# A Pathway-Specific Aggregate Biomarker Risk Score is Associated with Burden of Coronary Artery Disease and Predicts Near-term Risk of Myocardial Infarction and Death

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Subject Terms: biomarkers, mortality/survival

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## Abstract

**Background**: Inflammation, coagulation, and cell stress contribute to atherosclerosis and its adverse events. A biomarker risk score (BRS) based on the circulating levels of biomarkers C-reactive protein (CRP), fibrin degradation products (FDP), and heat shock protein-70 (HSP-70) representing these three pathways was a strong predictor of future outcomes. We investigated whether soluble urokinase activator receptor (suPAR), a marker of immune activation, is predictive of outcomes independent of the aforementioned markers, and whether its addition to a 3-BRS improves risk reclassification.

**Methods:** CRP, FDP, HSP-70 and suPAR were measured in 3278 patients undergoing coronary angiography. The BRS was calculated by counting the number of biomarkers above a cutoff determined using the Youden's index. Survival analyses were performed using models adjusted for traditional risk factors.

**Results:** A high suPAR level ≥3.5 ng/ml was associated with all-cause death and MI (HR 1.83 95%CI [1.43-2.35]) after adjustment for risk factors, CRP, FDP, and HSP70. Addition of suPAR to the 3-BRS significantly improved the C-statistic, IDI and NRI for the primary outcome. A BRS of 1, 2, 3 or 4 was associated with a 1.81, 2.59, 6.17, and 8.80-fold increase respectively in the risk of death and MI. The 4-BRS was also associated with severity of coronary artery disease and composite endpoints.

**Conclusion:** SuPAR is independently predictive of adverse outcomes, and its addition to a 3-BRS comprised of CRP, FDP and HSP-70 improved risk reclassification. The clinical utility of employing a 4-BRS for risk prediction and management of patients with coronary artery disease warrants further study.

Key Words: Biomarker, risk score, coronary artery disease, cardiovascular outcomes, prognosis

## Introduction

Coronary artery disease (CAD) continues to be the leading cause of mortality worldwide.[1] The mechanisms involved in the development of atherosclerosis and subsequent plaque rupture that precipitate acute coronary events and death are complex and involve several pathways including key contributions of inflammation, immune dysregulation, stress, and thrombosis.[2] Clinical tools such as the Framingham risk score predict long-term risk of outcomes in the healthy population,[3, 4] but fail to reliably prognosticate risk of adverse outcomes in patients with established CAD.[5]

We recently identified a simple non-invasive risk score based on the concept that the greater the number of pathways related to plaque rupture that are activated in a given patient, the greater the risk of actual plaque rupture.[6] This aggregate biomarker strategy for risk prediction was tested using the biomarkers C-reactive protein (CRP), representing inflammation, fibrin degradation products (FDP), representing the coagulation pathway, and heat shock protein-70 (HSP-70) representing cell stress. The biomarker risk score (BRS) strategy was highly successful in predicting risk of near-term MI and death in patients with suspected or established CAD.[6]

Soluble urokinase plasminogen activator receptor (suPAR) is a marker of immune activation and inflammation that appears to orchestrate cellular adhesion, migration, and proliferation during development of the atherosclerotic plaque.[7] Higher circulating levels of suPAR are associated with incident cardiovascular disease (CVD) in the healthy population[8,9], and we and others have found that it also predicts incident CVD events and chronic kidney disease in subjects with CAD.[10-12]

The purpose of the present investigation is to determine (1) whether suPAR levels are associated with outcomes independent of the aforementioned markers of inflammation (CRP), hypercoagulable state (FDP) and cellular stress (HSP70), and (2) whether addition of suPAR to the 3-BRS improves prediction of future adverse events.

## Methods

Study Population:

Subjects were recruited as a part of the Emory Cardiovascular Biobank and consisted of 3278 patients undergoing left heart catheterization for diagnosis of suspected CAD in Emory Healthcare hospitals between 2003 and 2009. Subjects with heart transplantation, severe valvular heart disease, congenital heart disease, severe anemia, recent blood transfusion, myocarditis, active inflammatory diseases, and cancer were excluded. Demographics, medical, smoking status, and risk factor prevalence were documented as previously described.[6] Smoking was classified as non-smoker or current smoker. Subjects were noted to have hypertension or dyslipidemia if they had a documented history or were on treatment. Acute MI at enrollment was defined using universal criteria.[13] Briefly, myocardial infarction was diagnosed with detection of a rise of cardiac troponin with either ischemic symptoms, dynamic EKG changes, or identification of an intracoronary thrombus by angiography. The study was approved by the Emory University Institutional Review Board. All subjects provided written informed consent.

Outcomes and Follow-up:

To minimize physician-imposed bias, we selected a composite endpoint consisting of hard outcomes of ischemic heart disease including all-cause death and non-fatal myocardial

infarction. Follow-up was conducted between 1 and 5 years for determination of the primary composite endpoint of all cause death and non-fatal MI, and the secondary endpoints of cardiovascular death, all cause death/MI/revascularization, and all cause death/MI/stroke. Follow-up data were collected by personnel blinded to the biomarker data through telephone interview, chart review and query of the Social Security Death Index and State records. Two independent cardiologists, both blinded to the clinical and biomarker data, adjudicated the cause of death. Cardiovascular death was defined as death attributable to an ischemic cardiovascular cause (i.e. fatal MI, stroke, or peripheral arterial disease) or sudden death due to an unknown but presumed cardiovascular cause in high-risk patients. Medical records were accessed to validate all self-reported events including MI.[13]

