Published in final edited form as:

Am J Cardiol. 2009 July 15; 104(2): 247–253. doi:10.1016/j.amjcard.2009.03.026.

Prognostic Value of Multiple Biomarkers in American Indians Free of Clinically Overt Cardiovascular Disease (From the Strong Heart Study)

Jorge R. Kizer, MD, MSc^{a,b}, Daniel G. Krauser, MD^a, Richard J. Rodeheffer, MD^c, John C. Burnett Jr., MD^d, Peter M. Okin, MD^a, Mary J. Roman, MD^a, Jason G. Umans, MD, PhD^e, Lyle G. Best, MD^f, Elisa T. Lee, PhD^g, and Richard B. Devereux, MD^a

^aDepartment of Medicine, Weill Cornell Medical Center, New York, NY.

^bDepartment of Public Health, Weill Cornell Medical Center, New York, NY.

^cDivision of Cardiovascular Diseases, Mayo Clinic and Foundation, Rochester, MN.

^dDepartments of Physiology and Internal Medicine, Mayo Clinic and Foundation, Rochester, MN.

^eMedStar Research Institute, Washington, DC.

^fMissouri Breaks Industries Research Inc., Timber Lake, SD.

^gCollege of Public Health, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Abstract

Several biomarkers have been documented, singly or jointly, to improve risk prediction, but the extent to which they improve prediction-model performance in populations with high prevalences of obesity and diabetes has not been specifically examined. We sought to evaluate the ability of various biomarkers to improve prediction-model performance for death and major cardiovascular (CVD) events in a high-risk population. The relations of 6 biomarkers with outcome were examined in 823 American Indians free of prevalent CVD or renal insufficiency, as were their contributions to risk prediction. In single-marker models adjusting for standard clinical and laboratory risk factors, 4 of 6 biomarkers significantly predicted mortality and major CVD events. In multi-marker models, these 4 biomarkers – urinary albumin/creatinine ratio (UACR), hemoglobin A1c (HbA1c), B-type natriuretic peptide (BNP), and fibrinogen – significantly predicted mortality, while 2 – UACR and fibrinogen – significantly predicted CVD. Based on its robust association in diabetic participants, UACR was the strongest predictor of mortality and CVD, individually improving model discrimination or classification in the entire cohort. Singly, all remaining biomarkers also improved risk classification for mortality, and enhanced average sensitivity for both mortality and CVD. Addition of one or more biomarkers to the single-marker UACR further improved discrimination or average sensitivity for these outcomes. In conclusion, biomarkers derived from diabetic cohorts, and novel biomarkers evaluated primarily in lower-risk populations, improve risk prediction in cohorts with prevalent obesity and diabetes. Risk-stratification of these populations with multi-marker models could enhance selection for aggressive medical or surgical approaches to prevention.

© 2009 Excerpta Medica, Inc. Published by Elsevier Inc. All rights reserved.

Address for Correspondence: Jorge R. Kizer, MD, MSc, Weill Cornell Medical Center, Box 222, 525 East 68th Street, New York, NY 10065. Tel: (212) 746-2642. Fax: (212) 746-8561. E-mail: jok2007@med.cornell.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Biomarkers; Cardiovascular Disease; Diabetes Mellitus; Obesity

Although type 2 diabetes mellitus has long been considered a coronary heart disease risk equivalent,¹ persons with this disorder manifest wide variation in risk of cardiovascular disease (CVD), which in large measure depends on the burden of associated risk factors.^{2,3} This has led to development of risk-prediction instruments to guide the intensity of primary prevention approaches,⁴ but these have primarily relied on traditional atherosclerosis risk factors, achieving only moderately good performance.⁵ In this context, a number of biochemical markers have been shown to independently predict CVD and mortality in a variety of settings.^{6–13} Some of these biomarkers, and notably natriuretic peptides among them, have even been reported, singly^{14,15} or in combination,¹⁶ to afford better risk-stratification than traditional risk factors. Among the more time-tested biomarkers, UACR and HbA1c have been widely evaluated in cohorts with type 2 diabetes mellitus, but much of the data supporting novel biomarkers come from white populations with low prevalences of obesity and type 2 diabetes mellitus. Moreover, the extent to which natriuretic peptides and other novel biomarkers improve risk prediction when considered jointly in populations with higher prevalences of obesity and diabetes, as are occurring in all industrialized societies, has been rarely examined. We sought to evaluate the individual and combined prognostic utility of several such biomarkers in the Strong Heart Study (SHS), where obesity and diabetes mellitus impose a particularly high burden.

