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Usefulness of the addition of Beta-2-microglobulin, Cystatin C and C-reactive protein to an Established Risk Factors Model to Improve Mortality Risk Prediction in Patients Undergoing Coronary Angiography

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Abstract

Evidence-based therapies are available to reduce the risk of death from cardiovascular disease, yet many patients go untreated. Novel methods are needed to identify those at highest risk of cardiovascular death. Here, the biomarkers beta-2-microglobulin, cystatin C and C-reactive protein were measured at baseline in a cohort of participants undergoing coronary angiography. Adjusted Cox proportional-hazards models were used to determine whether the biomarkers predicted all-cause and cardiovascular mortality. Additionally, improvements in risk reclassification and discrimination were evaluated by calculating the net reclassification improvement (NRI), C-index and the integrated discrimination improvement (IDI) with the addition of the biomarkers to a baseline model of risk factors for cardiovascular disease and death. During a median follow-up period of 5.6 years, there were 78 deaths among 470 participants. All biomarkers independently predicted future all-cause and cardiovascular mortality. A significant improvement in risk reclassification was observed for all-cause (NRI, 35.8%; $P=0.004$) and cardiovascular (NRI, 61.9%; $P=0.008$) mortality compared to the baseline risk factors model. Additionally, we observed significantly increased risk discrimination with a C-index of 0.777 (change in C-index [ΔC], 0.057; 95% CI, 0.016–0.097) and 0.826 (ΔC , 0.071; 95% CI, 0.010–0.133) for all-cause and cardiovascular mortality respectively. Improvements in risk discrimination were further supported using the integrated discrimination improvement index. In conclusion, we provide evidence that beta-2-microglobulin, cystatin C and C-reactive protein predict mortality and improve risk reclassification and discrimination for a high-risk cohort undergoing coronary angiography.

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Keywords

Angiography; Cardiovascular diseases; Proteins; Mortality

Introduction

The development and refinement of risk stratification tools and prognostication models has and will continue to significantly impact the treatment and prevention of cardiovascular disease. To date, these efforts have largely aimed to reclassify intermediate-risk patients either upwards into a subset where an intervention becomes clearly indicated or downward into a subset where it is likely that they can safely abstain from treatment. However, it is becoming increasingly clear that individuals felt to be at high-risk similarly can be re-stratified and may particularly benefit from appropriately intensified therapy¹. Especially with more expensive or invasive cardiovascular therapies, it is important to develop new tools to identify those truly at highest risk and most suitable for intervention and/or more intensive risk factor modification. To this end, we have previously identified a set of biomarkers that are preferentially expressed in patients with peripheral arterial disease, a group of patients at particularly elevated risk of major clinical events such as myocardial infarction and stroke². In the current study, we evaluated whether these biomarkers improve risk modeling in a cohort of patients undergoing coronary angiography.

Methods

The Genetic Determinants of Peripheral Arterial Disease (GenePAD) study consists of individuals who underwent an elective, non-emergent coronary angiogram for angina, shortness of breath or an abnormal stress test at Stanford University or Mount Sinai Medical Centers between January 1, 2004 and March 1, 2008^{3,4}. As previously detailed⁵, a sub-cohort of individuals was selected from the total cohort (n=1755) to characterize the role of biomarkers in cardiovascular disease. There were 470 patients with data on all biomarkers and relevant covariates included in the study. All individuals provided written informed consent. The GenePAD study was approved by the Stanford University and Mount Sinai School of Medicine Committees for the Protection of Human Subjects.

The biomarkers of interest were beta-2-microglobulin, cystatin C and C-reactive protein. Blood samples were collected on fasting participants while the patient was being prepped for scheduled coronary angiography. The biomarkers were measured with standard nephelometry using BNII-Nephelometry system (Dade Behring Inc.). The intra-assay and inter-assay coefficients of variation were <4.1% and <3.3% for beta-2-microglobulin, <4.4% and <5.7% for cystatin C, and <2.83% and <5.1% for C-reactive protein respectively.

The outcomes of interest in this analysis were death from any cause and from cardiovascular causes. Cardiovascular deaths were attributed to myocardial infarction, cardiac arrest, stroke, heart failure or aneurysm rupture. Ascertainment of mortality was achieved through phone or postal communication, medical record review and the Social Security Death Index. New mortalities were identified through March 31, 2012.