Identification of CAD and Severity Scoring:

Luminal narrowing of coronary arteries were quantified using a modified AHA/ACC classification of the coronaries.[14] Patients were classified as having non-significant CAD (visible plaque resulting in <50% luminal stenosis) or significant CAD (at least one major epicardial vessel with ≥ 50% stenosis). Normal coronaries were defined as those with no visual stenosis of major epicardial arteries and smooth appearance during angiography and without history of prior coronary artery bypass graft (CABG) surgery or angioplasty. Quantitative angiographic scoring was performed using the Gensini score that quantifies CAD severity by a nonlinear points system for degree of luminal narrowing and has been shown to have prognostic significance.[14, 15]

Sample Collection:

Fasting arterial blood samples for serum and plasma were drawn before angiography and stored at -80º C (mean, 4.9 years). Details of the biomarker assays have been previously described. [6, 11] Briefly, serum CRP and FDP measurements were determined using a sandwich immunoassay by FirstMark, Inc., San Diego, CA. Serum HSP-70 was measured with a sandwich enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minnesota) and optimized by FirstMark. Plasma suPAR levels were measured using commercially available kits (suPARnostic kit, Virogates, Copenhagen, Denmark). Minimum detectable CRP, FDP, HSP-70, and suPAR were 0.1 mg/L, 0.06 µg/ml, 0.313 ng/ml, and 0.1 ng/mL, respectively.

Statistical Analyses:

Continuous variables are presented as mean (± SD) or median (IQR) and categorical variables as proportions (%). The student's t-test and Chi-Square tests were used when appropriate. Mann-Whitney U or Kruskall Wallis non-parametric tests were performed on non-normally distributed variables. The relationship between biomarkers and outcomes was determined using the Cox proportional-hazards regression in unadjusted models and in models adjusted for established risk factors that include clinically relevant covariates for CVD outcomes (age, gender, race, history of hypertension, diabetes, dyslipidemia, previous MI, acute MI at presentation, estimated glomerular filtration rate (eGFR), Gensini score, body mass index (BMI), left ventricular ejection fraction (LVEF), history of CABG, smoking status, and the use of aspirin, clopidogrel, and statins). Fine and Gray's sub-distribution hazard model was used to analyze cardiovascular death outcome, considering non-cardiovascular death as the competing risk event.

The best discriminatory cutoff for each biomarker in association with the death/MI outcome was determined using the Youden's index (sensitivity – (1-specificity)) from the Receiver Operating Characteristic (ROC) analysis to identify "high" vs. "low" levels. The Youden index is a global measure of a biomarker's effectiveness that calculates to the maximum difference between sensitivity and 1-specificity.[16] Cut points for CRP, FDP, HSP-70, and suPAR were 3 mg/L, 1.0 μg/ml, 0.313 ng/ml, and 3.5 ng/ml, respectively, as described previously.[6, 11] A 3-BRS was derived by counting the number of biomarkers above respective cut-points.

Discrimination analysis for the prediction of each endpoint was calculated as the difference in C-statistic comparing the baseline model incorporating traditional risk factors (model 1), a second model including the 3 biomarkers (model 2), and lastly a model containing the 3 biomarkers in addition to suPAR levels (model 3). C-statistics, continuous net reclassification index (NRI) and integrated discrimination improvement (IDI) metrics comparing model 3 to models 1 and 2 were calculated using the R package survC1 and survIDINRI.[17-20]

Average annual event rates for each outcome measure were calculated by dividing the observed number of events by the observed event-specific number of person-years of follow-up. Interaction terms of each covariate on the association of the aggregate BRS and the primary end point were evaluated and demonstrated using a forest plot.[21] The independent association of the BRS with the presence of ≥50% stenosis in any major coronary artery was evaluated with a binary logistic regression model adjusting for known cardiovascular risk factors including age, gender, diabetes, hypertension, dyslipidemia, and smoking status. P values <0.05 from two-sided tests were considered statistically significant. Statistical analyses were performed with SAS (Version 9.3; SAS Institute, NC, USA).

## Results

Relationship between biomarker risk score and clinical risk factors:

The relationships between the categories of the BRS and the clinical and demographic factors are listed in Table#1. Although the absolute differences in most of the parameters were small, subjects with a higher BRS were more likely to be older, female, African American, diabetic, hypertensive, active smoker, and to have lower LVEF and lower eGFR. There was no significant difference in management strategy (i.e. medical vs. revascularization) between patients in different categories of the BRS.

Relationship of biomarker risk score with angiographic CAD:

Patients with higher BRS were more likely to have at least one epicardial vessel with ≥50% stenosis and higher burden of CAD as quantified by higher Gensini Score, Table#1. Conversely, visually normal coronary arteries were more likely to be present in those with a lower compared to a higher BRS. Compared to those with a BRS of 0 or 1, those with BRS ≥ 2 had 25% increased odds of having significant CAD (> 50% stenosis) (OR=1.25, p=0.013) independent of age, gender, diabetes, hypertension, dyslipidemia, and smoking status. Similarly, the BRS was an independent correlate of severity of CAD assessed by the Gensini score after adjustment for variables listed above.

