7-2013

Aggregate Risk Score Based on Markers of Inflammation, Cell Stress, and Coagulation Is an Independent Predictor of Adverse Cardiovascular Outcomes

Danny J. Eapen
Emory University

Pankaj Manocha
Emory University

Riyaz S. Patel
Emory University; Cardiff University

Muhammad Hammadah
Emory University

Emir Veledar
Department of Biostatistics, Florida International University; Emory University, eveledar@fiu.edu

See next page for additional authors

Authors
Danny J. Eapen, Pankaj Manocha, Riyaz S. Patel, Muhammad Hammadah, Emir Veledar, Christina Wassel, Ravi A. Nanjundappa, Sergey Sikora, Dylan Malayter, Peter W.F. Wilson, Laurence Sperling, Arshed A. Quyyumi, and Stephen E. Epstein

Published in final edited form as:
J Am Coll Cardiol. 2013 July 23; 62(4): 329–337. doi:10.1016/j.jacc.2013.03.072.

Aggregate Risk Score Based on Markers of Inflammation, Cell Stress, and Coagulation Is an Independent Predictor of Adverse Cardiovascular Outcomes

Danny J. Eapen, MD[*], **Pankaj Manocha, MD**[*], **Riyaz S. Patel, MD**[*,†], **Muhammad Hammadah, MD**[*], **Emir Veledar, PhD**[*], **Christina Wassel, PhD**[‡], **Ravi A. Nanjundappa, MD, MPH**[*], **Sergey Sikora, PhD**[§], **Dylan Malayter, BS**[§], **Peter W. F. Wilson, MD**[*], **Laurence Sperling, MD**[*], **Arshed A. Quyyumi, MD**[*], and **Stephen E. Epstein, MD**[||]
Atlanta, Georgia; Cardiff, United Kingdom; San Diego, California; and Washington, DC

Abstract

Objectives—This study sought to determine an aggregate, pathway-specific risk score for enhanced prediction of death and myocardial infarction (MI).

Background—Activation of inflammatory, coagulation, and cellular stress pathways contribute to atherosclerotic plaque rupture. We hypothesized that an aggregate risk score comprised of biomarkers involved in these different pathways—high-sensitivity C-reactive protein (CRP), fibrin degradation products (FDP), and heat shock protein 70 (HSP70) levels—would be a powerful predictor of death and MI.

Methods—Serum levels of CRP, FDP, and HSP70 were measured in 3,415 consecutive patients with suspected or confirmed coronary artery disease (CAD) undergoing cardiac catheterization. Survival analyses were performed with models adjusted for established risk factors.

Results—Median follow-up was 2.3 years. Hazard ratios (HRs) for all-cause death and MI based on cutpoints were as follows: CRP ≥3.0 mg/l, HR: 1.61; HSP70 >0.625 ng/ml, HR; 2.26; and FDP ≥1.0 μg/ml, HR: 1.62 ($p < 0.0001$ for all). An aggregate biomarker score between 0 and 3 was calculated based on these cutpoints. Compared with the group with a 0 score, HRs for all-cause death and MI were 1.83, 3.46, and 4.99 for those with scores of 1, 2, and 3, respectively (p for each: <0.001). Annual event rates were 16.3% for the 4.2% of patients with a score of 3 compared with 2.4% in 36.4% of patients with a score of 0. The C statistic and net reclassification improved ($p < 0.0001$) with the addition of the biomarker score.

Reprint requests and correspondence: Dr. Arshed A. Quyyumi, Cardiology Division, Emory University School of Medicine, 1462 Clifton Road N.E. Suite 507, Atlanta, Georgia 30322. aquyyum@emory.edu.
[*]Department of Medicine, Division of Cardiology, Emory University School of Medicine, Atlanta, Georgia
[†]Department of Medicine, Cardiff University, Cardiff, United Kingdom
[‡]Department of Family and Preventive Medicine, University of California San Diego, San Diego, California
[§]Division of GenWay Biotech, First-Mark, San Diego, California
[||]MedStar Heart Institute, Washington Hospital Center, and MedStar Health Research Institute, Washington, DC.
Drs. Quyyumi and Epstein have contributed equally to this paper.

Dr. Sikora and Mr. Malayter are employed by FirstMark, Division of GenWay Biotech. Drs. Epstein and Quyyumi are equity holders in GenWay Biotech and receive consulting fees. Dr. Wassel receives consulting fees from GenWay Biotech. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Conclusions—An aggregate score based on serum levels of CRP, FDP, and HSP70 is a predictor of future risk of death and MI in patients with suspected or known CAD.

Keywords

biomarker; C-reactive protein; fibrin degradation product; heat shock protein; myocardial infarction

Stable coronary artery disease (CAD) can lead to severe ischemic symptoms from stenosis-related coronary blood flow reduction, but plaque rupture leading to myocardial infarction (MI) and death is its most devastating complication. Although many patients with CAD never experience clinical plaque rupture, others may experience an early MI. Importantly, distinct genetic differences distinguish patients with stable CAD versus those who experience plaque rupture (1). Thus, signaling pathways predisposing to atherosclerosis probably differ from those contributing to plaque vulnerability. This distinction is likely to be crucial when considering strategies for identifying patients at risk of MI and death from plaque rupture.

The Framingham Risk Score and similar scores are widely used to assess absolute risk of adverse cardiac events in patients without known CAD (2,3); however, they do not reliably predict risk of plaque rupture (and consequent MI and/or death) in patients with already established CAD (4,5). Other biomarkers appear to predict such risk, but their associated hazard ratios (HRs) have been modest (6–8). Our purpose is to develop a robust, noninvasive, and simple biomarker strategy to identify CAD patients at increased risk of plaque rupture.

The strategy we explored derived from the concept that activation of multiple pathways, including inflammatory, stress-related, and coagulation pathways, each contribute to coronary plaque instability. Elevated levels of high-sensitivity C-reactive protein (CRP) reflect vascular inflammation and are associated with greater risk for subsequent cardiovascular disease (CVD) events, but the effects are modest (9,10). Heat shock proteins (HSPs), including HSP70, are highly conserved intracellular proteins that increase in response to stress, and may provide evidence of increased cellular stress, and thus, a predisposition to plaque rupture (11–13). Both fibrinogen and fibrin degradation products (FDP), end products in the coagulation cascade, have been associated with CAD development and severity (14,15). Moreover, D-dimer, a degradation product of fibrinogen and soluble fibrin monomers, has been associated with adverse cardiac events (16,17). We hypothesized that risk assessment would be markedly enhanced when the 3 biomarkers—CRP reflecting inflammation; HSP70, associated with increased cellular stress; and FDP, associated with coagulation cascade activation—are used in aggregate, that is, the risk of plaque rupture would be greater when biomarkers reflected activation of 2 or 3 pathways compared with activation of 0 or 1 pathways.

