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Biomarkers and degree of atherosclerosis are independently associated with incident atherosclerotic cardiovascular disease in a primary prevention cohort: The ARIC study

Anandita Agarwala[2], **Salim Virani**[1,3], **David Couper**[4], **Lloyd Chambless**[4], **Eric Boerwinkle**[5], **Brad C. Astor**[6], **Ron C. Hoogeveen**[2], **Joe Coresh**[7], **A. Richey Sharrett**[7], **Aaron R Folsom**[8], **Tom Mosley**[9], **Christie M. Ballantyne**[2,3], and **Vijay Nambi**[1,2,3,*]

[1]Michael E DeBakey Veterans Affairs Hospital

[2]Baylor College of Medicine

[3]Methodist DeBakey Heart and Vascular Center

[4]University of North Carolina at Chapel Hill School of Global Public Health

[5]University of Texas, School of Public Health, Houston

[6]University of Wisconsin School of Medicine and Public Health

[7]Johns Hopkins School of Public Health

[8]University of Minnesota, Division of Epidemiology and Community Health, Minneapolis, MN

[9]University of Mississippi

Abstract

Background and aims—Biomarkers and atherosclerosis imaging have been studied individually for association with incident cardiovascular disease (CVD); however, limited data exist on whether the biomarkers are associated with events with a similar magnitude in the presence of atherosclerosis. In this study, we assessed whether the presence of atherosclerosis as measured by carotid intima media thickness (cIMT) affects the association between biomarkers

*Corresponding author: Alkek Tower, Room A679B, 6565 Fannin Street, Houston, TX 77030. Tel: (713) 798 5800. vnambi@bcm.edu (V. Nambi).

Author contributions

All authors contributed substantially to the work presented in this paper.

Conflict of interest

A. Agarwala, None; S.S. Virani, None; D. Couper, None; B. Astor, None; T.H. Mosley, None; E. Boerwinkle, None; J. Coresh, None; R. Sharrett, None; A.R. Folsom, None; V. Nambi, Research collaboration with GE (no financial support), Modest, Research Grant; Along with Roche and Baylor College of Medicine we have filed a provisional patent (patent #61721475) entitled ” Biomarkers to Improve Prediction of Heart Failure Risk”, Modest, Other.

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known to be associated with coronary heart disease (CHD) and incident cardiovascular disease (CVD) in a primary prevention cohort.

Methods—8,127 participants from the ARIC study (4th visit, 1996–1998) were stratified as having minimal, mild, or substantial atherosclerosis by cIMT. Levels of C-reactive protein, lipoprotein-associated phospholipase A2, cardiac troponin T, N-terminal pro-brain natriuretic peptide, lipoprotein(a), cystatin C, and urine albumin to creatinine ratio were measured in each participant. Hazard ratios were used to determine the relationship between the biomarkers and incident CHD, stroke, and CVD in each category of atherosclerosis.

Results—While each of the biomarkers was significantly associated with risk of events overall, we found no significant differences noted in the strength of association of biomarkers with CHD, stroke, and CVD when analyzed by degree of atherosclerosis.

Conclusions—These findings suggest that the level of atherosclerosis does not significantly influence the association between biomarkers and CVD.

Keywords

Carotid intima media thickness; high sensitivity C-reactive protein; lipoprotein associated phospholipase A2; high sensitivity troponin T; NT-pro B-type natriuretic peptide; lipoprotein(a); cardiovascular disease; coronary heart disease; stroke

Introduction

Several biomarkers and atherosclerosis imaging measures have been evaluated for association with incident cardiovascular disease (CVD) and for value in predicting incident CVD. Imaging provides an assessment of atherosclerosis burden while biomarkers provide information related to the *in vivo* biochemical milieu.

Biomarkers associated positively with prevalent and incident cardiovascular disease including coronary heart disease and stroke include C-reactive protein (hs-CRP)[1–5], lipoprotein-associated phospholipase A2 (LpPLA2)[2,6,7], cardiac troponin T (cTnT)[8], N-terminal pro-brain natriuretic peptide (NT-proBNP)[9], lipoprotein(a) (Lp(a))[10–14], cystatin C[15–18], and urine albumin to creatinine ratio[9,19,20]. Additionally, atherosclerosis as assessed using imaging techniques such as carotid intima media thickness (cIMT), carotid artery plaque, and coronary artery calcium measured on a computed tomography (CT) scan, have also been shown to be associated positively with CVD and to improve coronary heart disease risk prediction[21–26]. Although many of these modalities have been studied individually, limited data exist on whether all of the biomarkers are associated with events with a similar magnitude in the presence of atherosclerosis and whether these imaging measures and biomarkers interact with each other in the determination of cardiovascular risk.

