harbor-University of California Los Angeles Research and Education Institute, Los Angeles, California, USA) to measure calcified plaque specifically for the MESA study (20). Both inter- and intra-observer agreements were very high ($\kappa \geq 0.90$) (22).

Incident CAC was defined as newly detectable CAC at the follow-up examinations in participants who were free of CAC at baseline (5). As previously reported (23), among participants with detectable CAC at baseline, the presence of CAC progression was defined

as annualized change of 10 Agatston units/year for participants with Agatston score >0 and <100 at baseline and annualized change in Agatston score 10%/year for participants with Agatston score ≥ 100 at baseline. The study design for CAC incidence and progression analysis is summarized in Fig 1. We also tested change in CAC as a continuous outcome variable by calculating its annualized change between baseline and follow examinations.

2.4. Covariates

Trained technicians collected information on age, sex, education, race/ethnicity, smoking status, and medication use (1). Diabetes mellitus was defined as fasting plasma glucose ≥ 126 mg/dl, self-report of physician-diagnosed diabetes, or use of hypoglycemic drugs. Glucose was measured by rate reflectance spectrophotometry on the Vitros analyzer (Johnson & Johnson Clinical Diagnostics, Rochester, New York, USA). Fasting total cholesterol was measured using a cholesterol oxidase method (Roche Diagnostics, Indianapolis, Indiana, USA). High-density lipoprotein (HDL) cholesterol was measured similarly after precipitation of non-HDL cholesterol with magnesium-dextran. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation (24). Resting blood pressure was the average of the last two of three measurements using a Dinamap automated oscillometric sphygmomanometer (model Pro 100, Critikon, Tampa, Florida, USA). Body mass index was calculated as weight in kilograms divided by height squared in meters.

2.5. Statistical Analysis

Baseline characteristics were compared among four groups according to CAC at baseline and CAC incidence/progression during follow-up, i.e., no CAC at baseline + no incident CAC, no CAC at baseline + incident CAC, detectable CAC at baseline + no progression, and detectable CAC at baseline + progression. Continuous and categorical variables were compared across these four groups using ANOVA and χ^2 tests, as appropriate.

Logistic regression was used to assess the association between seropositivity to the pathogens and CAC incidence and progression. We implemented three models for the adjustment for covariates. Model 1 was not adjusted for any potential confounders (unadjusted). Model 2 was adjusted for age, sex, and race/ethnicity. Model 3 was further adjusted for level of education, smoking status, diabetes, HDL and LDL cholesterol, systolic and diastolic blood pressure, and body mass index. Because Models 2 and 3 provided very similar results, we mainly demonstrated results from Models 1 and 3. We analyzed CAC incidence and progression separately as well as combined as previously conducted (23). For a change in CAC as a continuous outcome variable, we fitted robust linear regression, which iteratively down-weighted observations with large residuals (5). We did not incorporate baseline CAC score as a covariate due to concerns about potential bias (25).

To assess potential interaction by demographic variables, we repeated our analysis after stratifying by age ($<$ and ≥ 65 years), sex, and race/ethnicity. Interactions were assessed by the likelihood-ratio test for models with and without interaction terms of pathogens and these demographic variables. Given the data availability, our primary exposure was seropositivity to Chlamydia pneumoniae. Using a random sample of 873 participants, we additionally examined the other four pathogens and total number of their seropositivity (pathogen burden [data on all pathogens available in 860 participants]). Since CAC evaluation at visit 4 was conducted only in one-fifth of participants, our primary analysis was based on CT at visits 2 and 3. However, we repeated our analysis incorporating visit 4 CT scan when available, which provided us with more incidence/progression cases and also allowed us to estimate annualized change in CAC score from a least-square regression model using CT scans at three time points. All analyses were conducted using Stata 11.2 and

12.1 software (Stata Corp, College Station, TX), and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Study sample and baseline characteristics

Out of 2,942 participants who did not have detectable CAC at baseline, during the follow-up of average 2.4 years (2002-2005), 475 participants (16.1%) had incident CAC at visit 2 (average follow-up 1.6 years [range from 0.9 to 3.4 years]) or visit 3 (average follow-up 3.2 years [1.9 to 4.9 years]). Among 2,802 participants who had detectable CAC at baseline (prevalent CAC), CAC progression was observed in 1,537 participants (54.9%) at visit 2 or 3. Participants with prevalent CAC were likely to be older, male, whites, and former smokers and have higher blood pressure and diabetes compared with those without prevalent CAC at baseline (Table 1). Similarly, those who had incident or progressed CAC were likely to have higher cardiovascular risk profile (i.e., older age, male, former or current smokers, higher blood pressure, and higher prevalence of diabetes) as compared to their counterparts who stayed at baseline level of CAC score.