Clinical and demographic predictors of incident adverse outcomes:

Over a median follow-up of 2.3 years and a total of 7539 patient years of follow-up, 269 subjects died (8.2%), 116 had an MI (3.5%), 153 (4.7%) had cardiovascular death, 35 had stroke (1.1%), and 353 underwent revascularization (10.8%), Table#1. In a Cox proportional hazard

model for the composite endpoint of all cause death/MI adjusting for all the aforementioned demographic and clinical covariates, significant predictors were: age (years, HR: 1.02, 95% CI: 1.01-1.03)), eGFR (ml/min, HR: 0.99, 95% CI: 0.993-0.999), acute MI (HR: 1.97, 95% CI: 1.49-2.59), diabetes (HR: 1.65, 95% CI: 1.32-2.08), active smoking (HR: 1.42, 95% CI: 1.05-1.93), LVEF (%, HR: 0.98, 95% CI: 0.97-0.98), Gensini score (HR: 1.003, 95% CI: 1.001-1.005), aspirin use (HR=0.69, 95% CI: 0.46-0.85), and clopidogrel use (HR: 1.43, 95% CI: 1.11-1.84); p-value for all < 0.05. We believe that the elevated hazard associated with clopidogrel use is likely related to a high risk inherent to individuals taking this antiplatelet medication.

Relationship between biomarker risk score and outcomes:

Correlation analyses between the 4 biomarkers revealed weak but significant correlations, Table#2. An increased (above cutoff) level of each biomarker was independently associated with future risk of all cause death, cardiovascular death, composite of death and MI, combined death, MI, and revascularization, and composite of death, MI, and stroke after adjustment for the noted clinical covariates. Only elevated CRP was not significantly associated with future cardiovascular death, Table#3. Importantly, a high suPAR level was an independent predictor of all incident outcome measures in models adjusted for all aforementioned variables as well as a 3-BRS comprising of CRP, FDP, and HSP-70. For instance, a high suPAR level was independently associated with incident all-cause death/MI (HR=1.83, p<0.001), Table#3.

The four-BRS was associated with a graded increase in risk of each of the endpoints noted. When analyzed as a numeric scale in Cox proportional hazard models adjusting for all previously described variables, each 1 unit increase in the BRS was associated with 1.94-fold increased risk of all-cause death, 1.71-fold increased risk of cardiovascular death, 1.77-fold increased risk of

death/MI, 1.45-fold increase in the risk of death/MI/revascularization, and 1.72-fold increased risk of death/MI/stroke (p<0.001 for all). Comparison of hazard ratios for those with 1, 2, 3, and 4 elevated biomarkers compared to those with no elevated biomarker are presented in Table#3, and cumulative incidence plots in Figure#1. Thus, compared to those with a BRS of 0, those with a BRS of 4 had an 8.8-fold (p<0.001) increased odds of having death or MI, or a 6.76-fold (p<0.001) increased risk of cardiovascular death. Average annual event rates increased in a graded fashion across the categories of the BRS with the 2.6% of subjects with a BRS of 4 having on average 21% per year risk of death/MI compared to a 1.1% event rate in the 28% of subjects with a BRS of 0, Figure#2. Average annual event rates for each endpoint across categories of the BRS are presented in Figure#2.

Discrimination Testing:

The addition of suPAR to a model consisting of clinical covariates and the 3-BRS was associated with significant improvement in risk reclassification metrics and c-statistic with respect to the primary endpoint of all-cause death and MI, as well as the outcomes of all-cause death, cardiovascular death and the combined outcomes Table#4.

## Discussion

Several pathways are involved and act synergistically in the development of atherosclerotic plaque and its progression to the stage of plaque instability and rupture. We had previously identified a BRS comprised of CRP, FDP, and HSP-70 that predicted risk of incident MI and death but did not correlate with presence or severity of CAD.[6] The present study demonstrates that the circulating level of suPAR is an independent predictor of adverse CVD outcomes after

adjustment for all traditional cardiovascular risk factors and levels of CRP, FDP and HSP70. Moreover, the addition of suPAR to a 3-BRS based on the aforementioned biomarkers improves risk reclassification metrics of the C-statistic, NRI, and IDI. When comparing this 4-BRS strategy to our prior study involving 3 biomarkers, we found that only the 4-BRS and not the 3-BRS was independently associated with both presence and severity of CAD.

The Urokinase Plasminogen Activator (uPA) is a serine protease produced by smooth muscle cells, vascular endothelial cells, macrophages, monocytes and fibroblasts, and when bound to its receptor (uPAR), leads to the generation of plasmin.[7, 22] uPAR is involved in several functions including migration, adhesion, fibrinolysis, and cell proliferation.[23-25] Plasma suPAR reflects cellular shedding of uPAR, which is induced during inflammation; shedding appears to be free of circadian changes and is relatively stable during periods of acute stress.[9, 26] suPAR has been reported to predict incident CVD independent of the Framingham risk score in healthy populations free of CVD[27, 28] and in those with CVD. [10, 11]

CRP has been widely studied in populations free of CVD[29, 30] as well as cohorts with established CAD; it predicts incident CVD and MACE independent of traditional cardiovascular risk factors.[31, 32] Heat shock proteins are abundant intracellular proteins that aid in a cell's response to acute stress and are involved in protein folding and transport.[33] HSP-70 is one of the more extensively studied HSPs, yet its relationship with CAD has been quite controversial.[34] It appears that while lower levels are associated with long-term development of atherosclerotic plaque,[34] higher levels predispose to higher risk of plaque rupture and incident future outcomes.[6] Fibrin degradation products are measures of ongoing fibrin/fibrinogen degradation. Increased plasma FDP level predicts incident cardiovascular events in patients with peripheral vascular disease.[35]

Plasma D-dimer, which is one of the products included in the FDP analysis, is a marker of turnover of cross-linked fibrin; it was higher in those with CAD compared to healthy controls[36] and predicted adverse cardiovascular events in healthy individuals independent of cardiovascular risk factors.[37] Similarly, in the Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) study, the diabetics with CAD had higher D-dimer levels that were associated with an increased risk of cardiac events. [38] FDP ELISA assay used in this study detects the full complement of FDP components, including D-dimer as well as fragments D and E and any additional intermediate products from fibrin degradation.