The Cardiovascular Health Study (CHS) reported that a positive association between hs-CRP and incident CV events was found only when atherosclerosis (as measured by c-IMT/plaque) was present[27]. However, knowing whether other biomarkers are associated with CVD only in the presence of atherosclerosis is of interest and value in both clinical practice and in research as it may help us understand the implication if one test result is normal when

the other is abnormal. We sought to answer the question whether there is an interaction between biomarkers and levels of atherosclerosis as measured by carotid ultrasound on incident CVD. We hypothesized that the strength of the association between biomarkers and CVD would be significantly weaker in individuals with a lower burden of atherosclerosis, as defined by carotid IMT (cIMT) in the lowest quartile and absence of plaque, compared to those with a greater burden of atherosclerosis.

Materials and methods

Study design and study participants

The Atherosclerosis Risk in Communities (ARIC) study is a prospective study of the incidence of cardiovascular disease that includes 15,792 men and women between the ages of 45 and 64 who were recruited from four US communities (selected suburbs of Minneapolis, Minnesota; Washington County, Maryland; Forsyth County, North Carolina; Jackson, Mississippi) between the years of 1987 to 1989. Since the original visit, 4 additional visits have been completed. The 4th visit of the ARIC study (1996–1998) formed the baseline for this analysis. The institutional review boards of all participating centers approved the ARIC study and all participants provided informed consent.

Additional description of the ARIC study design is published elsewhere[28]. Of the 15,792 participants, we excluded those who did not participate in visit 4 (n= 4,136), those who described their race to be neither white or black (n= 31), blacks from the Minneapolis or Washington county cohorts (n= 38), participants with prevalent stroke or coronary heart disease (CHD) at visit 4 (n= 1,436), missing cIMT or plaque information at both visits 3 and 4 (n= 1,469), missing biomarkers (n= 445), or missing CHD[29,30] or stroke[31] risk score at visit 4 (n= 120), which resulted in a remaining cohort of 8,127 individuals who were included in the final analysis. Prevalent CHD was defined as self-reported myocardial infarction prior to visit 1, silent myocardial infarction (diagnosed by electrocardiogram), ARIC-documented myocardial infarction, or coronary revascularization occurring between visits 1 and 4. Incident endpoints through 2012 assessed included CHD (defined as definite or probable myocardial infarction or fatal CHD), stroke (ischemic/thrombotic), which together comprised the CVD endpoint after visit 4. Methods for ascertainment and diagnosis of incident CVD events have been described previously[32].

Cigarette smoking and the use of antihypertensive and lipid lowering medications were obtained from standardized questionnaires at visit 4. Hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg, prior diagnosis of hypertension by a physician, or the use of antihypertensive medications during the 2 weeks prior to participation. Diabetes was defined as a fasting plasma glucose level ≥126 mg/dL, a non-fasting plasma glucose level of ≥200 mg/dL, or a self-reported history of physician diagnosed diabetes or treatment for diabetes. Plasma total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides were measured using enzymatic measures. Low-density lipoprotein cholesterol (LDLC) was calculated using the Friedewald equation. Plasma hs-CRP was measured using a high sensitivity immunonephelometric assay using a BNII nephelometer (Siemens Healthcare Diagnostics, Deerfield, IL). The reliability coefficient in 421 blinded replicates was 0.99. Methods have been previously