Research Design and Methods

SHS is a population-based study of CVD and associated risk factors in 13 American-Indian communities in Arizona, Oklahoma, and North and South Dakota. Information about the population, methods, and enrollment procedures for the study has been reported previously.¹⁷ The current study focused on a subset of 1028 North and South Dakota participants in the 2nd SHS exam in whom serum BNP was measured. Participants underwent clinical and laboratory evaluation between 1993 and 1995, as previously described.¹⁷ Participants with clinically overt CVD (coronary heart disease, heart failure, stroke, or atrial fibrillation) or renal insufficiency (serum creatinine ≥ 2.0 mg/dL) at baseline were excluded, leaving 823 eligible individuals. Follow-up was 99% complete through 2005.

We selected 6 biomarkers reflecting different, if sometimes overlapping, pathogenetic pathways for major atherothrombosis-related events and mortality: HbA1c, a measure of glycemic dysregulation and advanced glycation end-product formation;⁹ UACR, a marker of glomerular, as well as generalized, endothelial damage;¹³ CRP and fibrinogen, molecules involved in inflammation, thrombosis, or both;^{6,7} PAI-1, a marker of excess adiposity that directly impairs fibrinolysis;⁸ and BNP, a hormone secreted by ventricular myocardium in response to increased wall stress.¹⁰ Our aim was to determine if these biomarkers would improve prognostication over clinical and laboratory variables obtained routinely in clinical practice.

Hypertension was defined as blood pressure $\geq 140/90$ mm Hg or anti-hypertensive therapy. *Diabetes mellitus* was defined as fasting glucose ≥ 126 mg/dl or glucose-lowering treatment. *Waist-hip ratio* was computed by dividing waist by hip circumference, and *body mass index* as the ratio of weight in kilograms to the square of height in meters.

The 2 endpoints considered were all-cause mortality and major CVD events, the latter comprising non-fatal myocardial infarction or stroke and CVD death.¹⁷ Deaths were classified

as attributable to CVD if caused by myocardial infarction, sudden cardiac death, stroke, or heart failure as determined by standardized review blinded to biomarker measurements.¹⁷

BNP was measured in 2004 by high-sensitivity non-competitive immunoradiometric assay (Shionogi Co. Ltd., Tokyo, Japan) on plasma (EDTA) samples maintained at -80°C since collection. The intra-assay and inter-assay coefficients of variation were both 8%. CRP, PAI-1 and fibrinogen concentrations were determined by ELISA^{18,19} or modification of the method of Clauss,²⁰ respectively, as reported previously. HbA1c was assessed by high-pressure liquid chromatography.²¹ Albuminuria was measured on a single spot urine sample and was expressed in relation to urinary creatinine (mg/g).¹⁷

Categorical variables were compared by the chi-square test, and continuous variables by the Wilcoxon rank-sum test. All biomarkers underwent logarithmic transformation to achieve normality. Adjusted relations between log-transformed biomarkers, standardized per unit change in standard deviation, and time-to-event were assessed using Cox models. Individual biomarkers were entered in multivariable models that included age, sex, waist-hip ratio, hypertension, diabetes, total cholesterol/HDL ratio, smoking status, and serum creatinine. Biomarkers significantly associated with outcome in these models were then selected for inclusion in multi-marker models. Assessment for multiplicative interaction involved inclusion of corresponding cross-product terms. For all covariates other than BNP, values were missing in <2.2% of the cohort; participants with missing values were excluded from relevant analyses.

Predictive accuracy was determined by assessing discrimination and reclassification. The c-statistic was calculated as a measure of discrimination. This measure is equivalent to the area under the receiver-operating-characteristic curve, a plot of the true-positive rate (sensitivity) against the false-positive rate (1 – specificity). The c-statistic gives the proportion of all subject pairs composed of one who develops the outcome and one who does not for which the model assigns a higher risk to the former than the latter.