3.2. Chlamydia pneumoniae in the entire cohort with CT at visits 2 and 3

In both unadjusted and adjusted models, the seropositivity to Chlamydia pneumoniae was not significantly associated with CAC incidence and progression overall (unadjusted odds ratio [OR] 1.11 [95% CI 0.88-1.39], $P=0.371$ and 1.14 [0.96-1.36], $P=0.135$, respectively) and in subgroups by age, sex, and race/ethnicity (Table 2). When we combined CAC incidence and progression, there was a significant association with the seropositivity to Chlamydia pneumoniae in unadjusted models for the entire sample (OR 1.17 [1.03-1.33], $P=0.016$), female (1.20 [1.01-1.43], $P=0.043$), and blacks (1.44 [1.09-1.90], $P=0.010$). However, these associations were no longer significant when we adjusted for traditional risk factors (point estimates of OR ranged from 1.04-1.14, all $P>0.19$). Of note, the adjustment solely for age diminished the significance of these estimates (OR 1.09 [0.95-1.24], $P=0.220$, for overall study population, 1.12 [0.93-1.34], $P=0.242$, for female, and 1.21 [0.90-1.61], $P=0.203$, for blacks). Similar results were observed when seropositivity to Chlamydia pneumoniae was treated as an ordinal variable based on the fluorescence intensity (Table 3). We did not observe any significant interactions between the seropositivity to Chlamydia pneumoniae and the demographic variables tested in either unadjusted or adjusted models (data not shown). When change in CAC score was treated as a continuous outcome variable in the entire study sample, we did not observe significant associations with Chlamydia pneumoniae in either unadjusted or adjusted models ($\beta=0.10$ for seropositive vs. seronegative, $P=0.062$ in Model 1, $\beta=0.05$, $P=0.302$ in Model 2, and $\beta=0.06$, $P=0.277$ in Model 3, respectively).

3.3. Five pathogens in the subsample with CT at visits 2 and 3

In 893 participants with information on additional pathogens, none of five pathogens (Chlamydia pneumoniae, Helicobacter pylori, cytomegalovirus, herpes simplex virus and hepatitis A virus) was significantly associated with CAC incidence or progression in adjusted models (Fig 2). The unadjusted models demonstrated similar results ($P>0.32$ for any positive associations). When we evaluated the accrual of seropositivity to these pathogens, we did not observe any dose-response relationships in either unadjusted or adjusted models (Table 3). Similarly, none of five pathogens were associated with increase in CAC change as a continuous outcome variable in either unadjusted or adjusted models ($\beta=0.03$, $[-0.04-0.10]$, $P=0.362$ for Chlamydia pneumoniae, $\beta=-0.02$, $[-0.09-0.04]$, $P=0.499$ for Helicobacter pylori, $\beta=-0.09$, $[-0.15--0.03]$, $P=0.005$ for cytomegalovirus, $\beta=-0.02$

$[-0.10-0.07]$, $P=0.718$ for herpes simplex virus, $=-0.06 [-0.12--0.001]$, $P=0.047$ for hepatitis A virus in unadjusted models).