described in the ARIC study[33]. Lp-PLA2 was measured by an automated Colorimetric Activity Method (CAM) assay (diaDexus Inc., South San Francisco, CA) using a Beckman Coulter (Olympus) AU400e autoanalyzer using a dual monoclonal antibody immunoassay that is standardized to recombinant Lp-PLA2[2,34]. Interassay precision was assessed using 2 controls of known concentration (low and high) in 40 separate assays; the interassay coefficients of variation were 12.7% and 9.6%, respectively. NT-proBNP was measured by using an electrochemiluminescent immunoassay on an automated Cobas e411 analyzer (Roche Diagnostics) with lower detection limit of 5 pg/mL. The reliability coefficient and the inter-assay coefficient of variation, were 1.00 and 9.9% and 1.00 and 6.7%, respectively. based on 418 blind-replicate quality control samples before and after removal of outliers (greater than 3 standard deviations). The interassay coefficient of variation, based on 83 runs, at NT-proBNP concentrations of 121.6 pg/mL and 4059.1 pg/mL were 6.97% and 6.76%, respectively. CTnT levels were measured with a high-sensitivity assay (Elecsys Troponin T; Roche Diagnostics, Indianapolis, Illinois, USA) using a Cobas e411 automated analyzer[35]. The lower and upper limits of detection of the cTnT assay are 3 and 10,000 ng/L, respectively, and the limit of quantitation is 13 ng/L. The reliability coefficient and interassay coefficient of variation, based on 418 blind quality control samples before and after removal of outliers (great than 3 standard deviations), were 0.98 and 23.1%, and 0.99 and 15%, respectively. The interassay coefficient of variation, based on 103 runs, at cTnT concentrations of 29 ng/L and 2378 ng/L were 6.2% and 2.6%, respectively. Details on variability and interassay reliability have been previously described in the ARIC study[36]. Cystatin C was measured by a particle-enhanced immunonephelometric assay with a BNII nephelometer (Siemens Healthcare Diagnostics, Deerfield, Illinois, USA)[37]. The reliability coefficient for 421 blinded replicates was 0.65 (0.94 after removing 10 pairs of outliers). The coefficient of variation was 6.6%. Urine albumin creatinine ratio was calculated from a random urine sample. The Jaffe method was used to measure urine creatinine and the nephelometric method (measured either on the Dade Behring BN100 or Beckman Image Nephelometer) was used to measure urine albumin[38]. Lp(a) levels were measured at visit 4 using an immunoturbidimetric assay using polyclonal antibodies directed against epitopes on apolipoprotein(a) (Denka Seiken, Coventry, United Kingdom)[11,39]. Other than the cholesterol markers and Lp(a) blood biomarker measurements were carried out using frozen, stored samples at a later date.

Ultrasound imaging

By design in the ARIC study, cIMT was performed in half of the participants at visit 3 and the remaining half at visit 4. Ultrasound images of the carotid arteries were obtained using a Biosound 2000II-SA ultrasound system and measured using standardized, centrally trained and certified readers. CIMT was assessed on both sides in 3 territories: the distal common carotid (1 cm proximal to the dilation of the carotid bulb), the carotid artery bifurcation (1 cm proximal to the flow divider), and the proximal internal carotid arteries (a 1 cm section of the internal carotid artery immediately distal to the flow divider). We used the mean IMT value averaged across the six sites imputing any missing sites from the observed sites. Methods for imputation have been described previously[40,41]. The presence of plaque was determined by trained readers based on the presence or absence of two out of the following three criteria: abnormal wall thickness (defined as CIMT >1.5 mm), abnormal shape

(protrusion into the lumen and loss of alignment with adjacent arterial wall boundary), and abnormal wall texture (brighter echoes than adjacent boundaries). Since cIMT was measured in visits 3 and 4, cIMT was stratified by visit in addition to gender as <25, 25–75, and >75th percentile. Significant burden of sub-clinical atherosclerosis consisted of cIMT values >75th percentile and presence of plaque. Mild sub-clinical atherosclerosis category consisted of cIMT values between the 25th-75th percentile or presence of plaque. Minimal sub-clinical atherosclerosis category consisted of cIMT values <25th percentile and absence of plaque. All subjects in our cohort with cIMT >75th percentile had plaque present. To estimate the reliability of the IMT measurements, from 278 pairs of scans performed up to a year apart and the estimated correlation between the scans was 0.56 for the mean right common carotid ITM and 0.55 for the left common carotid IMT. Methodology for the cIMT measurement along with its variation and reproducibility has been previously described in the ARIC study[42–47].