Reclassification refers to the model's ability to provide a revised predicted probability of the outcome that moves individuals across pre-specified risk categories adopted to guide the intensity of preventive therapies. Improvement in classification was evaluated formally by calculating net reclassification improvement (NRI)²² based on previously defined 10-year risk categories.¹ The NRI gives the proportion of persons correctly reassigned to higher or lower-risk categories depending on whether they do or do not develop the outcome. Model performance was also evaluated by computing integrated discrimination improvement (IDI),²² an index that does not depend on the choice of categories, and indicates the extent to which the new model improves average sensitivity without compromising average specificity.

All analyses were conducted with SPSS version 12.0 (SPSS Inc., Chicago, IL) or Stata version 10.0 (StataCorp, College Station, TX).

Results

Baseline characteristics are shown in Table 1. In the entire sample, over four in five were overweight or obese, more than half had the metabolic syndrome, and over a third had diabetes. Compared with non-diabetic participants, those with diabetes were more likely female, and to have hypertension, overweight/obesity, and hyperlipidemia, but were less frequently smokers. Plasma BNP did not differ between the two groups, but values of all other biomarkers were significantly higher in diabetic subjects.

During mean follow-up of 9.9 ± 2.8 years, 222 participants died (61 from ascertainable CVD causes), and 159 suffered non-fatal myocardial infarction ($n=83$) or stroke ($n=33$), or CVD death. Of these, 99 deaths and 75 CVD events occurred in diabetics, while 123 deaths and 84

CVD events took place in non-diabetics. Table 2 shows adjusted relationships between individual biomarkers and outcomes. For mortality, significant associations were present for BNP and fibrinogen, but not for CRP (marginal) or PAI-1, after adjustment for standard clinical and laboratory risk factors (basic model). In the case of UACR and HbA1c, but not the other 4 biomarkers, a significant interaction by diabetes status was uncovered, wherein each was significantly predictive of mortality among diabetics, but not non-diabetics (Table 2). When considered jointly in addition to basic-model covariates, all 4 biomarkers – BNP, fibrinogen, HbA1c and UACR – retained significant multivariable associations with mortality.

For major CVD events, BNP, fibrinogen, HbA1c, and UACR were again significantly associated with outcome after adjustment for standard covariates (Table 2), but no multiplicative interaction was detected between HbA1c and diabetes ($p=0.963$). UACR again exhibited a significant interaction with diabetes, such that this biomarker was independently predictive of CVD only among diabetics (Table 2). When all 4 biomarkers were included in the multivariable model, only fibrinogen and UACR retained significant associations with major CVD.

Table 3 presents the discriminative ability of biomarker models as compared with the basic model. Addition of HbA1c or UACR individually, but not BNP or fibrinogen, resulted in significant improvement in the c-statistic for all-cause mortality. UACR yielded the highest c-statistic of single biomarker models. Inclusion of all 4 biomarkers further improved the c-statistic (Table 3), which was nearly significantly different as compared to the single-marker UACR model. A more parsimonious model containing BNP, HbA1c and UACR, however, afforded virtually identical improvement as the complete model.

For major CVD, UACR led to the highest c-statistic among the single-marker models, but the improvement over the basic model fell short of statistical significance (Table 3). Addition of fibrinogen further increased the c-statistic, however, resulting in a multi-marker model with significantly better discrimination than the basic model.

Formal assessment of the proportion of participants reclassified correctly into higher or lower risk categories, or NRI, showed that all 4 significant single-marker models achieved more accurate classification for mortality than the basic model. The NRI was highest for UACR (7.8%, $p=0.004$), followed by fibrinogen (5.8%, $p=0.006$), HbA1c (5.3%, $p=0.024$), BNP (5.0%, $p=0.032$). The multi-marker model achieved superior reclassification (12.9%, $p<0.001$) relative to the basic model, details of which are provided in Table 4. The latter shows that 14 participants who died were correctly moved to a higher predicted-risk category by the multi-marker model, while 16 such participants were incorrectly moved to a lower-risk category. In turn, 105 subjects alive at follow-up's end were correctly assigned to a lower-risk category by the multi-marker model, as opposed to 27 such subjects incorrectly assigned a higher-risk level. This netted a substantial increase in participants (approximately 1 in 10) correctly reclassified by the multi-marker model, in this case to a lower-risk category. Moreover, the full multi-marker model, unlike more limited models with combinations of 2 or 3 biomarkers, was the only one to achieve significant improvement in reclassification versus the single-marker UACR model (6.2%, $p=0.040$). By contrast, neither the single-marker models nor the multi-marker model achieved significant NRIs for major CVD (all $p\geq 0.140$).