3.4. Sensitivity analyses incorporating CT at visit 4

A third CT scan was available on average 4.8 years (range from 3.9 to 6.8 years) after baseline scan in 1,117 participants (595 without and 522 with prevalent CAC at baseline), of which, 108 and 105 participants newly developed incident and progressed CAC after visit 2 and 3 CT scans, respectively (different 67 and 254 participants already had incident and progressed CAC at visit 2 or 3, respectively). With these data, we observed largely similar results. Specifically, the seropositivity to Chlamydia pneumonia was only significantly associated with combined outcome of CAC incidence and progression in unadjusted model (OR 1.17 [95% CI 1.03-1.32], $P=0.015$) but not in the adjusted model (1.03 [0.90-1.18], $P=0.664$). The association with respective CAC incidence and progression was not significant even in unadjusted models (OR 1.06 [95% CI 0.86-1.30], $P=0.599$ and 1.18 [0.99-1.41], $P=0.062$, respectively) and was even weaker in the adjusted models (1.00 [0.80-1.24], $P=0.977$ and 1.11 [0.92-1.33], $P=0.283$, respectively). Similarly, there was no significant association with change in CAC score estimated from regression models incorporating all available CT scans ($=0.10 [-0.01-0.22]$, $P=0.084$ for unadjusted and $=-0.01 [-0.22-0.20]$, $P=0.931$ for the adjusted model). The results for the other four pathogens in the subsample were much the same as primary analysis with visit 2 and 3 CT scans (data not shown).

5. Discussion

To our knowledge, this is the first population-based cohort study investigating the prospective relationship of Chlamydia pneumoniae and other four pathogens to CAC incidence and progression evaluated over mean follow-up of 3 years. Overall, our analyses with the entire MESA cohort did not demonstrate independent associations of Chlamydia pneumonia, one of most investigated pathogens in atherosclerosis (26), with CAC incidence and progression. We obtained similar null results in all of the subgroups according to age, sex, and race/ethnicity tested. In a random sample of ~900 participants, the seropositivity to five pathogens including Helicobacter pylori, cytomegalovirus, herpes simplex virus and hepatitis A virus as well as Chlamydia pneumonia and their accrual was not significantly associated with CAC incidence and progression. The results were similar when change in CAC score was treated as a continuous outcome variable.

Several potential mechanisms linking pathogens and calcification of artery have been reported. Specifically, pathogens stimulate human macrophages, endothelial cells and arterial smooth muscle cells (12) and induce production of cytokines and matrix metalloproteinases, which may be involved in the development of artery calcification (11, 13-15). Indeed, several cross-sectional studies report the positive association between pathogens and calcification of artery (16-18). However, our prospective study confirmed that there is no consistently significant association of Chlamydia pneumoniae and the other four pathogens with CAC incidence and progression, as previously shown in a larger cross-sectional study from the MESA (1). Our study has a few important advantages as compared to previous studies in terms of sample size (>5,000 participants) and prospective design. Of note, our results are consistent with a meta-analysis demonstrating that antibiotic therapy is not effective to improve prognosis in patients with coronary artery disease (26).

Our study does not completely rule out involvement of pathogens in CAC formation or other process of atherosclerosis. IgG antibodies were used to evaluate seropositivity to Chlamydia pneumoniae in the current study and may reflect past exposure rather than active chronic infection (16). Thus, pathogens may contribute to CAC development in the acute phase.

Indeed, some investigators suggest that *Chlamydia pneumoniae* IgA antibodies may be more strongly associated with atherosclerosis compared to IgG antibodies (27, 28). Nevertheless, atherosclerosis is a long-standing process, and it is important to confirm lack of association between chronic present or past infection of several pathogens and CAC. For *Helicobacter pylori*, cytomegalovirus, herpes simplex virus, and hepatitis A virus, there may be an issue of statistical power since seropositivity to these pathogens was only measured in ~900 participants. However, none of ORs of CAC incidence and progression for these pathogens exceeded 1.2, suggesting a weak association, if any, between these pathogens and CAC. Of note, the accrual of seropositivity to these pathogens was not associated with CAC incidence and progression either, despite that some studies have postulated pathogen burden, rather than individual pathogens, as a cardiovascular risk factor (1). There is a possibility that these pathogens contribute to CAC development over a longer term. However, we did not observe any stronger association when we limited our analysis to those with visit 4 CT scan obtained ~4 to ~7 years after baseline (data not shown). Since MESA exclusively enrolled those without clinical history of cardiovascular disease, it is possible that the pathogens are involved in CAC progression among those with prior cardiovascular disease. Nevertheless, given that other traditional risk factors (e.g., diabetes and lipids) contribute to CAC incidence and progression with data at MESA visits 2 and 3 (5), our findings suggest that the association of these pathogens with CAC is not as strong as that of standard risk factors. Our study does not preclude the possibility that these pathogens contribute to calcification of vasculature other than coronary artery or other pathogens play a role in the pathophysiology of CAC (15). Also, future prospective studies would be required for other measures of subclinical atherosclerosis such as carotid intima-media thickness.