Statistical analyses

Biomarkers were modeled as continuous variables and were log transformed. A Cox proportional hazards model was developed to estimate the hazards ratio for each biomarker separately for the incident events of CHD, stroke, and CVD. Models were then rerun, stratified by cIMT and plaque presence into 3 categories: minimal (negligible), mild, and substantial atherosclerosis. Multiplicative interactions between the biomarker and atherosclerosis level were evaluated in the overall model, using cross-product terms, and the p-value for each interaction was determined using the Wald test. The Cox proportional hazards model were analyzed using two different sets of covariates. Model 1 adjusted for age at visit 4, race, and sex. For CHD, model 2 adjusted for the ARIC 10-year CHD risk score[48]. For stroke, model 2 adjusted for the ARIC 10-year stroke risk score[31]. For CVD, model 2 adjusted for variables used in CHD as well as the stroke risk scores. As 14.7% of participants had imputed values additional similar analyses were also done after exclusion of imputed values for each of the outcomes overall and across each of the IMT categories. Additionally, interactions between each biomarker and the presence of carotid artery plaque overall and in each of the 3 IMT categories was assessed for CHD, stroke, and CVD.

Results

The mean age of the study participants was 63 years, with 59% females and 81% whites at visit 4. Forty three percent of the participants had hypertension while 13% had diabetes (table 1). The mean BMI of the study population was 28.3 ± 5.3 kg/m^2. During a median follow-up duration of 5448 days for CHD, 5458 for stroke, and 5424 for CVD, there were 620 incident CHD events, 366 stroke events, and 924 CVD events in the cohort. The association between cIMT/plaque and CHD, stroke, and CVD events was significant even after adjusting for demographics and the ARIC 10-year CHD and stroke scores (p<0.005).

Most of the biomarkers were positively and independently associated with CHD, stroke, and CVD after both minimal (model 1) (model 1 graphs are shown in the supplemental material as supplemental figures 1–3) and additional adjustment for other risk factors (model 2). The hazards ratio (HR) for overall events for 1 unit increment of the log transformed variables

ranged from 1.04 (0.96, 1.13) for log lipoprotein(a) to 2.34 (1.72, 3.18) for log cystatin c after adjustment for other risk factors (model 2). Log LpPLA2 activity and log of Lp(a) did not demonstrate a significant association with stroke (hazards ratios are shown in supplemental tables 1–7). Log cystatin C demonstrated the strongest association with CHD (HR 2.34, 95% CI 1.72, 3.18), stroke (HR 2.32, 95% CI 1.53, 3.50), and CVD (HR 2.27 95% CI 1.74, 2.95).

Incidence rates of CVD increased across levels of atherosclerosis (Table 2). Contrary to our hypothesis, the presence and degree of atherosclerosis did not significantly affect the associations in adjusted models between the biomarkers and CHD, stroke, or CVD (figures 1–3), with no significant multiplicative interactions ($p>0.05$). For example, though log cystatin c was independently associated with CHD (HR 2.34, 95% CI 1.78, 3.18), when stratified by degree of atherosclerosis the hazard ratio was 1.62 for minimal atherosclerosis, 2.38 for mild atherosclerosis, and 2.68 for substantial atherosclerosis with a p value for interaction of 0.72.

Of the participants used in the current analyses 14.7% had imputed cIMT values. After excluding participants with one or more imputed cIMT values (supplemental tables 9–11), the results were comparable to those in which the imputed cIMT values were included with the exception of log NT proBNP. The degree of atherosclerosis had a significant effect on CHD for log NT proBNP (HR 1.21, 95% CI 0.95, 1.54 for minimal atherosclerosis, HR 1.21, 95% CI 1.10, 1.36 for mild atherosclerosis, and HR 1.59, 95% CI 1.35, 1.89 for substantial atherosclerosis, p for interaction is 0.04, supplemental tables 9–11). As there is some controversy about cIMT as a marker of atherosclerosis we also assessed presence of carotid plaque alone as a marker of atherosclerosis and investigated interactions between each biomarker and the presence of plaque overall and within each of the 3 IMT categories for each of the 3 outcomes (CHD, stroke, and CVD). The interaction between the biomarkers and plaque was not significant in any of the models (supplemental tables 12–14).

Discussion

In this prospective population-based study, we investigated whether the burden of atherosclerosis, as measured by cIMT/ presence of plaque, modifies the associations between biomarkers and incident CVD events. Contrary to our hypothesis, the positive associations of biomarkers with incident CVD were not stronger in the presence of substantial burden of atherosclerosis. Studies have demonstrated the presence of plaque even with normal cIMT suggesting the importance of assessing for carotid plaque[48,49]. In light of these findings, we assessed for interaction between biomarkers and the presence or of plaque overall and in each of the three cIMT categories, however, we did not find that the association between biomarkers and incident CVD was strengthened in the presence of plaque.