At enrollment, participants provided information on all included covariates through a trained nurse or research assistant. Diabetes status was classified as use of insulin or oral hypoglycemic agents as ascertained by direct medication inventory. Total cholesterol and high-density lipoprotein (HDL) cholesterol were measured by standard assays using AU5400 Chemistry Immuno-Analyzer (Olympus Inc.). The glomerular filtration rate (GFR) was estimated using the Modification of Diet in Renal Disease method⁶. An experienced cardiologist who was blinded to participant details evaluated coronary angiograms.

Hemodynamically significant coronary artery disease (CAD) was defined as >60% stenosis^{7,8}.

Cumulative mortality for all-cause and cardiovascular mortality was calculated for each biomarker using the Kaplan-Meier method with the median level for each biomarker as the designated cut-off value between groups. Additionally, participants in the upper 50% for all three biomarkers were compared to those in the lower 50% for all three biomarkers.

Continuous variables with a right-skew (beta-2-microglobulin, cystatin C and C-reactive protein) were log-transformed to achieve a normal distribution. The association of biomarkers with death from all causes and death from cardiovascular causes was investigated using Cox proportional-hazards regression. Hazard ratios were expressed per 1-standard deviation change of the log biomarker level. Standard deviations were 6.4, 0.98 and 7.0 mg/L for beta-2-microglobulin, cystatin C and C-reactive protein respectively. Subgroup analysis was carried out for all-cause mortality according to CAD status. Due to limited numbers of cardiovascular mortalities (n=19) we elected not to undertake subgroup analysis on this outcome.

For all survival analyses the follow-up time was defined as the period between the enrollment interview and the last confirmed follow-up or date of death. If participants had a confirmed mortality of unknown cause they were excluded from the cardiovascular mortality analysis (n=48). Survival analyses were adjusted for age, sex, race, systolic blood pressure (SBP), body mass index (BMI), total cholesterol, HDL cholesterol, smoking history, use of lipid-lowering and anti-hypertensive medications, use of insulin or oral hypoglycemic agents and GFR. All variables were continuous except race (categorical), diabetes status, smoking, and use or nonuse of lipid-lowering and anti-hypertensive medications (dichotomous). Proportional-hazards assumptions were evaluated by Schoenfeld's residuals tests. Calibration was assessed on all models using the Gronnesby-Borgan test to evaluate goodness-of-fit ($P>0.05$) by comparing predicted mortalities with observed mortalities as described for survival analysis⁹.

The net reclassification improvement (NRI), C-index and integrated discrimination improvement (IDI) were evaluated to determine whether the biomarkers significantly improved risk reclassification and discrimination for all-cause and cardiovascular mortality when added to a baseline model. In this diverse population at high-risk for cardiovascular events, we used a baseline model consisting of risk factors for cardiovascular disease and death including age, sex, race, smoking history, BMI, SBP, use of lipid-lowering or anti-hypertensive medications, diabetes, total cholesterol, HDL cholesterol and GFR¹⁰⁻¹³. Additionally, secondary analyses were conducted using risk variables from the European SCORE risk model to evaluate model improvement against an established risk score¹². This model was established for cardiovascular mortality, and includes age, sex, smoking history, SBP and total cholesterol.

The NRI was used to evaluate the proportion of correct risk reclassification when adding biomarkers to the baseline model¹⁴. We utilized the category-free NRI as it has been suggested to be the most objective and reproducible measure of improvement in risk prediction especially when established *a priori* risk categories do not exist¹⁵. Furthermore, we calculated the NRI separately in participants with and without an event during follow-up.

The C-index was used to estimate improvements in model discrimination with the addition of the biomarkers. In survival analysis, the C-index interpretation is equivalent to the area under the ROC curve or c-statistic while allowing for censored data with a 1% increase indicating that the correct order of failure (e.g. mortality) would be correctly predicted in an

additional 1 in every 100 pairs of randomly selected individuals compared to the baseline model^{16,17}.

Model performance was further evaluated with the addition of the biomarkers using the IDI. The IDI compares two models according to the average difference in predicted risk between those who have the event and those who do not¹⁴. If the new model assigns a higher risk to those who will have a mortality and a lower risk to those who will not, as compared to the baseline model, the IDI will be > 0 . Therefore, the IDI can be interpreted as the average net improvement in the predicted risk of the outcome in the new model compared to the baseline model.