Turning to IDI, all 4 single-marker models for mortality led to significant increases in this index. The highest IDI was again seen for UACR (5.7%, $p<0.001$), followed by HbA1c (3.0%, $p<0.001$), fibrinogen (1.3%, $p=0.012$), and BNP (1.1%, $p=0.009$). Addition of HbA1c to the UACR single-marker model significantly increased IDI by another 2.3% ($p<0.001$). Individual addition of BNP or fibrinogen in the UACR-HbA1c dual-marker model did not significantly enhance IDI, but joint inclusion did (1.1%, $p=0.015$). Similarly, models assessing individual

biomarkers with major CVD as the endpoint yielded significantly increased IDIs: UACR (4.1%, $p<0.001$), fibrinogen (1.4%, $p=0.008$), HbA1c (0.9%, $p=0.044$), and BNP (0.8%, $p=0.031$). Although IDI was greater for the combined than the basic model (4.7%, $p<0.001$), this did not achieve significance compared to the single-marker UACR model (0.6%, $p=0.076$).

Discussion

In this population free of clinically overt CVD, but with high prevalences of obesity and diabetes mellitus, various established and novel biomarkers were confirmed as independent predictors of all-cause mortality and major CVD events. More important, these biomarkers, either singly or jointly, were shown to significantly improve risk prediction for these outcomes.

The foremost predictor of both mortality and major CVD was UACR. On the strength of its association with outcome in diabetic participants, UACR was the biomarker whose addition to risk-prediction equations for the entire cohort most improved their performance. HbA1c in turn emerged as the second strongest multivariable predictor of mortality, though not of major CVD, for the overall cohort, again based on its independent relationship in diabetics. Both UACR and HbA1c are well-established independent risk factors in populations with diabetes, but they have also been documented to predict adverse events in cohorts without diabetes.^{9, 13} The present study demonstrates the primacy of UACR, and to a lesser extent HbA1c, in risk-prediction for a cohort where diabetes is prevalent, even when these biomarkers could not be individually confirmed to be independent predictors of outcome in the non-diabetic subset. Importantly, the current findings extend previous observations regarding these 2 biomarkers by showing, principally for UACR, that their predictive utility is independent of novel neurohormonal or thrombo-inflammatory molecules with strong proven relations to outcome in other settings.

Among the novel biomarkers, both fibrinogen and BNP emerged as significant independent predictors of mortality and major CVD in single-marker models. These significant relationships also held in multi-marker models for mortality, but only fibrinogen retained significance in multi-marker prediction of CVD. Although neither fibrinogen nor BNP significantly improved the c-statistic for mortality over the basic model, both did significantly enhance classification and discrimination, as gauged by NRI and IDI. Moreover, addition of BNP to the UACR-HbA1c dual-marker model for mortality resulted in a nearly significant increase in the model's c-statistic. This was not the case for fibrinogen, but addition of both BNP and fibrinogen did achieve an incrementally greater IDI than the UACR-HbA1c model. Thus, all 4 biomarkers contributed significantly, if increasingly marginally, to the multi-marker model's enhanced IDI, corresponding to a net improvement of 9.1% in average sensitivity without an accompanying loss in average specificity. For major CVD, addition of fibrinogen to the UACR single-marker model led to significant improvement in the c-statistic over the basic model, which neither biomarker alone could quite demonstrate. Here, however, the contribution of fibrinogen over UACR to the multi-marker model's IDI of 4.7% failed to reach statistical significance.