Methods

Study population

Study participants were recruited as part of the Emory Cardiology Biobank (EMCAB), consisting of 3,763 consecutive patients enrolled before undergoing elective or emergent coronary angiograms across 3 Emory healthcare sites, between 2003 and 2009 (details in the Online Appendix).

Outcomes and follow-up

Record of death was obtained from the Social Security Death Index, and the cause of death adjudicated from medical records or direct contact was made with the patient's family member(s). Cardiac death was defined as death attributable to a cardiovascular cause or sudden death due to an unknown cause. Follow-up was conducted between 1 and 5 years to identify cases of MI and revascularization (defined as percutaneous coronary interventions or coronary artery bypass graft [CABG] surgery). MI and revascularization occurring within a month of enrollment were not included.

Identification of CAD and severity scoring

All coronary angiograms were scored for luminal narrowing using a modified American Heart Association/American College of Cardiology classification of the coronaries (18). Patients were designated as having either angiographically smooth normal coronary arteries, nonsignificant CAD (visible plaque resulting in <50% luminal stenosis), or significant CAD (at least 1 major epicardial vessel with ≥50% stenosis). Quantitative angiographic scoring was performed using the Gensini score, which quantifies CAD severity by a nonlinear points system for degree of luminal narrowing. The score has prognostic significance (19).

Sample collection

Fasting arterial blood samples for serum were drawn at cardiac catheterization and stored at −80°C (mean 4.9 years) before analysis by FirstMark, Inc. (San Diego, California) (Online Appendix). CRP and FDP levels were determined using a sandwich immunoassay. FDP components included fragments D and E, D-dimer, and additional intermediate cleavage products. HSP70 was measured with a sandwich enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minnesota) and optimized by FirstMark. Minimum detectable CRP, FDP, and HSP70 concentrations were 0.1 mg/l, 0.06 μg/ml, and 0.625 ng/ml, respectively.

Statistical analyses

Continuous variables are presented as mean ± SD, and categorical variables are presented as proportions (percentages). Student *t* test, 1-way analysis of variance, and Cochran-Mantel-Haenszel chi-square test were used as appropriate. Mann-Whitney *U* or Kruskall-Wallis nonparametric 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 included clinically relevant covariates for CVD outcomes (age at baseline, race, diagnosis of

hypertension, diabetes, dyslipidemia, use of statins, aspirin, clopidogrel, history of MI, acute MI at presentation, estimated glomerular filtration rate [eGFR; calculated using the Modification of Diet in Renal Disease equation], Gensini score, body mass index, left ventricular ejection fraction [LVEF], history of CABG, and smoking status). The proportional hazards assumption for Cox models was evaluated by plots of Schoenfeld residuals and formal testing (a chi-square test calculated as the sum of Schoenfeld residuals). No significant violations of the assumption were found.

Biomarkers were evaluated both as continuous (natural log transformed) per SD (Online Fig. 1) and as categorical variables based on cutpoints. Penalized B-splines within the Cox models were also used to assess the functional form of the association between each biomarker and events (20). We also took into consideration clinically relevant cutpoints (21). Through these evaluations, cutpoints were determined as 3 mg/l for CRP, 1.0 μg/ml for FDP, and 0.625 ng/ml for HSP70. The FDP cutpoint corresponded to the fourth quartile. Analyses were performed on all participants, and in subsets of those with and those without significant CAD.

The ability of the standard clinical model for predicting adverse events was calculated using the C statistic from Cox regression models before and after addition of the independently predictive biomarkers identified both individually and in aggregate (22). Using multivariate Cox models with the previously noted clinical covariates, continuous net reclassification improvement (NRI) and integrated discrimination improvement (IDI) metrics were calculated. Values of $p < 0.05$ from 2-sided tests were considered to indicate statistical significance. Further statistical methods are in the Online Appendix.

Results

Baseline characteristics of the 3,415 patients (age 63 ± 11 years) are shown in Table 1.

Relationship between biomarkers and prevalent CAD

Compared with patients with angiographically normal (smooth) coronary arteries, those with angiographic atherosclerosis had higher levels of HSP70, but FDP and CRP levels were not significantly different. FDP and HSP70, but not CRP levels, were significantly lower in patients with nonsignificant versus significant CAD (Online Table 1A). In univariate analysis, the Gensini CAD severity score was significantly higher in patients with elevated HSP70 and FDP levels (above their respective cutpoints), but not in those with an elevated CRP level (Online Tables 1B and 1C). However, none of these differences remained significant after multivariate adjustment with the aforementioned covariates (Online Table 2).

Clinical and demographic predictors of adverse outcomes

Over a median follow-up of 2.3 years, 283 patients died (8.3%) (150 were cardiac deaths [4.9%] and 122 had an MI [3.6%]), and 371 had revascularization (10.8%) (Table 1). Using Cox proportional hazard models that included all the aforementioned covariates, age (HR: 1.02, $p = 0.004$), diabetes (HR: 1.65, $p < 0.0001$), ever smoking (HR: 1.38, $p = 0.0046$), Gensini score (HR: 1.003, $p = 0.006$), aspirin use (HR: 0.62, $p = 0.001$), clopidogrel use

(HR: 1.51, p = 0.0008), acute MI at presentation (HR: 1.69, p <0.0001), eGFR (HR: 0.99, p < 0.0001), and LVEF (HR: 0.98, p < 0.0001) were all independent predictors of the combined outcomes of all-cause death and MI.

Relationships between individual biomarkers and outcomes

Cox proportional hazard regression models, adjusted for aforementioned covariates, performed for individual biomarkers (natural log transformed) demonstrated that HSP70 (HR: 1.14, p < 0.0001) and CRP (HR: 1.29, p < 0.0001), but not FDP (p = 0.90), were significantly associated with combined outcomes of death and MI. However, elevated levels (above cutpoints) of HSP70, FDP, and CRP were each associated with increased risk of all-cause death, cardiac deaths, the combined endpoint of cardiac or all-cause death and MI, and the combined endpoint of death, MI, and revascularization (Table 2). All 3 biomarkers predicted adverse CVD events in subgroups with nonsignificant CAD (<50% stenosis, n = 1,480) and in those with significant CAD (≥50% stenosis, n = 1,935) (Table 2).