Tests were considered significant if the two-sided P-value was < 0.05 . All analyses were performed using Stata version 12.0 (StataCorp, College Station, Texas). Study data were collected and managed using REDCap electronic data capture tools hosted at Stanford University¹⁸.

Results

Enrollment characteristics of the 470 individuals constituting the study sample are presented in Table 1. During a median follow-up period of 5.6 years there were 78 mortalities (17%) of which 19 were known to be from cardiovascular causes.

We observed increased cumulative all-cause mortality (Figure 1) and cardiovascular mortality (Supplementary Figure 1) among individuals with levels of beta-2-microglobulin, cystatin C or C-reactive protein that were greater than the study median. This relationship was most pronounced when comparing participants with measurements above the median for all biomarkers as compared to below the median for all biomarkers.

The adjusted hazard ratios for the association of all biomarkers with mortality are shown in Table 2. Higher levels of the biomarkers beta-2-microglobulin, cystatin C and C-reactive protein were significantly associated with increased all-cause and cardiovascular mortality during follow-up. The observed associations did not significantly differ according to gender or race ($P = 0.05$). We also conducted analyses using fasting glucose as an alternative measure of diabetes status, which yielded statistically similar results (data not shown). Schoenfeld's residuals tests demonstrated that the proportional hazards assumption was met for all models. Regression coefficients for the all-cause mortality analysis can be found in Supplementary Table 1.

In subgroup analysis, beta-2-microglobulin, cystatin C and C-reactive protein were predictive of all-cause mortality among individuals with CAD diagnosed at enrollment. Beta-2-microglobulin and C-reactive protein continued to significantly predict mortality risk among individuals without CAD while cystatin C demonstrated a borderline significance in this subgroup.

Assessment of calibration using the Grønnesby-Borgan statistic demonstrated good fit for all models with and without biomarkers ($P = 0.05$).

The category-free NRI showed significant improvement in the net proportion of risk reclassification for all models with the addition of beta-2-microglobulin, cystatin C and C-reactive protein, individually and combined, compared to the baseline risk factors model for both all-cause and cardiovascular mortality (Table 3).

Results for the C-index and IDI analyses are presented in Table 4. The baseline cardiovascular risk factors model had a C-index of 0.720 (95% CI, 0.660–0.780) and 0.755

(95% CI, 0.650–0.860) for all-cause and cardiovascular mortality, respectively. As compared to the baseline model, beta-2-microglobulin and C-reactive protein demonstrated significantly improved model risk discrimination for all-cause mortality. None of the three biomarkers significantly improved cardiovascular mortality risk discrimination individually using the C-index. However, the addition of all three biomarkers showed the largest magnitude of increased C-index for all-cause and cardiovascular mortality respectively.

The IDI demonstrated a significant average net improvement in the predicted risk of all-cause mortality with the individual addition of beta-2-microglobulin, cystatin C and C-reactive protein (Table 4). Only C-reactive protein significantly improved the IDI for cardiovascular mortality with cystatin C showing a borderline significance. The models including all three biomarkers demonstrated the largest IDI for all-cause mortality and for cardiovascular mortality.

The results of the addition of biomarkers to the model consisting of SCORE risk variables are presented in Supplementary Tables 2 and 3. These analyses demonstrated statistically significant improvement for all measures of risk discrimination and reclassification for all-cause mortality using the NRI, C-index and IDI. For cardiovascular mortality, all biomarkers significantly improved risk reclassification per the NRI, individually and combined, over the SCORE risk variables model. Estimated IDI values were consistent with improved discrimination but did not reach statistical significance. However, compared to the baseline model of SCORE variables, all biomarkers significantly improved the C-index with the three biomarker model resulting in a C-index of 0.806 ($P=0.007$).

Additionally, we examined the NRI, C-index and IDI according to CAD status for all cause mortality compared to the baseline risk factors model (Supplementary Tables 4 and 5). The addition of all three biomarkers significantly improved risk reclassification and discrimination among individuals both with and without CAD at enrollment ($P<0.05$).