Previous studies investigating the role of multiple biomarkers in populations without CVD have demonstrated only slight improvements in predictive potential (using C-statistic) when added to standard clinical models.[39-41] In contrast, our study establishes the value of a multi-marker aggregate score in a high risk population with suspected or established CAD, a group in which traditional risk scores such as Framingham have failed to identify risk of recurrent CVD events. We have shown that despite a statistically significant two-by-two correlation between the four biomarkers, the observed correlation coefficients were rather modest. This finding is compatible with the concept that these biomarkers act independently and do so with their predominant activities involving separate biological pathways. It is well known that the immune system, partly due to stimulation of inflammatory pathways, plays an important role in the pathogenesis of atherosclerosis and in the activation of pathways leading to plaque rupture.[42] Here we have shown that a BRS comprised of suPAR (a biomarker of immune system activation and of inflammation) in addition to CRP, FDP, and HSP-70 significantly predicts all major outcomes as well as the severity of CAD. Thus, whereas the HR for increase in risk of death and

MI was 5-fold greater in those who had 3 positive biomarkers compared to those with a BRS of 0 using the risk score described previously, the HR was 8.8-fold greater in those with 4 positive biomarkers using the 4 aggregate BRS.[6] This corresponds to an average annual event rate of 21% in these patients. Use of this BRS in conjunction with other readily available clinical factors could potentially guide clinicians in appropriate identification of patients at highest risk with use of more aggressive treatment options, risk factor control, and behavioral modification counseling. Conversely, identification of patients at very low risk could prevent unnecessary testing in this population. Further studies are needed to evaluate the effect of the use of this BRS on tailoring of medical therapy.

Strengths and Limitations:

Our study has several strengths. We enrolled individuals of both genders and races, those with acute MI, and patients with a range of LVEF, reflecting a population that is high risk yet typical of those undergoing cardiac catheterization. Biomarker evaluation was performed at one time point by the same lab personnel, which minimized variability. C-statistic, NRI and IDI were calculated using survival models, which allows for better model discrimination and overall predictive ability. Limitations of our study include a one-time measurement of biomarkers that may not reflect fluctuations in their levels over time. Despite rigorous attempts in controlling for confounding variables, our inability to adjust for a well-validated comorbidity index in this study further adds to limitations. Our results need to be further validated and should not be generalized to a population without suspected or known CAD. Furthermore, future studies are needed to assess cost-effectiveness of using this multi-marker BRS in routine clinical practice.

In conclusion, a 4-BRS representing inflammation, coagulation, cell stress, and immune pathways significantly predicts risk of MI and death, and significantly improves risk reclassification above and beyond a 3-BRS. Whether more aggressive medical management in individuals with high BRS would lead to a decrease in the BRS, and whether such a decrease, if it occurs, modifies subsequent risk of outcomes, remains to be studied.

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**Table 1. Baseline Characteristics**