Relationship between aggregate biomarker score and outcomes

There were significant but weak correlations between CRP and both HSP70 and FDP, and between HSP70 and FDP (Online Table 1B). For each patient, a score of 0 or 1 was assigned based on the presence or absence of elevated levels (above each cutpoint value) of each of the 3 biomarkers, and an aggregate risk score between 0 and 3 was calculated. Compared with those with 0 positive biomarkers (n = 1,248), patients with an elevated biomarker score were more often black, had lower eGFRs and LVEFs, and had a greater frequency of diabetes and smoking history (Table 1). Among the 4 groups stratified by biomarker positivity, there were no significant differences between the management strategy (medical management or revascularization) after index catheterization (Table 1).

In unadjusted analyses, an increasing biomarker score (0 to 3) was associated with increased risk of all-cause death (p for trend <0.0001), cardiac deaths (p for trend < 0.0001), MI (p for trend <0.0001), and revascularization (p for trend <0.0001) (Table 1). There was a stepwise decline in survival free of death (log rank p < 0.0001), death and MI events (log rank p < 0.0001), and the combined endpoint of death, MI, and revascularization (log rank p < 0.0001) with increasing biomarker risk score (Fig. 1).

The Cox proportional hazard regression model adjusting for all the previously described covariates revealed that for each 1 point increase in risk score, the HR increased 1.87 (p <0.0001) for all-cause death, 1.79 (p <0.0001) for cardiac death, 1.76 (p <0.0001) for combined outcomes of death and MI, and 1.49 (p < 0.0001) for death, MI, and revascularization. HRs of 1, 2, or 3 positive biomarkers compared with 0 are shown in Table 2 and Online Table 3, and the rates of annual CVD events in these aggregate risk score categories are shown in Figure 2. The score was also associated with individual events of MI (HR: 1.40, p = 0.0024) and revascularization (HR: 1.26, p = 0.0003) for each 1 point increase in risk score.

Discrimination testing

In the whole cohort and in the patients with nonsignificant and with significant CAD, the C statistic increased significantly for prediction of cardiac deaths (referent C statistic = 0.76, with added biomarker score, 0.80; p = 0.0002), all-cause death, for combined events of death and MI, and for death, MI, and revascularization, when all 3 biomarkers were incorporated into a model with the established risk factors both as a risk score or as categorical variables (Table 3).

The NRI of the biomarker score for all-cause death and the combined events of death and MI were 44% and 42%, respectively (Table 4). This corresponded to 14% and 13% rates of correctly reclassifying events and 30% and 29% rates of correctly reclassifying nonevents, respectively. The relative IDI for this model was 31% for deaths and 30% for death and MI. There was improvement in both subgroups with and without significant CAD, with a trend to greater NRI in those without significant CAD.

Subgroup analyses

Results were similar in the subgroups with either significant or nonsignificant CAD, including those with normal coronary arteries (Table 2). A score of 3 corresponded to an 18%/year risk of death and MI in the significant CAD group and a 14%/year risk in the nonsignificant CAD group (Fig. 2). Even after excluding patients with angiographically normal smooth coronary arteries and those with acute MI on presentation, our results remained unchanged.

We examined whether there was heterogeneity in the HRs based on age, sex, race, and presence of individual risk factors, presentation with acute MI, severity of CAD, and eGFR values. We found that aside from age (p = 0.003), eGFR (p = 0.03), and a diagnosis of hypertension (p = 0.009), there was no significant interaction (p > 0.05) among these factors and the predictive capacity of the risk score (Fig. 3).

Discussion

Multiple pathways contribute to the development of atherosclerotic plaque instability and thereby increase the likelihood that plaques will rupture or erode. We identified 3 circulating biomarkers (CRP, FDP, and HSP70) that are involved in signaling pathways that likely influence plaque instability (including inflammation, coagulation, and stress-induced cellular responses) and examined the hypothesis that each biomarker would predict risk, but the more biomarkers that were abnormal, the greater the expected risk. Our results support the validity of our underlying hypothesis. Among patients who underwent coronary angiography for suspected or established CAD, these 3 biomarkers were significant and independent predictors of risk of all-cause death, cardiac death, combined outcomes of death and MI, and death, MI, and revascularization. Importantly, an aggregate score based on the number of biomarkers that were abnormal was a more powerful predictor of higher risk. In comparison to patients with a 0 biomarker score, those who had a risk score of 3 (<5% of the population) experienced over a 5-fold increased risk of all-cause death or MI within 1 year, with an annual rate of more than 16%. The aggregate risk score significantly improved

discrimination of future death and MI risk over a standard clinical model, as evidenced by improvement in the C statistic and NRI.

Current evidence suggests that signaling pathways and their effector molecules involved in the development of plaque rupture are different from pathways involved in atherogenesis. Thus, a biomarker that is a predictor of adverse events emanating from the development of CAD in a population that is initially free of existing CAD is not necessarily a predictor of events in patients with established, or likely, CAD.

C-reactive protein

Many population-based studies in subjects free of known CAD have found that CRP adds to risk prediction above standard risk factor assessment (23,24). However, results remain unclear in patients with CAD. In a meta-analysis of 83 studies in patients with CAD, an elevated CRP posed an adjusted relative risk of only 1.19 (25). In our high-risk population, an elevated CRP level increased risk by a higher, but still modest, 1.6-fold.

Heat shock protein 70

Heat shock or stress proteins are highly conserved molecules that fulfill a range of functions, including cytoprotection and the intracellular assembly, stabilization, folding, and translocation of oligomeric proteins. Their synthesis can be induced by a range of cellular insults, including oxidative, hemodynamic, and inflammatory stress, all of which are associated with the development of CAD (11–13). The relation between cardiovascular outcomes and circulating HSP70 levels is controversial. In cross-sectional studies, elevated levels of HSP70 were associated with a lower prevalence of CAD and of carotid intimal thickness (26,27). These differences are most likely due to the fact that previous studies investigated risk of atherogenesis, whereas our present investigation focuses on whether the biomarkers are predictive of subsequent plaque rupture. HSP70 levels increase after an acute MI, indicating that acute MI can cause an increase in HSP70 (28,29). In our study, 12% of patients presented with an acute MI; however, our findings did not change after exclusion of this subset.

Fibrin degradation products

We employed FDP, and not D-dimer, to assess coagulation products. The immunoassay was designed to detect the full complement of fibrin and FDP (fibrin mono- and oligomers, fragments -X, -Y, and -E), providing an increased ability to measure coagulation-related products. Moreover, we found that FDP was not a predictor of adverse events when used as a continuous variable, but was an independent predictor using the cutoff value. This suggests that there is threshold value for FDP (>75th percentile of the population mean) above which it is associated with increased risk, and not with a continuous additive risk with increasing values.