The development and manifestation of CVD is a complex process that encompasses a number of factors including plaque development, plaque burden, and the risk of rupture or vulnerability. Imaging techniques such as cIMT and coronary artery calcium score (CAC score) can be used to estimate the burden of atherosclerosis and have been shown to be

associated with and to improve prediction of incident CHD[50–52]. While there have been and continue to be efforts in imaging the components and activity of plaques, the value of these techniques in prediction of incident CVD is not yet established.

On the other hand, although biomarkers have been associated with CVD[1–20] and provide a snapshot of the array of ongoing molecular processes, they generally do not provide specifics on the presence, composition, and stability of the plaques present. Hence we hypothesized that the presence of atherosclerosis may be required for the biomarkers to show an association with CVD. Our findings that biomarkers were positively associated with incident CVD whether or not atherosclerosis was present raise the question as to whether both of these variables, i.e. biomarkers and imaging (cIMT/plaque), which have previously shown independent association with incident events, may have additional value when combined; however this will need to be further explored. While the recent guidelines related to the management of hyperlipidemia[53] will limit the additional value of combining biomarkers and imaging in CVD risk prediction (since the population where additional risk stratification could impact management has considerably decreased) further exploration of our results may be of value in the future design and conduct of clinical studies.

Strengths of the present study include the fact that it was conducted in a large, well-characterized population based study whose stated goal is tracking and assessing cardiovascular disease incidence. We had information on several biomarkers and imaging, and the incident events were well characterized. Certain limitations also merit consideration: The meaning and value of cIMT that we included in our definition of atherosclerosis has been controversial. However, in the ARIC study both cIMT and plaque have been shown to be complementary and furthermore consensus documents such as the Mannheim consensus document and American Society of Echocardiography recommend the use of cIMT in addition to plaque and hence we used in our definition of atherosclerosis[48,54,55]. Furthermore, we did additional analyses to study the value of plaque alone and the results were for the most part the same. Another limitation is that by study design cIMT was performed at two different visits, three years apart. However we adjusted our analysis for the visit at which cIMT was done. Furthermore, although plaque information was utilized, it was only a qualitative assessment of presence or absence of plaque and no true quantification of plaque burden or assessment of characteristics of plaque, which would have been superior to test our hypothesis. However, technologies to accurately quantify plaque burden did not exist when ARIC ultrasound imaging was performed. Another consideration may be that atherosclerosis was assessed by ultrasound of the carotid and not CT scan based CAC. However, cIMT/ plaque presence has shown value in improving CHD risk prediction in the ARIC study[40] and has been shown to better associate with stroke (an end point in our analysis) than CAC[21]. Therefore, while future studies may consider the use of CAC, our analysis has value on its own. Another potential limitation is that both the biomarkers and atherosclerosis indices were measured once and may have changed during the long follow-up. Such misclassification would have tended to weaken observed risk factor associations and potentially limited our power to detect interactions with atherosclerosis. Finally, the lack of association of LpPLA2 and Lp(a) with some incident events, despite evidence of association in other studies including ARIC, must be considered. This may have been a result of the population included for this analysis, the longer follow-up time, or chance.

In conclusion, several CAD biomarkers are associated with CVD regardless of the level of atherosclerosis, as measured by cIMT and presence of plaque.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Whether atherosclerosis as evidenced by carotid ultrasound (cIMT, plaque) is required for biomarkers to be associated with CVD was tested in the ARIC study
- Associations between biomarkers and CVD were not affected by degree of atherosclerosis