Discussion

The key finding of this study is that the measurement and incorporation of beta-2-microglobulin, cystatin C and C-reactive protein into baseline risk models of cardiovascular disease and death significantly improved risk reclassification and discrimination in a high-risk group of patients undergoing coronary angiography. We show that all three biomarkers predict all-cause and cardiovascular mortality risk even when adjusting for a wide range of potential confounding factors. Importantly, these biomarkers predicted risk in a multi-ethnic cohort of both genders among individuals both with and without angiographic evidence of coronary artery disease, suggesting broad applicability in patients being considered for catheterization.

Novel treatment approaches have had a dramatic impact on cardiovascular outcomes over the last 30 years, with an approximate 30% reduction in cardiovascular mortality today compared to one generation ago¹⁹. However, cardiovascular disease remains by far the leading killer in the United States suggesting that many at-risk patients remain unidentified and untreated²⁰. Clearly, novel methods to detect those at highest risk are desired.

Historical risk-prediction algorithms have largely focused on ‘traditional’ risk factors, incorporating the risk associated with comorbidities that have been related to cardiovascular disease through epidemiological association studies (e.g. smoking, hypertension, dyslipidemia, etc.)²¹. However, it is now known that these established risk factors account for only a fraction of one’s lifetime risk of developing cardiovascular disease, with the balance being accounted for by other genetic and/or environmental factors which remain unidentified or unmeasured²². To better prognosticate risk of future events, other

biochemical markers that reflect perturbations in disease-related pathways that are independent of classical risk factors will need to be identified.

To this end, we have previously identified circulating beta-2-microglobulin as a factor strongly linked to both the presence and severity of peripheral arterial disease⁵. We hypothesized that this major histocompatibility complex-associated polypeptide is shed from cells in response to hypoxia, given its noncovalent association with the cell membrane, explaining why it might be elevated in individuals with atherosclerotic disease. Since that report, beta-2-microglobulin has been associated with other vascular phenotypes²³, and has been associated with clinical outcomes in several lower risk cohorts^{24–26}. In this report, we now extend these observations by associating elevated beta-2-microglobulin, along with cystatin C and C-reactive protein, with reduced long-term survival due to both all-cause and cardiovascular mortality in a high-risk cohort. The use of these biomarkers is conceptually attractive in that it may reflect derangements in three different pathological pathways including ischemic perfusion injury (beta-2-microglobulin)²⁷, renal insufficiency (cystatin C)²⁸ and inflammation (C-reactive protein)²⁹.

Individuals referred for coronary angiography are among the highest risk patients encountered in cardiovascular medicine. We hypothesize that additional stratification of this high-risk cohort may lead to more effective and appropriate interventions while offering useful prognostic information to the individual patient. Finally, we believe that the fact that these biomarkers predict mortality risk regardless of whether or not significant CAD is identified during angiography is a very important point, as they may capture microvascular dysfunction which cannot be appreciated on an angiogram.

As we examined the biomarkers in a high-risk group, our findings are not generalizable to lower risk populations. Additionally, reliance on patient report to define the cause of death potentially introduces error into the cardiovascular mortality analysis and limited our ability to ascertain the cause of death in all cases. These findings will need to be reproduced in a confirmatory cohort and larger studies will be needed to determine the optimal biomarker model. Finally, randomized prospective trials examining improvements in outcomes will be necessary to determine whether the addition of biomarkers to clinical models should be used to guide clinical intervention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Ambrose JA, Srikanth S. Vulnerable plaques and patients: improving prediction of future coronary events. *Am J Med.* 2010; 123:10–16. [PubMed: 20102983]
2. Wilson AM, Kimura E, Harada RK, Nair N, Narasimhan B, Meng XY, Zhang F, Beck KR, Olin JW, Fung ET, Cooke JP. Beta2-microglobulin as a biomarker in peripheral arterial disease: proteomic profiling and clinical studies. *Circulation.* 2007; 116:1396–1403. [PubMed: 17724262]
3. Sadrzadeh Rafie AH, Stefanick ML, Sims ST, Phan T, Higgins M, Gabriel A, Assimes T, Narasimhan B, Nead KT, Myers J, Olin J, Cooke JP. Sex differences in the prevalence of peripheral artery disease in patients undergoing coronary catheterization. *Vasc Med.* 2010; 15:443–450. [PubMed: 21183651]