Previous studies have only examined the value of D-dimer levels with respect to long-term outcomes. In population-based studies, D-dimer levels predicted adverse cardiovascular events, but was not always independent of CRP (16,30). Importantly, in the BARI 2D (Bypass Angioplasty Revascularization Investigation 2 Diabetes) study, the diabetics with

CAD had higher D-dimer levels that were associated with an increased risk of cardiac events (31).

Use of multiple biomarkers

Previous studies examining the role of multiple biomarkers in populations free of established CAD have demonstrated only slight improvement in predictive capacity (using C statistic) when added to standard clinical models (6–8). In contrast, our study establishes the value of a multimarker aggregate score in a population with suspected or established CAD, a group in which conventional risk scores such as the Framingham Risk Score have failed to identify risk of recurrent cardiac events.

The measured biomarkers were not associated with either the presence or severity of CAD after adjustment for risk factors, again emphasizing the concept that a biomarker that reflects plaque instability is not necessarily useful for identifying presence or severity of coronary plaque. There was minimal heterogeneity in the value of the biomarker risk score based on clinical variables, risk factors, medication use, and LVEF. Patients presenting with acute MI, which constituted 12% of our cohort, also had similar risk prediction compared with those without MI at presentation, and exclusion of these patients did not significantly alter risk prediction. Approximately two-thirds of our CAD patients underwent revascularization, and the remaining were treated with medical therapy upon discharge after cardiac catheterization; however, the rate of revascularization was similar between the various biomarker risk score groups. Including the revascularization strategy in the Cox regression model did not alter the overall HRs for the biomarker risk score.

Clinical cardiac events that include death, MI, and revascularization potentially result from complex kinetic interactions between the magnitude of vascular stenoses and factors that characterize plaque instability (32). The stenotic severity, reflected by the Gensini score, drives symptoms and some revascularization procedures, whereas plaque instability leads to death and MI events. Our results are in agreement with this concept because the Gensini score and the biomarkers were all independent predictors of outcome.

Study strengths

We enrolled consecutive individuals, including women (35% of total cohort), blacks, those with acute MI, and patients with a range of LVEFs, reflecting a population that is typical of those undergoing cardiac catheterization. This is different from many biomarker studies that are conducted retrospectively on highly select populations enrolled in clinical trials. Assays were performed at 2 time points by the same laboratory personnel, which minimized variability. C statistics, NRI, and IDI were calculated using survival models that allowed for better model discrimination and overall predictive ability.

Study limitations

Limitations of our study include a 1-time measurement of biomarkers that may not reflect levels at future time points. We have not studied other biomarkers, such as myeloperoxidase, or myocardial specific markers, such as troponin and brain natriuretic peptide. Our results need to be further validated and should not be extrapolated to the general population without

suspected or known CAD. Whether more aggressive management in patients with an elevated biomarker risk score will modify the score, and whether that will reflect lower risk remains unknown and needs further investigation.

Although we characterized these biomarkers as representatives of specific biologic pathways, signaling molecules are often involved in multiple pathways that interact with each other. For example, HSP70 pathways independent of cellular stress may also contribute to its biological actions (11–13,33). Our intent, therefore, was to use these 3 biomarkers as probable reflectors of activation of multiple pathways commonly associated with vulnerable plaque.

Conclusions

We have demonstrated that a strategy using an aggregate risk score consisting of 3 biomarkers (individually involved in inflammation, coagulation, and stress-induced cellular responses) identifies patients with suspected or with established CAD who are at increased risk of experiencing death and other adverse cardiac outcomes in the near and medium term. Whether treatment aimed at reducing activity of these pathways can positively alter the disease course remains to be determined and could be addressed in an adequately powered randomized interventional trial based on biomarker evaluation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank the members of the EMCAB Team, Emory Clinical Cardiovascular Research Institute (ECCRI), and Atlanta Clinical and Translational Science Institute for recruitment of participants, compilation of data, and preparation of samples. Sample measurements were conducted by FirstMark, Division of Genway Biotech Inc.

Funding for collection and management of samples was received from the Robert W. Woodruff Health Sciences Center Fund, Atlanta, Georgia; Emory Heart and Vascular Center, Atlanta, Georgia; Katz Family Foundation Preventive Cardiology Grant, Atlanta, Georgia; and NIH Grant UL1 RR025008 from the Clinical and Translational Science Award program (NIH Grant R01HL089650-02). Sample measurements were conducted by FirstMark, Division of Genway Biotech Inc, San Diego, California.

Abbreviations and Acronyms

CABG	coronary artery bypass grafting
CAD	coronary artery disease
CRP	C-reactive protein
CVD	cardiovascular disease
eGFR	estimated glomerular filtration rate
FDP	fibrin degradation products
HSP70	heat shock protein 70

IDI	integrated discrimination improvement
LVEF	left ventricular ejection fraction
MI	myocardial infarction
NRI	net reclassification improvement

