Materials and Methods

Study design and patient population. The Dallas Heart Study (DHS) is a probability-based sample of Dallas County residents aged 30-65 years, including intentional oversampling of black individuals to comprise approximately 50% of the cohort. The study design, participant selection and phenotypic characterization have been described.\textsuperscript{1} Briefly, a total of 6101 subjects aged 18 to 65 were enrolled in DHS upon completion of the first study visit, consisting of an in-home interview and measurements of blood pressure, heart rate, and body mass. Fasting venous blood was collected from participants aged 30-65 years during DHS visit 2 (n=3557), and all participants in the DHS for whom stored blood samples were available for measurement of SDMA and ADMA (n=3523) were included in the present study. The study protocol was approved by the University of Texas Southwestern Medical Center Institutional Review Board, and written informed consent was provided by all study participants.

Measurement of SDMA, ADMA and other biomarkers. Venous blood was collected into ethylenediaminetetraacetic acid tubes and stored at 4°C for <4 hours. After centrifugation, plasma was stored at <-70°C. N-terminal pro-brain-type natriuretic peptide (NT-proBNP), high-sensitivity C-reactive protein (CRP), and high-sensitivity cardiac troponin T (cTnT) were measured using established assays, as described.\textsuperscript{2-4} SDMA and ADMA were measured using a well-validated high-throughput liquid chromatographic-tandem mass spectrometric (LC-MS/MS) assay,\textsuperscript{5,6} described in detail elsewhere.\textsuperscript{7}
**Imaging and coronary artery calcium (CAC) classification.** CAC was measured using a single electron beam-computed tomography (EBCT) scanner, as described (n=2678), and was quantified by the method of Agatston and colleagues. The final CAC score was calculated as the average of two consecutive measurements. In accordance with previous reports, CAC scores between 0 and ≤10 Agatston units were classified as no detectable coronary calcium, and CAC scores >10 Agatston units were classified as CAC positive. Aortic wall thickness (AWT) was measured by software-based quantitative analysis of 6 transverse MRI slices of the infrarenal aorta, reporting the average wall thickness of the six slices (n=2455), as described.

**Definition of variables.** Race/ethnicity and smoking status were self-reported. Hypertension was defined as systolic blood pressure ≥ 140 mm Hg or diastolic ≥90 mm Hg based on the average of 5 measurements on the first study visit, or current use of antihypertensive drugs. Hypercholesterolemia was defined as fasting low-density lipoprotein (LDL) cholesterol ≥4.1 mmol/L (≥160 mg/dL) or current use of statins. Diabetes mellitus was defined as fasting plasma glucose ≥7 mmol/L (≥126 mg/dL), or random glucose ≥11.1 mmol/L (≥200 mg/dL), or current use of antihyperglycemic drugs. Metabolic Syndrome was defined according to the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP III) criteria. Prior coronary heart disease (CHD) was defined as self-reported history of MI or revascularization. Cardiovascular disease (CVD) was defined as prior CHD or self-reported history of chronic heart failure (CHF) or stroke. Glomerular filtration rate (GFR) was estimated using the Modification of Diet and Renal Disease (MDRD) formula, and chronic kidney disease (CKD) stages were defined according to the National Kidney Foundation (NKF) Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines.
Mortality assessment. Data from the National Center for Health Statistics National Death Index was used to determine overall and cause-specific participant mortality. Deaths including International Statistical Classification of Diseases, 10\textsuperscript{th} Revision codes I00-I99 were classified as cardiovascular.}\textsuperscript{14}

Statistical analyses. Categorical variables are reported as proportions and continuous variables as medians and interquartile ranges (IQR). Separate analyses were conducted for SDMA and ADMA. Participants were divided into quintiles based on SDMA and ADMA levels respectively. Trends across quintiles were assessed using the Jonckheere-Terpstra trend test.\textsuperscript{15} Unadjusted Kaplan-Meier curves across quintiles were constructed for all-cause and cardiovascular mortality. After ensuring that the assumptions of Cox proportional hazard modelling were met via Schoenfeld residuals, multivariable Cox proportional modelling was used to assess associations between outcomes of interest and quintiles of SDMA and ADMA after multivariable adjustments. A series of Cox proportional hazards models were used: model 1 adjusted for age, race, and sex; model 2 further adjusted for traditional cardiovascular risk factors (diabetes, BMI, hypertension, hypercholesterolemia, history of myocardial infarction or chronic heart failure, and current smoking); model 3 further adjusted for estimated renal function; model 4 further adjusted for high-sensitivity CRP and NT-proBNP; and model 5 further adjusted for high-sensitivity cTnT. SDMA and ADMA were also analyzed as continuous variables, using log-transformed values because of the right-skewed distribution of both biomarkers. In analyses of SDMA and ADMA as continuous variables, hazard ratios were calculated for change by one standard deviation or one log unit. The appropriateness of log-transformations was confirmed
after restricted cubic splines were used to assess the non-linearity of SDMA and ADMA in the adjusted models. To assess model discrimination, we constructed Harrell’s c-statistics for all models. The c-statistics for models with and without SDMA and ADMA were compared with bootstrapping. The integrated discrimination index (IDI) was calculated for the addition of SDMA or ADMA to each model. The IDI represents the difference between the improvement in average sensitivity and any increase in the average 1-specificity when a variable is added to a model, and is used to estimate the proportion of patients who are reclassified to a different risk category based on addition of the new biomarker to existing risk models. Calibration was assessed by D’Agostino’s and Nam’s modification of the Hosmer-Lemeshow calibration chi-squared statistic. Statistical analyses were performed using SAS version 9.2 (SAS Institute Inc, Cary, North Carolina). All statistical tests reported are two-tailed, with alpha=0.05.
References


2. de Lemos JA, McGuire DK, Khera A, Das SR, Murphy SA, Omland T, Drazner MH. Screening the population for left ventricular hypertrophy and left ventricular systolic dysfunction using natriuretic peptides: Results from the dallas heart study. *Am Heart J*. 2009;157:746-753 e742