The superior diagnostic performance of the multi-marker model for mortality than major CVD (c-statistic=0.730 vs. 0.701) may be partly explained by less misclassification of death than non-fatal CVD, and is consistent with findings in other studies.²³ Yet the proportion of deaths attributed to CVD causes was relatively modest given that a majority of deaths in patients with diabetes or other atherosclerosis risk factors are CVD-related.²⁴ This is attributable to exclusion of prevalent CVD from our study sample, but may also reflect limitations of cause-of-death ascertainment at a population level. Still, the independent relationship of HbA1c observed in the multi-marker model for mortality but not CVD may predominantly relate to this biomarker's ability to predict not only macrovascular, but also, principally, microvascular

complications.²⁵ In the case of BNP, the relationship with mortality likely reflects an increased burden of subclinical heart failure, and is all the more notable in this population because obesity lowers BNP level.¹⁰

The increased predictive accuracy of multi-marker models demonstrated here may partly owe to a relatively modest predictive ability of the basic model as compared to some,²³ but not other,¹⁶ leaner populations. This highlights potential shortcomings of traditional risk factors for risk prediction in populations with prevalent obesity and diabetes mellitus, and suggests that multi-marker models could be useful in improving selection of such individuals for intensification of therapy. To be sure, the high prevalence of atherosclerosis risk factors in this cohort, and particularly diabetes mellitus, would often already designate them as eligible for primary preventive therapies. But even among diabetics without overt CVD, appropriateness of aggressive interventions such as bariatric surgery remains unclear, especially when body mass index is between 30 and 35 kg/m², as does the target for LDL lowering.²⁴

Using previously defined 10-year coronary risk categories to assess mortality in this high-risk cohort, the multi-marker model enhanced overall classification, which was driven by downgrading to lower risk strata. Based on the risk thresholds selected, should cost-benefit considerations determine, for example, that a <20% 10-year risk of death would make a highly aggressive intervention such as bariatric surgery unwarranted, the multi-marker model would correctly down-classify a net 66 individuals at the expense of incorrectly up-classifying a net 13 subjects (Table 4). Although the benefit would lie in withholding low-yield or potentially inappropriate use of an intervention, reclassification performance depends on the specific risk thresholds chosen. Indeed, the use of risk strata defined for low-risk populations likely explains the slightly worse up-classification observed for mortality, and the lack of significant NRI for CVD events. For a major surgical intervention, however, thresholds above 20% might be appropriate. Defining such thresholds would require further investigation of benefits and cost-effectiveness of the procedure.

It is notable that despite supportive data from previous studies, PAI-1 did not exhibit significant relations with mortality or major CVD. Relations of CRP to these outcomes were marginally non-significant, and were not meaningfully influenced by exclusion of the 16% of participants with CRP>10 mg/L. These results are consistent with prior findings from SHS with respect to incident diabetes²⁶ or CVD,¹⁸ and signals differences in the prognostic properties of biomarkers when excess adiposity and insulin resistance are widespread.

Several limitations warrant consideration. First, availability of BNP measurements was limited to a subset of the SHS cohort, leaving a study sample of moderate size. Although this prevented detailed examination of biomarker relations in subgroups defined by diabetes status or for individual CVD endpoints, the high rates of events and long follow-up provided a substantial number of total events for evaluation of overall risk prediction. The biomarkers studied here, however, together with other candidate molecules, will require assessment in larger cohorts where differences in diabetes-defined subgroups can be more adequately examined and diabetes-specific models developed. Such larger samples will also be necessary for appropriate determination of optimal clinical cutpoints involving the biomarkers in question. Second, findings in this population of American Indians may or may not be applicable to other ethnic groups. Nevertheless, prior reports from SHS have yielded risk factor associations that have been consistent with other populations.

Acknowledgements

The authors thank the Strong Heart Study participants, staff, and coordinators. The views expressed in this article are those of the authors and do not necessarily reflect those of the Indian Health Service.

This study was supported by grants U01-HL41642, U01-HL41652, U01-HL41654, U01-HL65521 and by awards K23-HL070854 (to Dr. Kizer) and R01-HL55502 (to Dr. Rodeheffer) from the National Heart, Lung, and Blood Institute; and by grant M10RR0047 (GCRC) from the National Institutes of Health.