References

1. Reilly MP, Li M, He J, et al. Identification of ADAMTS7 as a novel locus for coronary atherosclerosis and association of ABO with myocardial infarction in the presence of coronary atherosclerosis: two genome-wide association studies. Lancet. 2011; 377:383–92. [PubMed: 21239051]
2. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation. 1998; 97:1837–47. [PubMed: 9603539]
3. Pencina MJ, D'Agostino RB Sr, Larson MG, Massaro JM, Vasan RS. Predicting the 30-year risk of cardiovascular disease: the Framingham Heart Study. Circulation. 2009; 119:3078–84. [PubMed: 19506114]
4. Shlipak MG, Ix JH, Bibbins-Domingo K, Lin F, Whooley MA. Biomarkers to predict recurrent cardiovascular disease: the Heart and Soul Study. Am J Med. 2008; 121:50–7. [PubMed: 18187073]
5. Hill J, Heslop C, Man SF, et al. Circulating surfactant protein-D and the risk of cardiovascular morbidity and mortality. Eur Heart J. 2011; 32:1918–25. [PubMed: 21653561]
6. St-Pierre AC, Cantin B, Bergeron J, et al. Inflammatory markers and long-term risk of ischemic heart disease in men: a 13-year follow-up of the Quebec Cardiovascular Study. Atherosclerosis. 2005; 182:315–21. [PubMed: 16159604]
7. Wang TJ, Gona P, Larson MG, et al. Multiple biomarkers for the prediction of first major cardiovascular events and death. N Engl J Med. 2006; 355:2631–9. [PubMed: 17182988]
8. Kim HC, Greenland P, Rossouw JE, et al. Multimarker prediction of coronary heart disease risk: the Women's Health Initiative. J Am Coll Cardiol. 2010; 55:2080–91. [PubMed: 20447530]
9. Ridker PM, Buring JE, Shih H, Matia M, Hennekens CH. Prospective study of C-reactive protein and the risk for future cardiovascular events among apparently healthy women. Circulation. 1998; 98:731–3. [PubMed: 9727541]
10. Shah T, Casas JP, Cooper JA, et al. Critical appraisal of CRP measurement for the prediction of coronary heart disease events: new data and systematic review of 31 prospective cohorts. Int J Epidemiol. 2009; 38:217–31. [PubMed: 18930961]
11. Delogu G, Signore M, Mechelli A, Famularo G. Heat shock proteins and their role in heart injury. Curr Opin Crit Care. 2002; 8:411–6. [PubMed: 12357108]
12. Djordjevic A, Adzic M, Djordjevic J, Radojcic MB. Stress type dependence of expression and cytoplasmic-nuclear partitioning of glucocorticoid receptor, hsp90 and hsp70 in Wistar rat brain. Neuro-psychobiology. 2009; 59:213–21.
13. Wilhide ME, Tranter M, Ren X, et al. Identification of a NF-kappaB cardioprotective gene program: NF-kappaB regulation of Hsp70. 1 contributes to cardioprotection after permanent coronary occlusion. J Mol Cell Cardiol. 2011; 51:82–9. [PubMed: 21439970]
14. Kannel WB, Wolf PA, Castelli WP, D'Agostino RB. Fibrinogen and risk of cardiovascular disease. The Framingham Study JAMA. 1987; 258:1183–6.
15. Tataru MC, Heinrich J, Junker R, et al. D-dimers in relation to the severity of arteriosclerosis in patients with stable angina pectoris after myocardial infarction. Eur Heart J. 1999; 20:1493–502. [PubMed: 10493848]
16. Wannamethee SG, Whincup PH, Shaper AG, Rumley A, Lennon L, Lowe GD. Circulating inflammatory and hemostatic biomarkers are associated with risk of myocardial infarction and

coronary death, but not angina pectoris, in older men. J Thromb Haemost. 2009; 7:1605–11. [PubMed: 19682232]

17. Zacharowski K, Zacharowski P, Reingruber S, Petzelbauer P. Fibrin(ogen) and its fragments in the pathophysiology and treatment of myocardial infarction. J Mol Med (Berl). 2006; 84:469–77. [PubMed: 16733765]
18. Austen WG, Edwards JE, Frye RL, et al. A reporting system on patients evaluated for coronary artery disease. Report of the Ad Hoc Committee for Grading of Coronary Artery Disease, Council on Cardiovascular Surgery, American Heart Association. Circulation. 1975; 51 (Suppl 4):5–40. [PubMed: 1116248]
19. Gensini, G. Coronary Arteriography. New York, NY: Futura Publishing Co; 1975.
20. Eilers PHC, Marx BD. Flexible smoothing with B-splines and penalties. Stat Sci. 1996; 11:89–102.
21. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation. 2003; 107:363–9. [PubMed: 12551853]
22. Pencina MJ, D'Agostino RB Sr, D'Agostino RB Jr, Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med. 2008; 27:157–72. [PubMed: 17569110]
23. Ridker PM. High-sensitivity C-reactive protein, vascular imaging, and vulnerable plaque: more evidence to support trials of antiinflammatory therapy for cardiovascular risk reduction. Circ Cardiovasc Imaging. 2011; 4:195–7. [PubMed: 21586741]
24. Shah KB, Kop WJ, Christenson RH, et al. Lack of diagnostic and prognostic utility of circulating plasma myeloperoxidase concentrations in patients presenting with dyspnea. Clin Chem. 2009; 55:59–67. [PubMed: 18988754]
25. Hemingway H, Philipson P, Chen R, et al. Evaluating the quality of research into a single prognostic biomarker: a systematic review and meta-analysis of 83 studies of C-reactive protein in stable coronary artery disease. PLoS Med. 2010; 7:e1000286. [PubMed: 20532236]
26. Zhu J, Quyyumi AA, Wu H, et al. Increased serum levels of heat shock protein 70 are associated with low risk of coronary artery disease. Arterioscler Thromb Vasc Biol. 2003; 23:1055–9. [PubMed: 12730089]
27. Pockley AG, Georgiades A, Thulin T, de Faire U, Frostegard J. Serum heat shock protein 70 levels predict the development of atherosclerosis in subjects with established hypertension. Hypertension. 2003; 42:235–8. [PubMed: 12900429]
28. Zhang X, Xu Z, Zhou L, et al. Plasma levels of Hsp70 and anti-Hsp70 antibody predict risk of acute coronary syndrome. Cell Stress Chaperones. 2010; 15:675–86. [PubMed: 20300983]
29. Dybdahl B, Slordahl SA, Waage A, Kierulf P, Espevik T, Sundan A. Myocardial ischaemia and the inflammatory response: release of heat shock protein 70 after myocardial infarction. Heart. 2005; 91:299–304. [PubMed: 15710705]
30. Empana JP, Canoui-Poitrine F, Luc G, et al. Contribution of novel biomarkers to incident stable angina and acute coronary syndrome: the PRIME Study. Eur Heart J. 2008; 29:1966–74. [PubMed: 18621771]
31. Sobel BE, Hardison RM, Genuth S, et al. Profibrinolytic, antithrombotic, and antiinflammatory effects of an insulin-sensitizing strategy in patients in the Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) trial. Circulation. 2011; 124:695–703. [PubMed: 21768545]
32. Diamond GA, Kaul S. From here to eternity: a unified kinetic model for the pathophysiology of atherosclerotic events. Am J Med. 2007; 120:5–11. [PubMed: 17208070]
33. Xu Q, Wick G. The role of heat shock proteins in protection and pathophysiology of the arterial wall. Mol Med Today. 1996; 2:372–9. [PubMed: 8885256]

APPENDIX

For supplemental information and supplemental tables, please see the online version of this article.