4. Wilson AM, Sadrzadeh-Rafie AH, Myers J, Assimes T, Nead KT, Higgins M, Gabriel A, Olin J, Cooke JP. Low lifetime recreational activity is a risk factor for peripheral arterial disease. *J Vasc Surg.* 2011; 54:427–432. 432 e421–424. [PubMed: 21664093]
5. Fung ET, Wilson AM, Zhang F, Harris N, Edwards KA, Olin JW, Cooke JP. A biomarker panel for peripheral arterial disease. *Vasc Med.* 2008; 13:217–224. [PubMed: 18687758]
6. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med.* 1999; 130:461–470. [PubMed: 10075613]
7. Tonino PA, Fearon WF, De Bruyne B, Oldroyd KG, Leeser MA, Ver Lee PN, Maccarthy PA, Van't Veer M, Pijls NH. Angiographic versus functional severity of coronary artery stenoses in the FAME study fractional flow reserve versus angiography in multivessel evaluation. *J Am Coll Cardiol.* 2010; 55:2816–2821. [PubMed: 20579537]
8. Atar D, Ramanujam PS, Saunamaki K, Haunso S. Assessment of coronary artery stenosis pressure gradient by quantitative coronary arteriography in patients with coronary artery disease. *Clin Physiol.* 1994; 14:23–35. [PubMed: 8149707]
9. McGeechan K, Macaskill P, Irwig L, Liew G, Wong TY. Assessing new biomarkers and predictive models for use in clinical practice: a clinician's guide. *Arch Intern Med.* 2008; 168:2304–2310. [PubMed: 19029492]
10. D'Agostino RB Sr, Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, Kannel WB. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation.* 2008; 117:743–753. [PubMed: 18212285]
11. Henry RM, Kostense PJ, Bos G, Dekker JM, Nijpels G, Heine RJ, Bouter LM, Stehouwer CD. Mild renal insufficiency is associated with increased cardiovascular mortality: The Hoorn Study. *Kidney Int.* 2002; 62:1402–1407. [PubMed: 12234312]
12. Conroy RM, Pyorala K, Fitzgerald AP, Sans S, Menotti A, De Backer G, De Bacquer D, Ducimetiere P, Jousilahti P, Keil U, Njolstad I, Oganov RG, Thomsen T, Tunstall-Pedoe H, Tverdal A, Wedel H, Whincup P, Wilhelmsen L, Graham IM. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. *Eur Heart J.* 2003; 24:987–1003. [PubMed: 12788299]
13. Stamler J, Vaccaro O, Neaton JD, Wentworth D. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care.* 1993; 16:434–444. [PubMed: 8432214]
14. 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; 27:157–172. discussion 207–112. [PubMed: 17569110]
15. Pencina MJ, D'Agostino RB Sr, Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. *Stat Med.* 2011; 30:11–21. [PubMed: 21204120]
16. Harrell FE Jr, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Stat Med.* 1996; 15:361–387. [PubMed: 8668867]
17. Pencina MJ, D'Agostino RB. Overall C as a measure of discrimination in survival analysis: model specific population value and confidence interval estimation. *Stat Med.* 2004; 23:2109–2123. [PubMed: 15211606]
18. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform.* 2009; 42:377–381. [PubMed: 18929686]
19. Capewell S, Morrison CE, McMurray JJ. Contribution of modern cardiovascular treatment and risk factor changes to the decline in coronary heart disease mortality in Scotland between 1975 and 1994. *Heart.* 1999; 81:380–386. [PubMed: 10092564]
20. Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Soliman EZ, Sorlie

PD, Sotoodehnia N, Turan TN, Virani SS, Wong ND, Woo D, Turner MB. Executive summary: heart disease and stroke statistics--2012 update: a report from the American Heart Association. *Circulation*. 2012; 125:188–197. [PubMed: 22215894]