References

1. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 2001;285:2486–2497. [PubMed: 11368702]
2. Howard BV, Best LG, Galloway JM, Howard WJ, Jones K, Lee ET, Ratner RE, Resnick HE, Devereux RB. Coronary heart disease risk equivalence in diabetes depends on concomitant risk factors. *Diabetes Care* 2006;29:391–397. [PubMed: 16443893]
3. Folsom AR, Chambless LE, Duncan BB, Gilbert AC, Pankow JS. Prediction of coronary heart disease in middle-aged adults with diabetes. *Diabetes Care* 2003;26:2777–2784. [PubMed: 14514579]
4. Stevens RJ, Kothari V, Adler AI, Stratton IM. The UKPDS risk engine: a model for the risk of coronary heart disease in Type II diabetes (UKPDS 56). *Clin Sci (Lond)* 2001;101:671–679. [PubMed: 11724655]
5. Yang X, So WY, Kong AP, Ma RC, Ko GT, Ho CS, Lam CW, Cockram CS, Chan JC, Tong PC. Development and validation of a total coronary heart disease risk score in type 2 diabetes mellitus. *Am J Cardiol* 2008;101:596–601. [PubMed: 18308005]
6. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 2000;342:836–843. [PubMed: 10733371]
7. Danesh J, Lewington S, Thompson SG, Lowe GD, Collins R, Kostis JB, Wilson AC, Folsom AR, Wu K, Benderly M, Goldbourt U, Willett J, Kiechl S, Yarnell JW, Sweetnam PM, Elwood PC, Cushman M, Psaty BM, Tracy RP, Tybjaerg-Hansen A, Haverkate F, de Maat MP, Fowkes FG, Lee AJ, Smith FB, Salomaa V, Harald K, Rasi R, Vahtera E, Jousilahti P, Pekkanen J, D'Agostino R, Kannel WB, Wilson PW, Tofler G, Arocha-Pinango CL, Rodriguez-Larralde A, Nagy E, Mijares M, Espinosa R, Rodriguez-Roa E, Ryder E, Diez-Ewald MP, Campos G, Fernandez V, Torres E, Marchioli R, Valagussa F, Rosengren A, Wilhelmsen L, Lappas G, Eriksson H, Cremer P, Nagel D, Curb JD, Rodriguez B, Yano K, Salonen JT, Nyyssönen K, Tuomainen TP, Hedblad B, Lind P, Loewel H, Koenig W, Meade TW, Cooper JA, De Stavola B, Knottenbelt C, Miller GJ, Bauer KA, Rosenberg RD, Sato S, Kitamura A, Naito Y, Palosuo T, Ducimetiere P, Amouyel P, Arveiler D, Evans AE, Ferrieres J, Juhan-Vague I, Bingham A, Schulte H, Assmann G, Cantin B, Lamarche B, Despres JP, Dagenais GR, Tunstall-Pedoe H, Woodward M, Ben-Shlomo Y, Davey Smith G, Palmieri V, Yeh JL, Rudnicka A, Ridker P, Rodeghiero F, Tosto A, Shepherd J, Ford I. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. *JAMA* 2005;294:1799–1809. [PubMed: 16219884]
8. Thogersen AM, Jansson JH, Boman K, Nilsson TK, Weinehall L, Huhtasaari F, Hallmans G. High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardial infarction in both men and women: evidence for the fibrinolytic system as an independent primary risk factor. *Circulation* 1998;98:2241–2247. [PubMed: 9826309]
9. Khaw KT, Wareham N, Bingham S, Luben R, Welch A, Day N. Association of hemoglobin A1c with cardiovascular disease and mortality in adults: the European prospective investigation into cancer in Norfolk. *Ann Intern Med* 2004;141:413–420. [PubMed: 15381514]
10. Daniels LB, Maisel AS. Natriuretic peptides. *J Am Coll Cardiol* 2007;50:2357–2368. [PubMed: 18154959]
11. Wang TJ, Larson MG, Levy D, Benjamin EJ, Leip EP, Omland T, Wolf PA, Vasan RS. Plasma natriuretic peptide levels and the risk of cardiovascular events and death. *N Engl J Med* 2004;350:655–663. [PubMed: 14960742]
12. Gerstein HC, Mann JF, Yi Q, Zinman B, Dinneen SF, Hoogwerf B, Halle JP, Young J, Rashkow A, Joyce C, Nawaz S, Yusuf S. Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. *JAMA* 2001;286:421–426. [PubMed: 11466120]