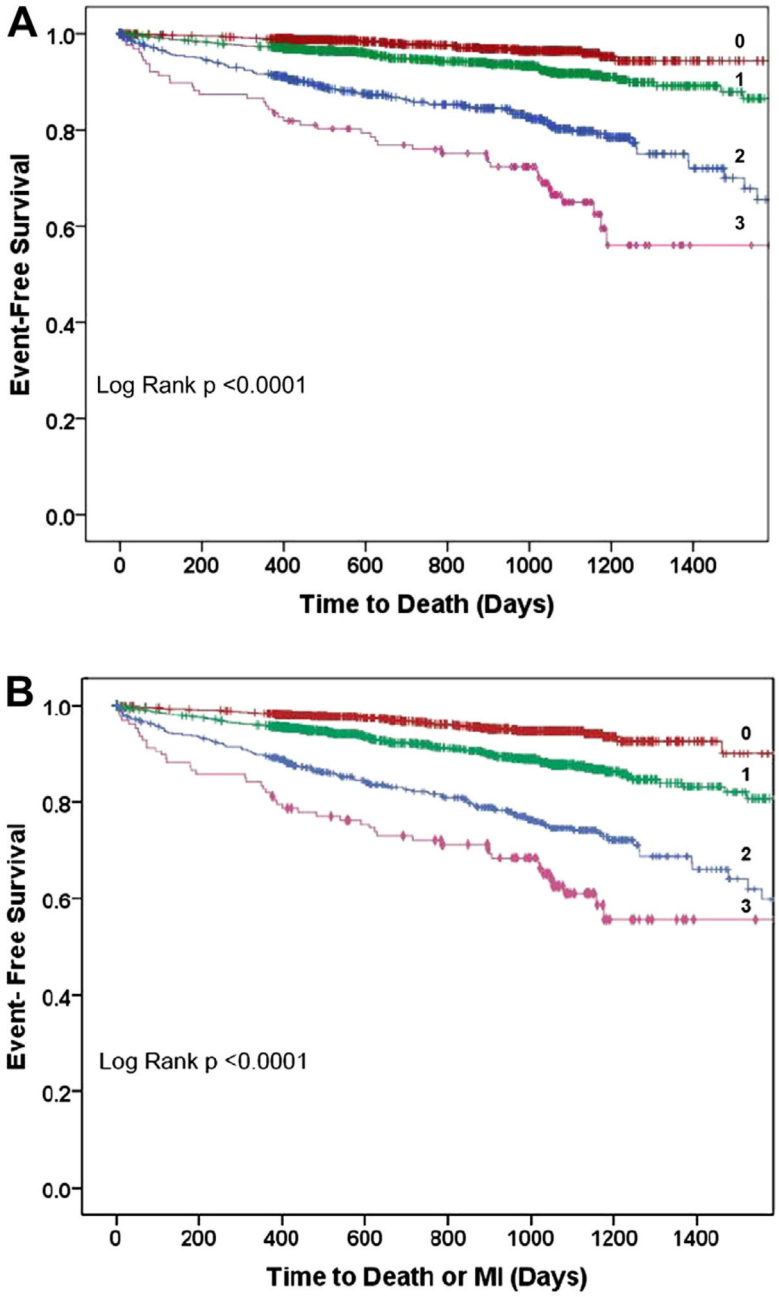

Figure 1. Kaplan-Meier Survival

Survival curves for biomarker risk score for **(A)** death and **(B)** death and myocardial infarction (MI). Number of positive biomarkers are listed adjacent to each survival curve.

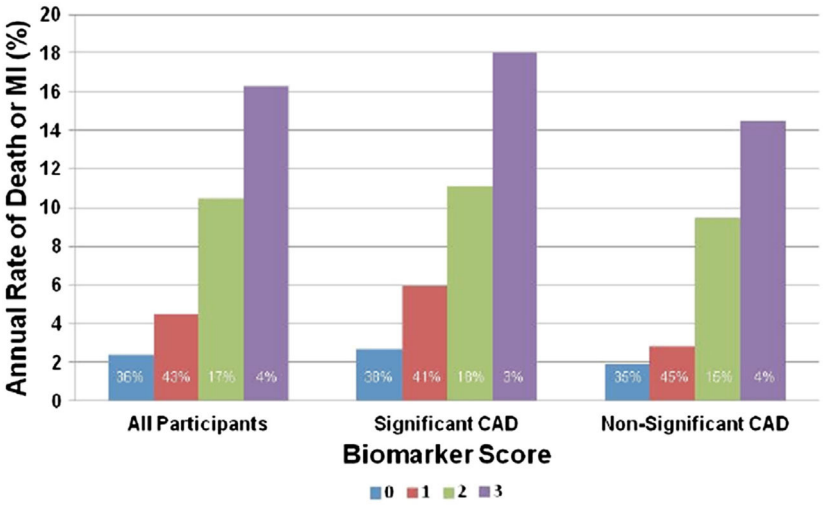

Figure 2. Annual Rate of Death and MI

Rate of death and MI grouped by coronary artery disease (CAD) status and by biomarker score. Percent of patients within each group listed in individual bars. Abbreviation as in Figure 1.