| | Total | Number of elevated biomarkers | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | |
| **Baseline Characteristics** | **N=3278** | **N=923** | **N=1141** | **N=796** | **N=332** | **N=86** | **P value for Trend** |
| **Demographics** | | | | | | | |
| Age (years) | 63±12 | 62±11 | 62±13 | 64±12 | 66±11 | 66±11 | <0.001 |
| Male Gender, N (%) | 2105 (64%) | 686 (74%) | 740 (65%) | 437 (55%) | 189 (57%) | 53 (62%) | <0.001 |
| Caucasian, N (%) | 2709 (83%) | 793 (86%) | 939 (82%) | 647 (81%) | 264 (79%) | 66 (78%) | 0.015 |
| Systolic BP (mmHg) | 137±23 | 136±20 | 137±23 | 138±23 | 138±26 | 132±24 | 0.062 |
| Diastolic BP (mmHg) | 76±12 | 76±11 | 76±12 | 75±12 | 75±13 | 74±11 | 0.045 |
| BMI (kg/m2) | 30±6 | 29±5 | 30±6 | 30±7 | 30±7 | 28±6 | <0.001 |
| **Comorbidities** | | | | | | | |
| Acute MI on presentation, N (%) | 389 (11%) | 57 (6.3%) | 141 (12.5%) | 110 (14%) | 60 (18%) | 1 (1.2%) | <0.001 |
| History of prior MI, N (%) | 981 (31%) | 251 (28%) | 319 (29%) | 259 (33%) | 124 (38%) | 28 (34%) | 0.001 |
| Diabetes, N (%) | 1033 (31%) | 199 (22%) | 329 (29%) | 316 (40%) | 146 (44%) | 43 (50%) | <0.001 |
| Hypertension, N (%) | 2362 (72%) | 632 (68%) | 818 (72%) | 597 (75%) | 255 (77%) | 60 (70%) | 0.010 |
| Dyslipidemia, N (%) | 2291 (70%) | 660 (71%) | 805 (71%) | 550 (69%) | 229 (69%) | 47 (55%) | 0.024 |
| Current Smoking, N (%) | 484 (15%) | 90 (10%) | 191 (17%) | 143 (18%) | 53 (17%) | 7 (8.4%) | <0.001 |
| History of prior CABG, N (%) | 721 (22%) | 192 (21%) | 233 (20%) | 169 (21%) | 96 (29%) | 31 (36%) | <0.001 |
| History of prior Angioplasty, N (%) | 1342 (42%) | 382 (42%) | 462 (41%) | 328 (42%) | 137 (42%) | 33 (39%) | 0.963 |
| LVEF (%) | 53±12 | 55±11 | 54±12 | 52±14 | 50±14 | 44±18 | <0.001 |
| **Medications** | | | | | | | |
| Statin use, N (%) | 2378 (72%) | 696 (75%) | 831 (73%) | 568 (71%) | 231 (70%) | 52 (60%) | 0.016 |
| Aspirin use, N (%) | 2652 (81%) | 749 (81%) | 931 (82%) | 637 (80%) | 272 (82%) | 63 (73%) | 0.371 |
| Clopidogrel use, N (%) | 1516 (46%) | 412 (45%) | 532 (47%) | 353 (44%) | 183 (55%) | 36 (42%) | 0.010 |
| Beta Blocker use, N (%) | 2075 (63%) | 523 (57%) | 720 (63%) | 538 (68%) | 235 (71%) | 59 (69%) | <0.001 |
| ACE-inh/ARB use, N (%) | 2042 (62%) | 536 (58%) | 748 (67%) | 504 (63%) | 208 (63%) | 46 (53%) | 0.004 |
| **Angiographic findings** | | | | | | | |
| Gensini Angiographic Score [median (IQR)] | 12 (0-50) | 11 (0-50) | 11 (0-48) | 12 (0-48) | 20 (2.1-61) | 20 (1.4-85) | 0.001 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ≥50% epicardial vessel stenosis, N (%) | 2026 (66%) | 547 (63%) | 706 (66%) | 494 (68%) | 225 (73%) | 54 (68%) | 0.024 |
| Visually normal coronaries, N (%) | 656 (20%) | 208 (22.5%) | 240 (21%) | 155 (19%) | 44 (13%) | 9 (11%) | 0.001 |
| **Laboratory values** | | | | | | | |
| GFR (ml/min) | 76±47 | 81±44 | 82±49 | 71±49 | 60±44 | 54±43 | <0.001 |
| LDL (mg/dl) | 99±37 | 98±35 | 102±39 | 100±38 | 96±39 | 97±35 | 0.070 |
| HDL (mg/dl) | 42±13 | 43±12 | 42±13 | 41±12 | 40±13 | 42±15 | 0.002 |
| **Biomarkers** | | | | | | | |
| CRP (mg/l) [median (IQR)] | 3.0 (1.2-7.6) | 1.1 (0.6-1.9) | 3.4 (1.45-6.9) | 6.0 (3.2-10) | 9.6 (4.7-18) | 8.9 (4.9-22) | <0.001 |
| HSP-70 (ng/ml) [median (IQR)] | 0.0 (0.0-0.0) | 0.0 (0.0-0.0) | 0.0 (0.0-0.0) | 0.0 (0.0-8.0) | 4.0 (0.0-189.2) | 264 (106-635) | <0.001 |
| FDP (µg/ml) [median (IQR)] | 0.5 (0.3-0.8) | 0.4 (0.3-0.5) | 0.5 (0.3-0.7) | 0.6 (0.4-1.0) | 1.2 (0.7-2.1) | 1.7 (1.2-4.6) | <0.001 |
| suPAR (ng/ml) [median (IQR)] | 3.0 (2.3-3.9) | 2.4 (2.0-2.9) | 2.8 (2.2-3.4) | 3.9 (3.0-4.9) | 4.5 (3.7-6.0) | 5.3 (4.1-7.1) | <0.001 |
| **Management Strategy** | | | | | | | 0.539 |
| Medical Management, N (%) | 1863 (57%) | 551 (60%) | 632 (55%) | 443 (56%) | 188 (57%) | 49 (57%) | -- |
| Revascularization, N (%) | 1260 (38%) | 337 (36%) | 449 (39.4%) | 316 (40%) | 127 (38.3%) | 31 (36%) | -- |
| Other, N (%) | 155 (4.7%) | 35 (3.8%) | 60 (5.3%) | 37 (4.6%) | 17 (5.1%) | 6 (7%) | -- |
| **Follow-up Events** | | | | | | | |
| MI, N (%) | 116 (3%) | 16 (2%) | 40 (3%) | 25 (3%) | 29 (9%) | 6 (7%) | <0.001 |
| All-cause death, N (%) | 269 (8%) | 21 (2%) | 46 (4%) | 79 (10%) | 86 (26%) | 37 (43%) | <0.001 |
| Cardiovascular death, N (%) | 153 (5%) | 14 (1%) | 27 (2%) | 43 (5%) | 48 (14%) | 21 (24%) | <0.001 |
| All-cause death/MI, N (%) | 363 (11%) | 34 (4%) | 82 (7%) | 98 (12%) | 109 (33%) | 40 (46%) | <0.001 |
| All-cause death/MI/revascularization, N (%) | 628 (19%) | 92 (10%) | 187 (16%) | 167 (21%) | 138 (42%) | 44 (51%) | <0.001 |
| All-cause death/MI/stroke, N (%) | 392 (12%) | 39 (4%) | 91 (8%) | 108 (14%) | 113 (34%) | 41 (48%) | <0.001 |

**Table 2. Associations between biomarkers**

| | HSP-70 | FDP | CRP |
|---|---|---|---|
| **suPAR** | | | |
| **r*** | 0.16 | 0.27 | 0.27 |
| **p** | <0.001 | <0.001 | <0.001 |
| **CRP** | | | |
| **r** | 0.072 | 0.22 | |
| **p** | <0.001 | <0.001 | |
| **FDP** | | | |
| **r** | 0.18 | | |
| **p** | <0.001 | | |

*R value represents correlation coefficient of the Spearman bivariate correlation test.
Statistically significant direct two-by-two correlations were observed between biomarkers.