21. Dent TH. Predicting the risk of coronary heart disease I. The use of conventional risk markers. *Atherosclerosis*. 2010; 213:345–351. [PubMed: 20637467]
22. Leeper NJ, Kullo IJ, Cooke JP. Genetics of peripheral artery disease. *Circulation*. 2012; 125:3220–3228. [PubMed: 22733336]
23. Kals J, Zagura M, Serg M, Kampus P, Zilmer K, Unt E, Lieberg J, Eha J, Peetsalu A, Zilmer M. beta2-microglobulin, a novel biomarker of peripheral arterial disease, independently predicts aortic stiffness in these patients. *Scand J Clin Lab Invest*. 2011; 71:257–263. [PubMed: 21314441]
24. Amighi J, Hoke M, Mlekusch W, Schlager O, Exner M, Haumer M, Pernicka E, Koppensteiner R, Minar E, Rumpold H, Schillinger M, Wagner O. Beta 2 microglobulin and the risk for cardiovascular events in patients with asymptomatic carotid atherosclerosis. *Stroke*. 2011; 42:1826–1833. [PubMed: 21546482]
25. Shinkai S, Chaves PH, Fujiwara Y, Watanabe S, Shibata H, Yoshida H, Suzuki T. Beta2-microglobulin for risk stratification of total mortality in the elderly population: comparison with cystatin C and C-reactive protein. *Arch Intern Med*. 2008; 168:200–206. [PubMed: 18227369]
26. Astor BC, Shafi T, Hoogeveen RC, Matsushita K, Ballantyne CM, Inker LA, Coresh J. Novel markers of kidney function as predictors of ESRD, cardiovascular disease, and mortality in the general population. *Am J Kidney Dis*. 2012; 59:653–662. [PubMed: 22305758]
27. Kalawski R, Majewski M, Kaszkowiak E, Wysocki H, Siminiak T. Transcardiac release of soluble adhesion molecules during coronary artery bypass grafting: effects of crystalloid and blood cardioplegia. *Chest*. 2003; 123:1355–1360. [PubMed: 12740247]
28. Coll E, Botey A, Alvarez L, Poch E, Quinto L, Saurina A, Vera M, Piera C, Darnell A. Serum cystatin C as a new marker for noninvasive estimation of glomerular filtration rate and as a marker for early renal impairment. *Am J Kidney Dis*. 2000; 36:29–34. [PubMed: 10873868]
29. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO 3rd, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL, Rifai N, Smith SC Jr, Taubert K, Tracy RP, Vinicor F. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation*. 2003; 107:499–511. [PubMed: 12551878]

Figure 1.

Frames A-C represent cumulative mortality in the upper 50% of biomarker levels (red) as compared to the bottom 50% of biomarker levels (blue) for Beta-2-microglobulin (median, 1.88 mg/L) Cystatin C, (median, 0.72 mg/L) and C-reactive protein (median, 1.60 mg/L). Frame D represents those individuals in the upper 50% of all three biomarkers (red) as compared to those individuals in the bottom 50% of all three biomarkers (blue).

Table 1

Baseline study population characteristics (n=470)

Characteristic	Value
Age, mean (years)	67 ± 10
Female	226 (48%)
Caucasian	253 (54%)
Black	77 (16%)
Hispanic	58 (12%)
Asian	33 (7%)
Other *	49 (10%)
Systolic blood pressure, mean (mm Hg)	141 ± 22
Body mass index, mean (kg/m ²)	29 ± 6
Lipids, mean (mg/dl)	
Total cholesterol	145 ± 38
High-density lipoprotein cholesterol	42 ± 13
Ever smoker	267 (57%)
Use of cholesterol lowering medication	301 (64%)
Use of antihypertensive medication	391 (83%)
Use of insulin or oral hypoglycemics	146 (31%)
Glomerular filtration rate, mean (mL/min/1.73m ²)	79 ± 37
Biomarker levels, median (mg/L) (IQR)	
Beta-2-microglobulin	1.88 (1.50–2.57)
Cystatin C	0.72 (0.61–0.93)
C-reactive protein	1.60 (0.60–4.30)
Coronary artery disease †	219 (47%)

* Includes Asian-Indian, Pakistani, Middle Eastern and Pacific Islander

† Defined as >60% stenosis on coronary angiography

All mean values are presented ± the standard deviation

IQR, interquartile range; No., number

Table 2

Adjusted hazard ratios per standard deviation increase in log biomarker level

All-cause mortality		HR	95%CI		P-value
			Lower	Upper	
Beta-2-microglobulin	Overall	1.80	1.38	2.34	<0.001
	CAD only	1.75	1.20	2.56	0.004
	Non CAD	1.96	1.24	3.10	0.004
Cystatin C	Overall	1.74	1.31	2.29	<0.001
	CAD only	1.79	1.20	2.65	0.004
	Non CAD	1.61	0.98	2.63	0.060
C-reactive protein	Overall	1.70	1.37	2.10	<0.001
	CAD only	1.67	1.28	2.17	<0.001
	Non CAD	1.66	1.04	2.66	0.035
Cardiovascular mortality					
		HR	95%CI		P-value
			Lower	Upper	
Beta-2-microglobulin	Overall	2.25	1.34	3.77	0.002
	Overall	2.35	1.40	3.93	0.001
C-reactive protein	Overall	1.96	1.24	3.09	0.004