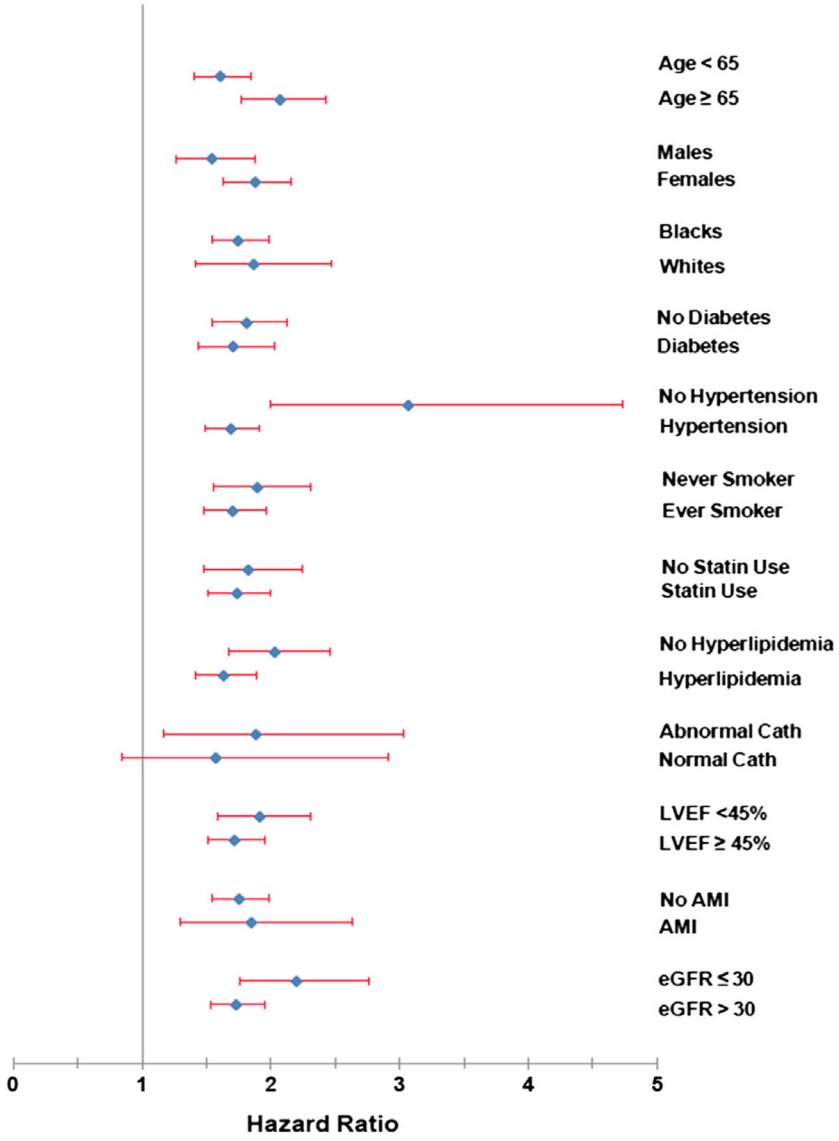

Figure 3. Forest Plot of Interaction With Cardiovascular Risk Factors for 1 Unit of Biomarker Risk Score for Outcomes of Death and MI

AMI = acute myocardial infarction; eGFR = estimated glomerular filtration rate; LVEF = left ventricular ejection fraction.

J Am Coll Cardiol. Author manuscript; available in PMC 2014 July 23.

Table 1

Baseline Demographics, Clinical Characteristics, and Biomarker Measurements

		No. of Positive Biomarkers				
Characteristics	**Entire Cohort (n = 3,415)**	**0 (n = 1,248)**	**1 (n = 1,475)**	**2 (n = 565)**	**3 (n = 127)**	**p Value**
Age (yrs)	63 ± 11	64 ± 11	62 ± 13	65 ± 12	65 ± 11	<0.0001
Male	65	71	61	62	60	<0.0001
Caucasian	82	87	81	80	74	<0.0001
BMI (kg/m^2)	30 ± 6	29 ± 6	30 ± 7	30 ± 7	28 ± 6	<0.0001
Systolic BP (mm Hg)	137 ± 23	137 ± 22	138 ± 23	137 ± 25	135 ± 24	0.343
Diastolic BP (mm Hg)	76 ± 12	76 ± 12	76 ± 12	76 ± 12	74 ± 13	0.350
LDL (mg/dl)	99 ± 38	96 ± 35	102 ± 39	100 ± 39	98 ± 35	0.001
HDL (mg/dl)	42 ± 13	42 ± 13	42 ± 13	40 ± 12	42 ± 15	0.016
Glucose (mg/dl)	122 ± 45	116 ± 35	124 ± 46	131 ± 56	126 ± 57	<0.0001
Catheterization: visually normal	9	11	10	6	6	0.001
Catheterization: >50% stenosis	67	67	65	72	64	0.240
Catheterization: Gensini score	39 ± 59	41 ± 61	35 ± 53	47 ± 66	44 ± 61	0.001
LVEF	53 ± 13	55 ± 11	54 ± 13	51 ± 13	47 ± 18	<0.0001
eGFR (ml/min)	77 ± 47	78 ± 43	81 ± 50	69 ± 48	61 ± 47	<0.0001
History of DM	33	26	35	39	45	<0.0001
History of HTN	92	89	93	92	93	0.019
History of dyslipidemia	70	73	70	68	54	<0.0001
Ever smoked	59	56	60	61	67	0.002
AMI on presentation	12	7	15	17	2	<0.0001
History of previous MI	31	29	30	37	30	0.024
On statin	72	75	72	72	61	0.005
On ARB or ACE-I	62	59	66	60	60	0.248
On aspirin	81	81	81	84	72	0.424

Characteristics	Entire Cohort (n = 3,415)	No. of Positive Biomarkers				p Value
		0 (n = 1,248)	1 (n = 1,475)	2 (n = 565)	3 (n = 127)	
On clopidogrel	46	45	45	53	45	0.048
On beta-blocker	63	58	65	67	72	<0.0001
CRP (mg/l)	7.2 ± 13	1.3 ± 0.8	8 ± 12	15 ± 20	18 ± 24	<0.0001
HSP70 (ng/ml)	82 ± 513	0.0 ± 0.0	30 ± 140	224 ± 829	839 ± 1714	<0.0001
FDP (μg/ml)	3.7 ± 16	0.5 ± 0.2	2.4 ± 12	8.9 ± 25	26 ± 42	<0.0001
Management: medical	57.4	58.8	56.7	55.6	61.2	0.444
Management: revascularization	40.9	39.4	41.4	43.2	38.8	0.271
Management: other	1.7	1.8	1.9	1.3	0	0.203
Follow-up: MI	3.6	1.6	3.9	6.4	5.9	<0.0001
Follow-up: revascularization	10.8	7.2	11.7	15.4	13.4	<0.0001
Follow-up: all-cause death	8.3	3.1	6.4	18.4	34.6	<0.0001

Values are mean ± SD or %.

ACE-I = angiotensin-converting enzyme inhibitor; AMI = acute myocardial infarction; ARB = angiotensin-receptor blocker; BMI = body mass index; BP = blood pressure; CRP = C-reactive protein; DM = diabetes mellitus; eGFR = estimated glomerular filtration rate; HDL = high-density lipoprotein; HSP70 = heat shock protein 70; HTN = hypertension; LDL = low-density lipoprotein; LVEF = left ventricular ejection fraction.

J Am Coll Cardiol. Author manuscript; available in PMC 2014 July 23.