Some other limitations as well as strengths of this study should be mentioned. The follow-up CT scan was not conducted at the same visit in every participant, namely, one-half at visit 2 and the rest at visit 3. However, the selection was conducted randomly, and the difference of on average ~1.5 year between visit 2 and 3 CT scans does not seem to induce strong selection bias. Of note, our findings were consistent when we incorporated visit 4 CT data obtained in a fifth of participants as a second follow-up (third CT scan including baseline) ~5 years after baseline. Scanners were changed between baseline and follow-up at 3 of the 6 sites (5). This is why we used phantom-adjusted CAC score. Also, we observed similar findings when we limited our analysis to those who had their CAC evaluated with the same scanners (data not shown). Several other definitions for CAC progression have been proposed (5, 29) and may provide slightly different results. Nevertheless, we confirmed the lack of positive association using change in CAC score as a continuous outcome, which did not rely on any specific thresholds or definitions. Strengths of this study include measurement of seropositivity to *Chlamydia pneumoniae* in the entire cohort with sample size of ~6,000 subjects and longitudinal design with repeated CT scans. Inclusion of four racial/ethnic groups from six US communities and the homogeneity of our results would support the generalizability of our findings to a broad range of populations.

In conclusion, our prospective study with repeated CTs in ~6,000 individuals did not demonstrate significant association of seropositivity to *Chlamydia pneumoniae* with CAC incidence and progression. Similarly, we did not observe significant prospective associations of *Helicobacter pylori*, cytomegalovirus, herpes simplex virus, and hepatitis A virus and their accrual with CAC in our subsample. Taken altogether, it is unlikely that these pathogens play important roles in the development of CAC.

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Highlights of “The prospective association of pathogens with development of coronary artery calcium: the Multi-Ethnic Study of Atherosclerosis (MESA)”

1. First prospective study of the association between pathogens and CAC development.
2. No independent associations of Chlamydia pneumonia with CAC development.
3. Similar null results in all of the subgroups according to age, sex, and race.
4. No independent associations for H. pylori, CMV, HSV and hepatitis A virus.
5. Unlikely that these pathogens play important roles in the development and progression of CAC.

Fig 1.
Study design for CAC incidence and progression analysis

Fig 2.

Adjusted odds ratio (95% CI) of CAC incidence and progression at visits 2 and 3 according to seropositivity to individual pathogens in a random sample (n=873)

Table 1
Baseline characteristics according to CAC status at baseline and during follow-up (visits 2 and 3)

Variable	Agatston CAC score at baseline			
	0 (not prevalent)		>0 (prevalent)	
	CAC incidence during follow-up		CAC progression during follow-up	
	–	+	–	+
N	2,467	475	1,265	1,537
Age in years (mean)	57 (9)	61 (9)	65 (10)	67 (9)
Male (%)	36	45	57	60
Ethnic background (%)				
Whites	34	37	45	46
African-American	31	31	22	24
Chinese	13	8	12	12
Hispanic	23	25	21	19
Cigarette smoking status (%) ^a				
Never	58	50	46	43
Former	30	37	42	44
Current	12	13	11	13
Triglycerides (mean, mg/dl) ^a	124(86)	135 (76)	132 (77)	139 (98)
HDL cholesterol (mean, mg/dl) ^a	53 (15)	50 (14)	50 (14)	49 (14)
LDL cholesterol (mean, mg/dl) ^a	116 (30)	119 (31)	119 (31)	118 (32)
Hypertension (%)	32	47	47	59
Systolic blood pressure (mean, mm/Hg) ^a	121 (20)	127 (21)	128(20)	132 (22)
Diastolic blood pressure (mean, mm/Hg) ^a	71 (10)	73 (10)	72 (10)	73 (10)
Diabetes (%) ^a	8	13	9	17
Body mass index (mean, kg/m ²)	28 (6)	29 (6)	28 (5)	28 (5)
Educational level (%) ^a				
<12 years completed	16	19	17	17
12-16 years completed	46	46	47	47
>16 years completed	38	35	37	36

Values are expressed as mean (SD) or proportion; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

^aMissing values (number missing): Cigarette smoking status, 14; Triglycerides, 8; HDL cholesterol, 11; LDL cholesterol, 80; Systolic blood pressure, 2; Diastolic blood pressure, 2; Diabetes 6; Education, 15. Diastolic blood pressure, 1; Diabetes 18; Education, 200.