13. Kistorp C, Raymond I, Pedersen F, Gustafsson F, Faber J, Hildebrandt P. N-terminal pro-brain natriuretic peptide, C-reactive protein, and urinary albumin levels as predictors of mortality and cardiovascular events in older adults. *JAMA* 2005;293:1609–1616. [PubMed: 15811980]
14. Blankenberg S, McQueen MJ, Smieja M, Pogue J, Balion C, Lonn E, Rupprecht HJ, Bickel C, Tiret L, Cambien F, Gerstein H, Munzel T, Yusuf S. Comparative impact of multiple biomarkers and N-Terminal pro-brain natriuretic peptide in the context of conventional risk factors for the prediction of recurrent cardiovascular events in the Heart Outcomes Prevention Evaluation (HOPE) Study. *Circulation* 2006;114:201–208. [PubMed: 16831981]
15. Cook NR, Buring JE, Ridker PM. The effect of including C-reactive protein in cardiovascular risk prediction models for women. *Ann Intern Med* 2006;145:21–29. [PubMed: 16818925]
16. Zethelius B, Berglund L, Sundstrom J, Ingelsson E, Basu S, Larsson A, Venge P, Arnlov J. Use of multiple biomarkers to improve the prediction of death from cardiovascular causes. *N Engl J Med* 2008;358:2107–2116. [PubMed: 18480203]
17. Lee ET, Welty TK, Fabsitz R, Cowan LD, Le NA, Oopik AJ, Cucchiara AJ, Savage PJ, Howard BV. The Strong Heart Study. A study of cardiovascular disease in American Indians: design and methods. *Am J Epidemiol* 1990;132:1141–1155. [PubMed: 2260546]
18. Best LG, Zhang Y, Lee ET, Yeh JL, Cowan L, Palmieri V, Roman M, Devereux RB, Fabsitz RR, Tracy RP, Robbins D, Davidson M, Ahmed A, Howard BV. C-reactive protein as a predictor of cardiovascular risk in a population with a high prevalence of diabetes: the Strong Heart Study. *Circulation* 2005;112:1289–1295. [PubMed: 16116058]
19. Zhang Y, Howard BV, Cowan LD, Welty TK, Schaefer CF, Wild RA, Yeh J, Lee ET. Associations of postmenopausal hormone therapy with markers of hemostasis and inflammation and lipid profiles in diabetic and nondiabetic american Indian women: the strong heart study. *J Womens Health (Larchmt)* 2004;13:155–163. [PubMed: 15072729]
20. Palmieri V, Celentano A, Roman MJ, de Simone G, Best L, Lewis MR, Robbins DC, Fabsitz RR, Howard BV, Devereux RB. Relation of fibrinogen to cardiovascular events is independent of preclinical cardiovascular disease: the Strong Heart Study. *Am Heart J* 2003;145:467–474. [PubMed: 12660670]
21. Lu WQ, Resnick HE, Jablonski KA, Jain AK, Jones KL, Robbins DC, Howard BV. Effects of glycaemic control on cardiovascular disease in diabetic American Indians: the Strong Heart Study. *Diabet Med* 2004;21:311–317. [PubMed: 15049931]
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;27:157–172. [PubMed: 17569110]discussion 207–212.
23. Wang TJ, Gona P, Larson MG, Tofler GH, Levy D, Newton-Cheh C, Jacques PF, Rifai N, Selhub J, Robins SJ, Benjamin EJ, D'Agostino RB, Vasan RS. Multiple biomarkers for the prediction of first major cardiovascular events and death. *N Engl J Med* 2006;355:2631–2639. [PubMed: 17182988]
24. Standards of medical care in diabetes--2009. *Diabetes Care* 2009;32:S13–S61. [PubMed: 19118286]
25. Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, Hadden D, Turner RC, Holman RR. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ* 2000;321:405–412. [PubMed: 10938048]
26. Davidson M, Zhu J, Lu W, Tracy RP, Robbins DC, Resnick HE, Ruotolo G, Howard BV. Plasminogen activator inhibitor-1 and the risk of Type 2 diabetes mellitus in American Indians: the Strong Heart Study. *Diabet Med* 2006;23:1158–1159. [PubMed: 16978385]