Table 2

Hazard Ratios for All-Cause Death, Combined Endpoints of Death and MI, and Combined Endpoints of Death, MI, and Revascularization According to Biomarker Levels

Variables	All Participants HR (95% CI); p Value	Significant CAD HR (95% CI); p Value	Nonsignificant CAD HR (95% CI); p Value
All-Cause Death			
All biomarkers in same model			
CRP ≥3.0 mg/l	1.80 (1.36–2.38); <0.0001	1.60 (1.13–2.27); 0.0087	2.36 (1.46–3.81); 0.0005
HSP70 at ≥0.625 ng/ml	2.02 (1.51–2.70); <0.0001	1.86 (1.28–2.70); 0.0012	2.29 (1.42–3.69); 0.0007
FDP ≥1.0 μg/ml	1.91 (1.46–2.49); <0.0001	1.60 (1.13–2.27); 0.0087	1.74 (1.11–2.71); 0.0152
Continuous biomarker risk score	1.87 (1.63–2.15); <0.0001	1.74 (1.45–2.08); <0.0001	2.07 (1.65–2.60); <0.0001
Categorical biomarker risk score			
1 vs. 0 markers	1.70 (1.16–2.50); 0.0065	1.76 (1.09–2.84); 0.0198	1.62 (0.84–3.13); 0.1520
2 vs. 0 markers	3.69 (2.52–5.41); <0.0001	3.26 (2.01–5.27); <0.0001	4.58 (2.39–8.80); <0.0001
3 vs. 0 markers	5.79 (3.63–9.22); <0.0001	4.89 (2.64–9.06); <0.0001	7.07 (3.33–15.03); <0.0001
Cardiac Death			
All biomarkers in same model			
CRP ≥3.0 mg/l	1.76 (1.19–2.60); 0.0049	1.59 (0.98–2.56); 0.0587	2.37 (1.17–4.81); 0.0164
HSP70 ≥0.625 ng/ml	1.69 (1.13–2.53); 0.0102	1.46 (0.88–2.41); 0.1436	2.17 (1.07–4.40); 0.0327
FDP ≥1.0 μg/ml	2.00 (1.38–2.89); 0.0003	2.06 (1.29–3.27); 0.0023	1.69 (0.89–3.21); 0.1072
Continuous biomarker risk score	1.79 (1.47–2.17); <0.0001	1.68 (1.32–2.14); <0.0001	1.97 (1.41–2.75); <0.0001
Categorical biomarker risk score			
1 vs. 0 markers	1.53 (0.90–2.58); 0.1155	1.54 (0.80–2.97); 0.1939	1.58 (0.64–3.90); 0.3255
2 vs. 0 markers	3.02 (1.77–5.15); <0.0001	2.94 (1.52–5.66); 0.0013	3.18 (1.24–8.17); 0.0163
3 vs. 0 markers	5.24 (2.79–9.86); <0.0001	4.26 (1.88–9.66); 0.0005	7.36 (2.56–21.17); 0.0002
All-Cause Death or MI			
All biomarkers in same model			
CRP ≥3.0 mg/l	1.61 (1.28–2.03); <0.0001	1.55 (1.17–2.06); 0.0023	1.93 (1.27–2.93); 0.0019
HSP70 ≥0.625 ng/ml	2.26 (1.77–2.90); <0.0001	2.32 (1.70–3.15); <0.0001	2.18 (1.42–3.33); 0.0003
FDP ≥1.0 μg/ml	1.62 (1.28–2.04); <0.0001	1.50 (1.12–2.00); 0.0067	1.77 (1.18–2.66); 0.0059
Continuous biomarker risk score	1.76 (1.56–1.98); <0.0001	1.70 (1.46–1.97); <0.0001	1.92 (1.57–2.36); <0.0001
Categorical biomarker risk score			
1 vs. 0 markers	1.83 (1.33–2.51); 0.0002	2.03 (1.38–2.99); 0.0003	1.50 (0.86–2.61); 0.15
2 vs. 0 markers	3.46 (2.51–4.78); <0.0001	3.31 (2.22–4.92); <0.0001	3.93 (2.25–6.87); <0.0001
3 vs. 0 markers	4.99 (3.31–7.53); <0.0001	4.80 (2.81–8.21); <0.0001	5.62 (2.86–11.06); <0.0001
All-Cause Death, MI, or Revascularization			
All biomarkers in same model			
CRP ≥3.0 mg/l	1.26 (1.06–1.49); 0.0084	1.13 (0.93–1.38); 0.22	1.86 (1.32–2.60); 0.0003
HSP70 ≥0.625 ng/ml	1.97 (1.64–2.36); <0.0001	2.01 (1.62–2.50); <0.0001	1.95 (1.37–2.78); 0.0002
FDP ≥1.0 μg/ml	1.47 (1.22–1.76); <0.0001	1.32 (1.05–1.65); 0.0172	1.76 (1.26–2.46); 0.0010
Continuous biomarker risk score	1.49 (1.36–1.63); <0.0001	1.39 (1.25–1.55); <0.0001	1.84 (1.55–2.18); <0.0001

Variables	All Participants HR (95% CI); p Value	Significant CAD HR (95% CI); p Value	Nonsignificant CAD HR (95% CI); p Value
Categorical biomarker risk score			
1 vs. 0 markers	1.59 (1.29–1.97); <0.0001	1.60 (1.26–2.04); 0.0001	1.69 (1.10–2.59); 0.0177
2 vs. 0 markers	2.44 (1.94–3.06); <0.0001	2.11 (1.62–2.75); <0.0001	3.68 (2.33–5.79); <0.0001
3 vs. 0 markers	3.05 (2.19–4.23); <0.0001	2.48 (1.63–3.77); <0.0001	5.41 (3.04–9.63); <0.0001

Values are hazard ratios (HRs) and 95% confidence intervals (CI).

Abbreviations as in Table 1.

Table 3

C-Statistic for Cox Regression Models Predicting Major Adverse Cardiovascular Events

Variables	All Participants C Statistic; p Value	Significant CAD C Statistic; p Value	Nonsignificant CAD C statistic; p Value
All-cause death			
Established risk factors	0.722; referent	0.734; referent	0.755; referent
Established risk factors + score	0.784; <0.0001	0.783; <0.0001	0.815; 0.001
All-cause death and MI			
Established risk factors	0.694; referent	0.703; referent	0.708; referent
Established risk factors + score	0.750; <0.0001	0.744; <0.0001	0.773; 0.001
All-cause death, MI, and Revascularization			
Established risk factors	0.677; referent	0.653; referent	0.691; referent
Established risk factors + score	0.707; <0.0001	0.671; 0.009	0.751; 0.0006

Abbreviations as in Table 1.

Table 4

NRI and IDI for Death and MI Using Biomarker Score

Variables	All Participants	Significant CAD	Nonsignificant CAD
Events correctly reclassified	13%	6%	16%
Nonevents correctly reclassified	29%	26%	29%
NRI	42%	33%	45%
IDI	0.03	0.03	0.03

IDI = integrated discrimination improvement; NRI = net reclassification improvement; other abbreviation as in Table 1.