Data were adjusted for age, sex, race, smoking history, body mass index, systolic blood pressure, use of lipid-lowering or anti-hypertensive medications, diabetes, total cholesterol, high-density lipoprotein cholesterol and glomerular filtration rate

CAD, coronary artery disease; CI, confidence interval; HR, hazard ratio; SD, standard deviation

Table 3

Category-free net reclassification improvement over baseline risk factors

All-cause mortality

Model	Overall		NRI Mortalities	NRI Non-mortalities
	NRI	P-value		
Baseline risk factors (BRF) *	ref	1.0 (ref)	ref	ref
BRF + Beta-2-microglobulin	25.0%	0.044	0.0%	25.0%
BRF + Cystatin C	27.0%	0.029	0.0%	27.0%
BRF + C-reactive protein	45.0%	<0.001	23.1%	21.9%
BRF + all biomarkers	35.8%	0.004	10.3%	25.5%

Cardiovascular mortality

Model	Overall		NRI Mortalities	NRI Non-mortalities
	NRI	P-value		
Baseline risk factors (BRF)	ref	1.0 (ref)	ref	ref
BRF + Beta-2-microglobulin	54.9%	0.019	26.3%	28.5%
BRF + Cystatin C	72.9%	0.002	47.4%	25.6%
BRF + C-reactive protein	66.0%	0.005	47.4%	18.6%
BRF + all biomarkers	61.9%	0.008	36.8%	25.1%

* Age, gender, race, smoking history, body mass index, systolic blood pressure, use of lipid-lowering medication, use of anti-hypertensive medication, diabetes status, total cholesterol, high-density lipoprotein cholesterol and glomerular filtration rate

NRI, net reclassification improvement; ref, reference

Table 4

C-index and integrated discrimination improvement over baseline risk factors

All-cause mortality					
Model	C-Index			IDI	
	C [†]	ΔC (95% CI)	P-value	IDI (95% CI)	P-value
Baseline risk factors (BRF) *	0.720	ref	ref (1.0)	ref	ref (1.0)
BRF + Beta-2-microglobulin	0.756	0.036 (0.007–0.065)	0.016	1.9% (0.3–3.5%)	0.017
BRF + Cystatin C	0.745	0.025 (–0.001–0.050)	0.061	1.6% (0.3–2.8%)	0.018
BRF + C-reactive protein	0.756	0.036 (0.001–0.072)	0.046	4.0% (1.9–6.1%)	<0.001
BRF + all biomarkers	0.777	0.057 (0.016–0.097)	0.006	5.1% (2.6–7.6%)	<0.001
Cardiovascular mortality					
Model	C-Index			IDI	
	C [†]	ΔC (95% CI)	P-value	IDI (95% CI)	P-value
Baseline risk factors (BRF)	0.755	ref	ref (1.0)	ref	ref (1.0)
BRF + Beta-2-microglobulin	0.813	0.058 (–0.003–0.118)	0.062	2.1% (–0.2–4.4%)	0.077
BRF + Cystatin C	0.814	0.059 (–0.001–0.118)	0.055	2.9% (–0.1–5.9%)	0.056
BRF + C-reactive protein	0.796	0.041 (–0.017–0.099)	0.166	1.8% (0.1–3.5%)	0.042
BRF + all biomarkers	0.826	0.071 (0.010–0.133)	0.023	3.8% (–0.1–7.8%)	0.058

* Age, gender, race, smoking history, body mass index, systolic blood pressure, use of lipid-lowering medication, use of anti-hypertensive medication, diabetes status, total cholesterol, high-density lipoprotein cholesterol and glomerular filtration rate

[†]The C-index for all models was significantly greater than the null hypothesis of 0.5 at P < 0.001

C, C-index; CI, confidence interval; ΔC, change in C-index from the reference model; IDI, integrated discrimination improvement; ref, reference