**Table 3. Association between major cardiovascular events and the biomarkers individually and as the BRS.**

| | All-Cause Death<br>HR (95% CI); p-value | Cardiovascular Death*<br>HR (95% CI); p-value | All-Cause Death and MI<br>HR (95% CI); p-value | All-Cause Death, MI, Revascularization<br>HR (95% CI); p-value | All-Cause Death, MI, Stroke<br>HR (95% CI); p-value |
|---|---|---|---|---|---|
| **All biomarkers in same model** | | | | | |
| **CRP ≥3.0 mg/L** | 1.69 (1.26-2.27), p<0.001 | 1.57 (0.39-6.38), p=0.53 | 1.58 (1.24-2.02), p<0.001 | 1.31 (1.09-1.56), p=0.003 | 1.58 (1.25-2.00), p<0.001 |
| **HSP70 > 0.313ng/mL** | 1.98 (1.46-2.67), p<0.001 | 1.79 (1.17-2.73), p=0.01 | 2.22 (1.71-2.87), p<0.001 | 1.94 (1.60-2.35), p<0.001 | 1.99 (1.56-2.55), p<0.001 |
| **FDP ≥ 1.0 ug/mL** | 1.87 (1.42-2.46), p<0.001 | 1.79 (1.25-2.58), p=0.002 | 1.57 (1.23-1.99), p<0.001 | 1.41 (1.16-1.70), p<0.001 | 1.60 (1.27-2.01), p<0.001 |
| **suPAR ≥ 3.5 ng/ml** | 2.31 (1.71-3.13), p<0.001 | 2.12 (1.37-3.26), p<0.001 | 1.83 (1.43-2.35), p<0.001 | 1.29 (1.07-1.55), p=0.006 | 1.78 (1.40-2.25), p<0.001 |
| **Categorical** | | | | | |
| **1 vs 0 markers** | 1.68 (0.96-2.93), p=0.065 | 1.59 (0.79-3.19), p=0.20 | 1.81 (1.19-2.76), p=0.005 | 1.63 (1.25-2.11), p<0.001 | 1.74 (1.18-2.59), p=0.005 |
| **2 vs 0 markers*** | 3.40 (2.0-5.76), p<0.001 | 2.52 (1.27-5.01), p=0.01 | 2.59 (1.70-3.94). p<0.001 | 1.93 (1.47-2.54), p<0.001 | 2.49 (1.68-3.69), p<0.001 |
| **3 vs 0 markers*** | 7.59 (4.46-12.91), p<0.001 | 5.60 (2.72-11.56), p<0.001 | 6.17 (4.05-9.40), p<0.001 | 3.68 (2.75-4.91), p<0.001 | 5.60 (3.76-8.34), p<0.001 |
| **4 vs 0 markers*** | 11.87 (6.5-21.65), p<0.001 | 6.76 (2.81-16.26), p<0.001 | 8.8 (5.32-14.57), p<0.001 | 4.17 (2.82-6.17), p<0.001 | 7.99 (4.93-12.93), p<0.001 |

Analyses were adjusted for age, gender, race, body mass index, glomerular filtration rate, acute myocardial infarction, history of previous myocardial infarction, hypertension, dyslipidemia, diabetes, left ventricular ejection fraction, history of coronary bypass graft surgery, history of coronary angioplasty, active smoking, Gensini angiographic severity score, aspirin use, Statin use, and Clopidogrel use. *Fine and Gray's sub-distribution hazard model was used to analyze cardiovascular death outcome, considering non-cardiovascular death as the competing risk event. Since no patients with 0 elevated markers experienced cardiovascular death in the data, patients with 0 or 1 elevated marker was treated as the reference category for the analysis of cardiovascular death.

**Table 4. Discrimination analysis of the biomarker risk score with major cardiovascular events.**

| Model | C-statistic (95% CI) | ΔC-Statistic (95%CI) | Continuous NRI (95% CI) | Relative IDI (95% CI) |
|---|---|---|---|---|
| | ***All-cause Death/MI*** | | | |
| **3 Biomarker Risk Score** | 0.71 (0.67-0.75) | - | - | - |
| **3 Biomarker Risk Score + suPAR** | 0.74 (0.70-0.78) | 0.03 (0.01, 0.05) | 0.60 (0.28-0.96) | 0.10 (0.03-0.17) |
| | ***All-cause Death*** | | | |
| **3 Biomarker Risk Score** | 0.71 (0.67-0.75) | - | - | - |
| **3 Biomarker Risk Score + suPAR** | 0.74 (0.70-0.78) | 0.03 (0.01,0.06) | 0.61 (0.27-0.99) | 0.11 (0.03-0.19) |
| | ***Cardiovascular Death*** | | | |
| **3 Biomarker Risk Score** | 0.71 (0.66-0.76) | - | - | - |
| **3 Biomarker Risk Score + suPAR** | 0.73 (0.68-0.79) | 0.02 (0.00-0.05) | 0.60 (0.13-0.86) | 0.08 (0.01-0.16) |
| | ***All-Cause Death/MI/Revascularization*** | | | |
| **3 Biomarker Risk Score** | 0.66 (0.63-0.69) | - | - | - |
| **3 Biomarker Risk Score + suPAR** | 0.67 (0.64-0.70) | 0.01 (0.00-0.02) | 0.57 (0.26-0.83) | 0.05 (0.01-0.09) |
| | ***All-Cause Death/MI/Stroke*** | | | |
| **3 Biomarker Risk Score** | 0.70 (0.66-0.75) | - | - | - |
| **3 Biomarker Risk Score + suPAR** | 0.73 (0.70-0.77) | 0.03 (0.01-0.05) | 0.60 (0.10-0.99) | 0.10 (0.02-0.16) |