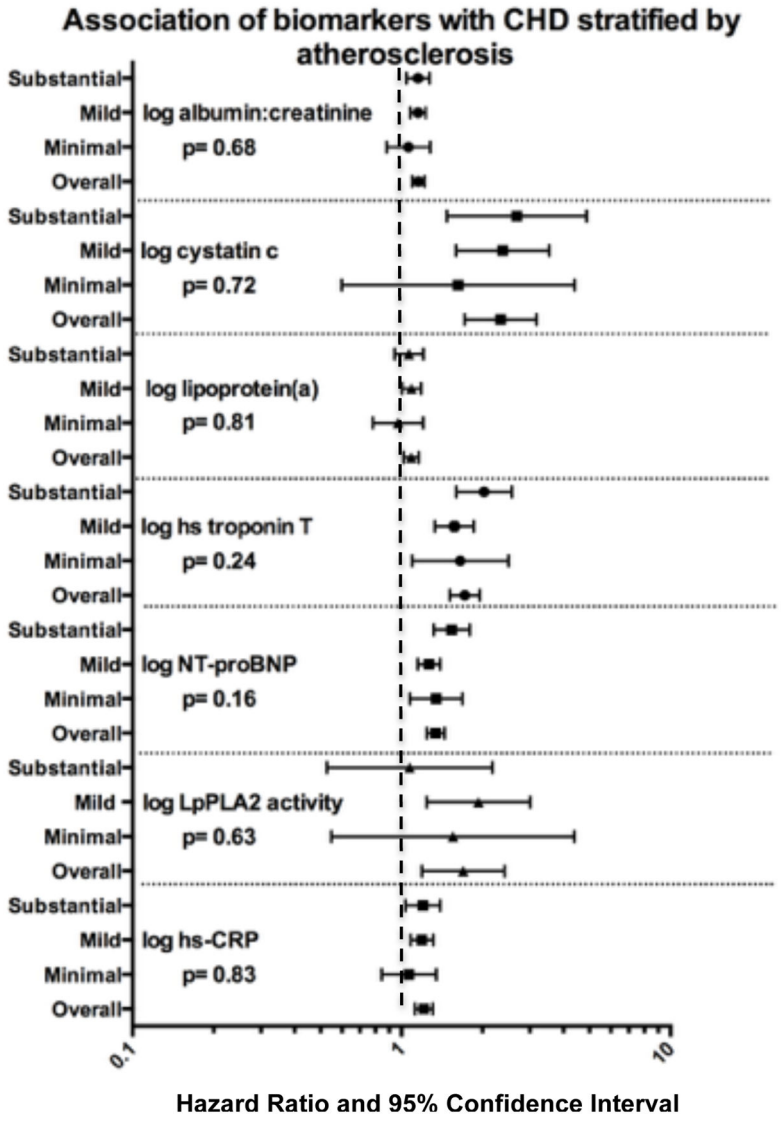

Fig. 1. The relationship between biomarkers and incident coronary heart disease stratified by degree of atherosclerosis (Model 2), ARIC 1996 to 2012

p value is for the interaction term between each biomarker and degree of atherosclerosis.