Table 2
Odds ratios (ORs) for CAC incidence and progression according to seropositivity to Chlamydia pneumonia in the entire cohort (n=5,744) of the Multi-Ethnic Study of Atherosclerosis

Subgroups	CAC incidence				CAC progression				CAC incidence & progression ^a			
	Unadjusted		Adjusted ^a		Unadjusted		Adjusted ^b		Unadjusted		Adjusted ^b	
	N	OR (95% CI)	N	OR (95% CI)	N	OR (95% CI)	N	OR (95% CI)	N	OR (95% CI)	N	OR (95% CI)
<i>Overall</i>												
Seronegative	767	ref	753	ref	665	ref	650	ref	1432	ref	1403	ref
Seropositive	2,175	1.11	2,145	1.06	2,137	1.14	2,098	1.07	4312	1.17 ^c	4,243	1.04
		(0.88 - 1.39)		(0.83 - 1.35)		(0.96 - 1.36)		(0.89 - 1.29)		(1.03 - 1.33)		(0.90 - 1.19)
<i>Gender</i>												
<i>Male</i>												
Seronegative	221	ref	214	ref	328	ref	321	ref	549	ref	535	ref
Seropositive	876	0.79	863	0.86	1,312	1.07	1,283	1.00	2,188	0.98	2146	0.93
		(0.55 - 1.14)		(0.59 - 1.27)		(0.84 - 1.37)		(0.78 - 1.29)		(0.81 - 1.19)		(0.76 - 1.14)
<i>Female</i>												
Seronegative	546	1	539	1	337	1	329	1	883	1	868	1
Seropositive	1,299	1.28	1,282	1.24	825	1.19	815	1.14	2,124	1.20 ^c	2,097	1.14
		(0.95 - 1.72)		(0.91 - 1.70)		(0.92 - 1.53)		(0.86 - 1.49)		(1.01 - 1.43)		(0.94 - 1.38)
<i>Ethnic background</i>												
<i>White</i>												

Subgroups	CAC incidence			CAC progression			CAC incidence & progression ^a		
	Unadjusted		Adjusted ^a	Unadjusted		Adjusted ^b	Unadjusted		Adjusted ^b
	N	OR (95% CI)	N	OR (95% CI)	N	OR (95% CI)	N	OR (95% CI)	N
Seronegative	325	ref	320	ref	386	ref	376	ref	711
Seropositive	681	1.19	672	1.14	881	1.11	867	1.15	1,539
		(0.84 - 1.70)		(0.78 - 1.67)		(0.88 - 1.42)		(0.96 - 1.39)	
Black									
Seronegative	200	ref	196	ref	108	ref	108	ref	304
Seropositive	711	1.40	702	1.26	533	1.22	525	1.44 ^c	1,227
		(0.89 - 2.21)		(0.78 - 2.04)		(0.81 - 1.85)		(1.09 - 1.90)	
Chinese									
Seronegative	59	ref	56	ref	45	ref	44	ref	100
Seropositive	292	0.56	288	0.42	290	1.14	283	1.03	571
		(0.26 - 1.22)		(0.18 - 1.02)		(0.61 - 2.15)		(0.66 - 1.61)	
Hispanic									
Seronegative	183	ref	181	ref	126	ref	122	ref	303
Seropositive	491	1.08	483	01.05	433	1.17	423	1.23	906
		(0.67 - 1.72)		(0.64 - 1.72)		(0.79 - 1.75)		(0.93 - 1.63)	
Age									
65 years									