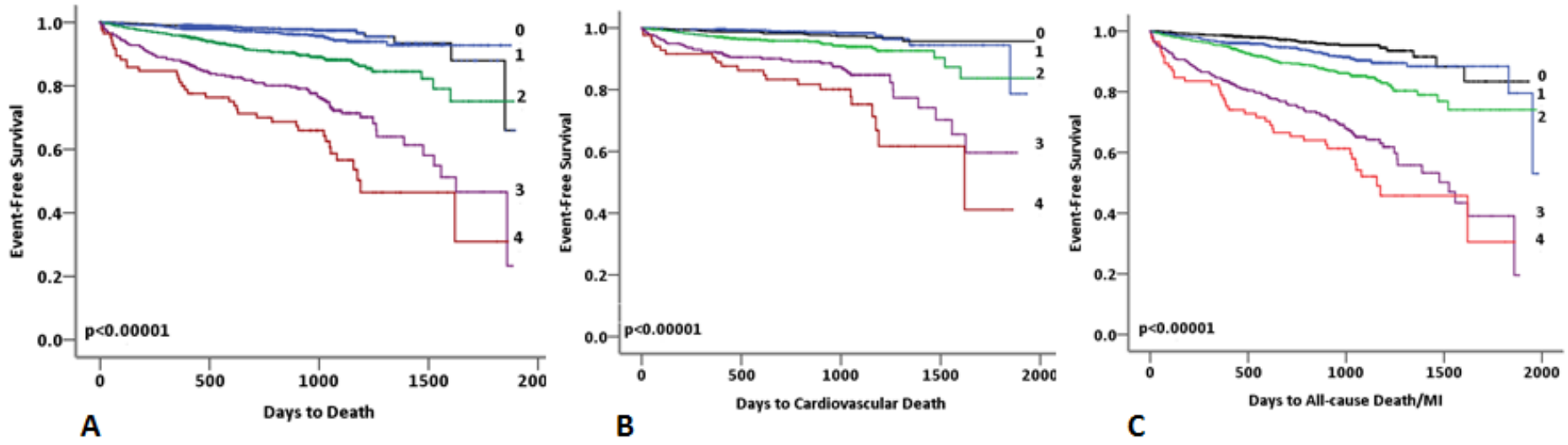
*Baseline model comprised of age, gender, race, body mass index, a history of smoking, glomerular filtration rate, history of myocardial infarction, history of revascularization (coronary bypass graft or percutaneous coronary intervention), hypertension, dyslipidemia, diabetes, left ventricular ejection fraction, presence of obstructive coronary artery disease, aspirin use, statin use, and clopidogrel use.

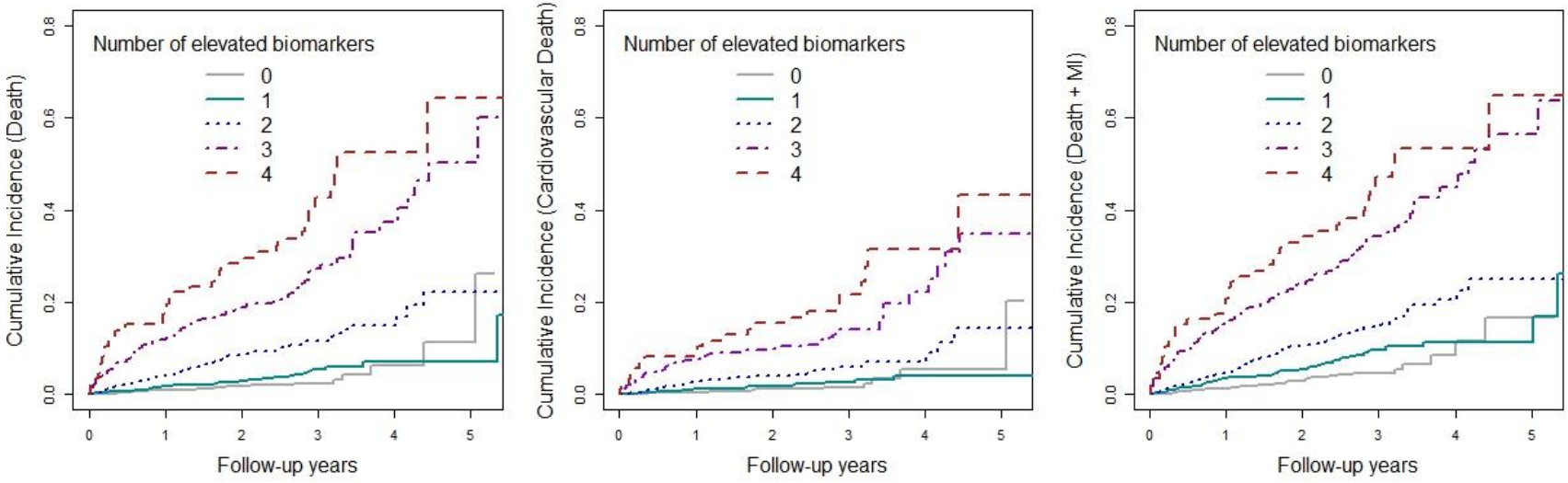
**Figure Legends:**

**Figure 1.** Cumulative incidence plots for all-cause death (A), cardiovascular death (B), and all-cause death/MI (C) per category of the biomarker risk score.