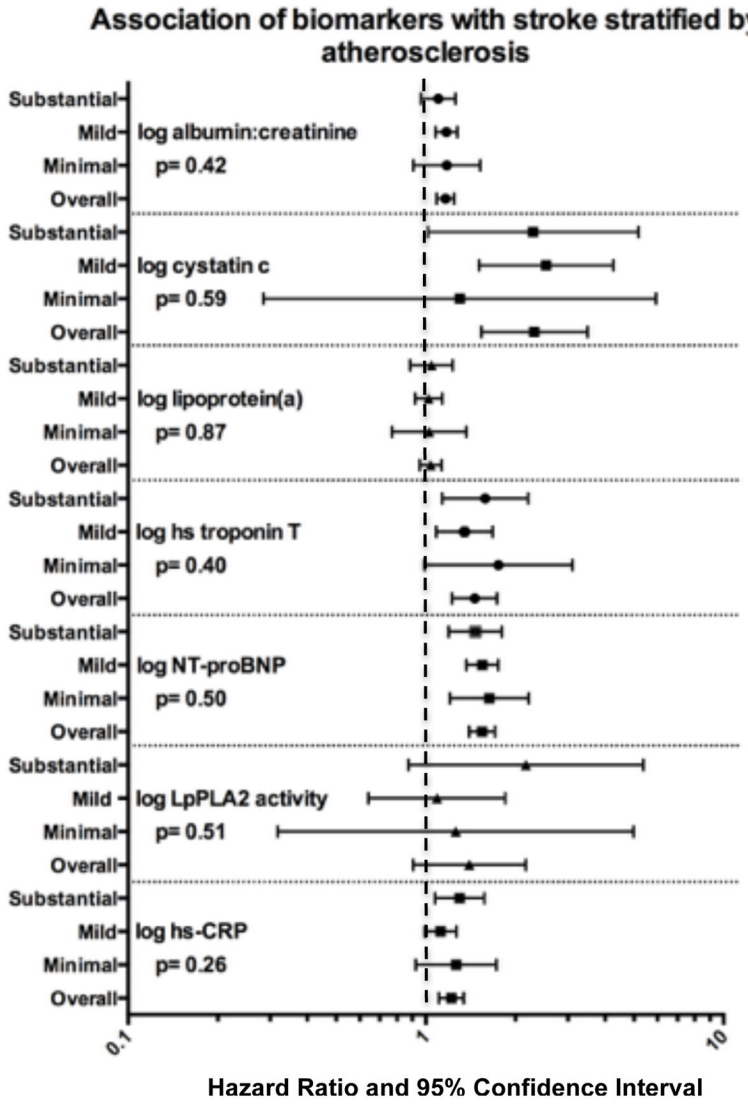

Fig. 2. The relationship between biomarkers and incident stroke stratified by degree of atherosclerosis (Model 2), ARIC 1996 to 2012

p value is for the interaction term between each biomarker and degree of atherosclerosis.

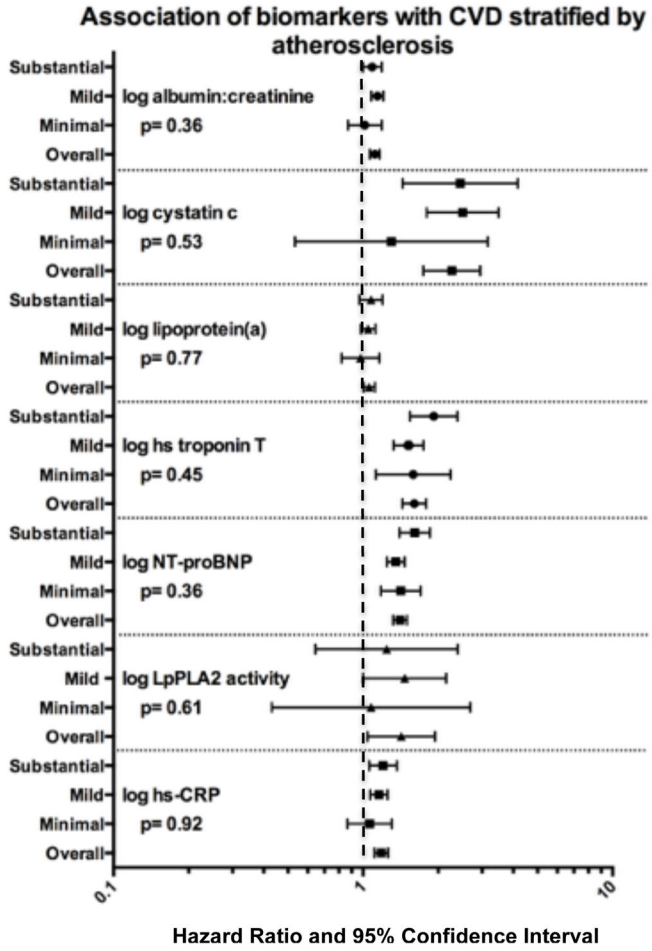

Fig. 3. The relationship between biomarkers and incident cardiovascular events stratified by degree of atherosclerosis (Model 2), ARIC 1996 to 2012

p value is for the interaction term between each biomarker and degree of atherosclerosis.

Table 1

Demographics and baseline characteristics

N	8,127
Age (years, mean ± SD)	62.5 ± 5.6
% Female	58.9%
% Caucasian	80.7%
% African American	19.3%
Hypertension (N)	43.3%
Diabetes (N)	13.4%
Total Cholesterol (mg/dL, mean ± SD)	202.2 ± 36.3
LDL-C (mg/dL, mean ± SD)	123.2 ± 33.1
HDL-C (mg/dL, mean ± SD)	51.4 ± 17.0
Triglycerides (mg/dL, mean ± SD)	141.1 ± 86.0
BMI (kg/m^2, mean ± SD)	28.3 ± 5.3
% Smokers (current and ever)	56.7%
% Statin users	8.2%

Table 2

Degree of atherosclerosis and incidence rate of CVD, ARIC 1996 to 2012

Degree of atherosclerosis	Subjects at risk	Incidence rate, 95% CI (per 1,000 person-years)
Minimal	1,708	3.82 (3.12, 4.68)
Mild	5,366	8.24 (7.60, 8.93)
Substantial	1,053	18.94 (16.68, 21.52)