CAC incidence				CAC progression				CAC incidence & progression ^a							
Subgroups	N	Unadjusted		Adjusted ^a		Unadjusted		Adjusted ^b		Unadjusted		Adjusted ^b			
		OR	(95% CI)	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)		
Seronegative	166	ref		ref		367	ref		ref		533	ref		523	ref
Seropositive	603	1.01		0.92		1,299	1.20		1.09		1,902	1.13		1,874	1.03
		(0.68 - 1.52)		(0.60 - 1.41)		(0.95 - 1.52)		(0.85 - 1.40)		(0.93 - 1.37)		(0.83 - 1.26)			
<65 years															
Seronegative	601	ref		ref		298	ref		ref		899	ref		880	ref
Seropositive	1,572	1.08		1.12		838	1.05		0.99		2,410	1.09		2369	1.03
		(0.82 - 1.43)		(0.83 - 1.50)		(0.80 - 1.37)		(0.74 - 1.31)		(0.91 - 1.30)		(0.85 - 1.24)			

^aCAC incidence and progression were modeled together.

^bAdjusted for age, sex, ethnic background, education, smoking status, diabetes, HDL and LDL cholesterol, systolic and diastolic blood pressure, and body mass index.

^cP < 0.05.

Table 3
Odds ratios (ORs) for CAC incidence and progression according to fluorescence intensity of positivity to Chlamydia pneumonia in the entire cohort (n=5,744) of the Multi-Ethnic Study of Atherosclerosis

fluorescence intensity	CAC incidence			CAC progression			CAC incidence & progression ^a		
	Unadjusted		Adjusted ^b	Unadjusted		Adjusted ^b	Unadjusted		Adjusted ^b
	N	ORs (95% CI)	N	ORs (95% CI)	N	ORs (95% CI)	N	ORs (95% CI)	N
Negative	767	1	753	1	665	1	1,432	1	1,403
		(Reference)		(Reference)		(Reference)		(Reference)	
1+	540	0.95	528	0.97	448	1.10	441	1.07	988
		(0.69 - 1.29)		(0.70 - 1.35)		(0.86 - 1.40)		(0.83 - 1.37)	
								(0.85 - 1.20)	(0.83 - 1.20)
2+	1,237	1.15	1,227	1.09	1,199	1.19	1,177	1.11	2,436
		(0.90 - 1.48)		(0.84 - 1.42)		(0.99 - 1.44)		(0.90 - 1.35)	
								(1.05 - 1.38)	(0.91 - 1.23)
3+	398	1.20	390	1.09	490	1.06	480	0.99	888
		(0.87 - 1.66)		(0.77 - 1.54)		(0.84 - 1.34)		(0.77 - 1.27)	
								(1.06 - 1.50)	(0.85 - 1.24)

CAC, coronary artery calcium incidence and progression; 95% CI, 95% confidence intervals.

^aCAC incidence and progression modeled together.

^bAdjusted for age, sex, ethnic background, education, smoking status, diabetes, HDL and LDL cholesterol, systolic and diastolic blood pressure, and body mass index.

Odds ratios (ORs) for CAC incidence and progression according to seropositivity to pathogen burden^a in a random sample (n=860) of the Multi-Ethnic Study of Atherosclerosis

Table 4

Pathogen burden ^a	CAC incidence			CAC progression			CAC incidence & progression ^b		
	Unadjusted	Adjusted ^c	ORs	Unadjusted	Adjusted ^c	ORs	Unadjusted	Adjusted ^c	ORs
	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)
N	N	N	N	N	N	N	N	N	N
0 and 1	51	1	50	1	47	1	46	1	96
2	84	1.28	81	0.96	58	1.04	57	0.91	142
3	99	0.62	96	0.49	91	1.27	90	1.04	190
4	130	0.66	127	0.57	85	0.93	85	0.67	215
5	128	0.85	125	0.67	87	1.12	87	0.81	215

CAC, coronary artery calcium incidence and progression; 95% CI, 95% confidence intervals.

^aPathogen burden is defined as the total number of pathogens (Chlamydia pneumoniae, Helicobacter pylori, cytomegalovirus, herpes simplex virus and hepatitis A virus) with seropositivity.

^bCAC incidence and progression modeled together.

^cAdjusted for age, sex, ethnic background, education, smoking status, diabetes, HDL and LDL cholesterol, systolic and diastolic blood pressure, and body mass index.