**Figure 2.** Annual event rates of major cardiovascular events in each category of the biomarker risk score. Percent of patients within each biomarker risk category is listed beside each score.

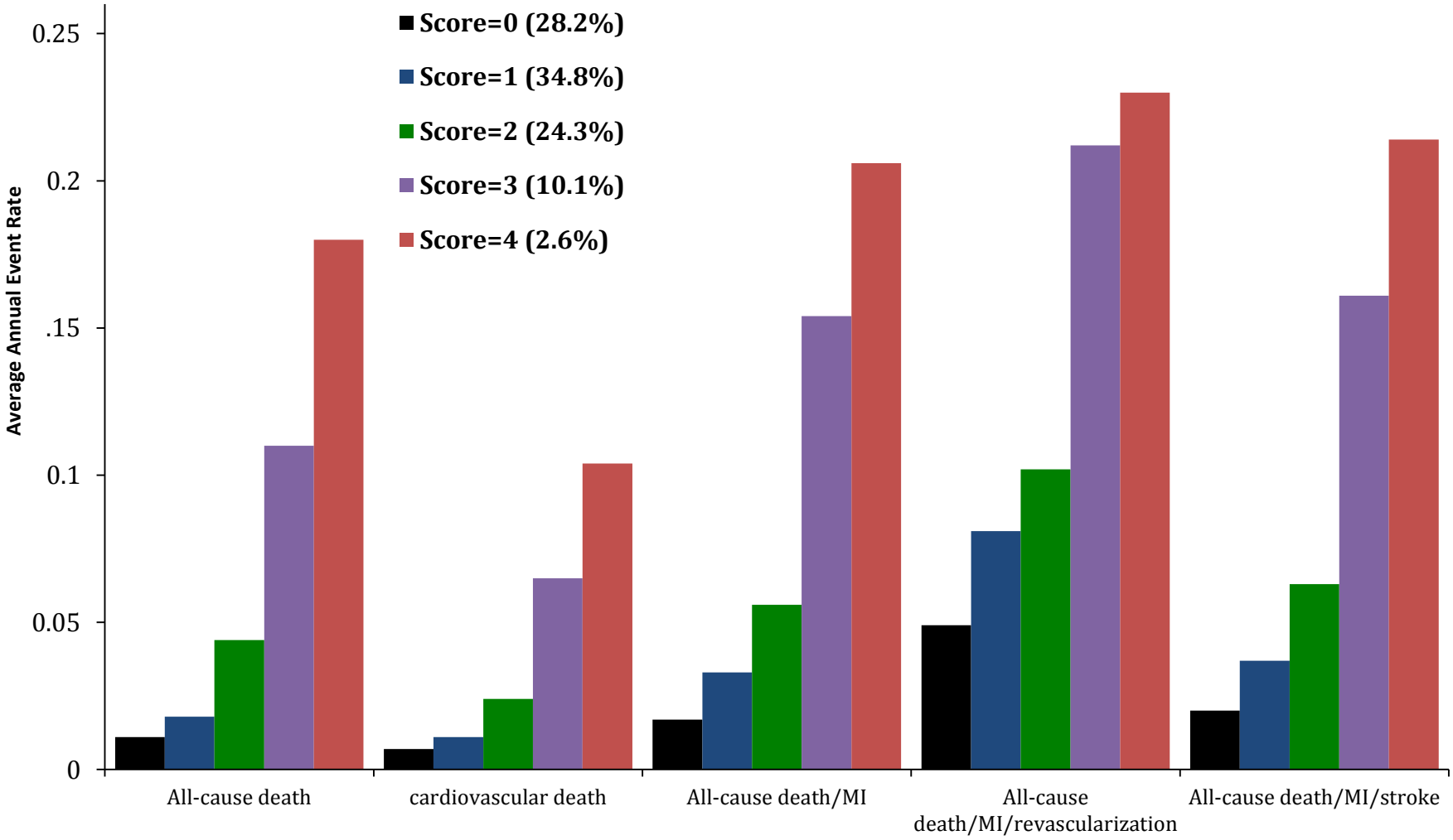
**Figure 1**


Event-Free Survival
1.0
0.8
0.6
0.4
0.2
0.0
p<0.00001
0
500
1000
1500
2000
Days to Death
A
0
1
2
3
4
Event-Free Survival
p<0.00001
Days to Cardiovascular Death
B
Event-Free Survival
p<0.00001
Days to All-cause Death/MI
C


Cumulative Incidence (Death)
Number of elevated biomarkers
0
1
2
3
4
Follow-up years
Cumulative Incidence (Cardiovascular Death)
Number of elevated biomarkers
Follow-up years
Cumulative Incidence (Death + MI)
Number of elevated biomarkers
Follow-up years

A B C

**Figure 2**


Score=0 (28.2%)
Score=1 (34.8%)
Score=2 (24.3%)
Score=3 (10.1%)
Score=4 (2.6%)
Average Annual Event Rate
0.25
0.2
.15
0.1
0.05
0
All-cause death
cardiovascular death
All-cause death/MI
All-cause death/MI/revascularization
All-cause death/MI/stroke