Table 1

Baseline Characteristics

Characteristic	Entire cohort (n=823)	Diabetes Mellitus		p value
		Yes (n=286)	No (n=537)	
Age (years)	59.1±7.7	59.5±7.3	58.9±7.9	0.108
Women	62.1%	71.7%	57.0%	<0.001
Hypertension	34.1%	41.6%	30.2%	0.001
Current smoking	44.6%	40.2%	46.9%	0.065
Body mass index (kg/m ²)	29.8±5.6	31.2±5.4	29.1±5.6	<0.001
Overweight or Obese	81.6%	89.1%	77.6%	<0.001
Obese	44.5%	56.3%	38.2%	<0.001
Waist-hip ratio	0.96±0.07	0.98±0.06	0.95±0.08	<0.001
Waist circumference (cm)	102.7±13.4	107.1±12.2	100.3±13.5	<0.001
Men	101.1±12.1	106.9±11.8	99.1±11.6	<0.001
Women	103.6±14.1	107.1±12.3	101.3±14.7	<0.001
Serum total cholesterol/HDL ratio	5.2±2.1	5.7±2.3	4.9±1.9	<0.001
Metabolic syndrome	55.5%	86.4%	39.1%	<0.001
Serum creatinine (mg/dL)	0.9±0.2	0.9±0.2	0.9±0.2	0.014
Plasma B-type natriuretic peptide (pg/mL)	20.0 (7.8–52.1)	18.7 (8.2–51.7)	20.6 (7.8–52.5)	0.834
Serum C-reactive protein (mg/L)	3.7 (2.0–6.7)	4.40 (2.60–7.40)	3.4 (1.8–6.0)	<0.001
Serum fibrinogen (mg/dL)	335 (300–378)	349 (304–393)	332 (296–369)	<0.001
Serum plasminogen activator inhibitor-1 (ng/mL)	39 (24–60)	44 (30–72)	36 (22–54)	<0.001
Hemoglobin A1c	5.5% (5.0–7.2)	8.3% (6.4–10.1)	5.1% (4.8–5.6)	<0.001
Urine albumin/creatinine ratio (mg/g)	9.1 (5.1–25.4)	25.0 (8.9–92.0)	6.7 (4.3–12.7)	<0.001

Unless noted, values expressed as mean ± standard deviation or median (interquartile range); categorical variables compared using Chi-square, continuous variables compared using Wilcoxon test.

Table 2
Relations of Biomarkers to All-Cause Mortality and Major Cardiovascular Events

Biomarker	All-Cause Mortality		p Value	Major Cardiovascular Events		P Value
	Hazard Ratio (95% Confidence Interval) per Standard Deviation [‡] Log Increase [‡]			Hazard Ratio (95% Confidence Interval) per Standard Deviation [‡] Log Increase [‡]		
B-type natriuretic peptide						
Age- and sex-adjusted model	1.22 (1.07–1.40)		0.003	1.19 (1.02–1.39)		0.029
Basic multivariable model [†]	1.22 (1.07–1.40)		0.004	1.20 (1.02–1.41)		0.026
Basic model [‡] + other biomarkers [‡]	1.17 (1.01–1.35)		0.038	1.12 (0.95–1.32)		0.183
C-reactive protein						
Age- and sex-adjusted model	1.19 (1.04–1.35)		0.011	1.23 (1.06–1.43)		0.008
Basic multivariable model [†]	1.14 (0.99–1.30)		0.065	1.16 (0.99–1.36)		0.066
Basic model [‡] + other biomarkers [‡]	1.00 (0.85–1.17)		0.987	1.05 (0.87–1.28)		0.615
Fibrinogen						
Age- and sex-adjusted model	1.34 (1.17–1.53)		<0.001	1.39 (1.19–1.63)		<0.001
Basic multivariable model [†]	1.27 (1.10–1.46)		0.001	1.27 (1.08–1.50)		0.004
Basic model [‡] + other biomarkers [‡]	1.16 (1.00–1.35)		0.044	1.18 (1.00–1.40)		0.049
Plasminogen activator inhibitor 1						
Age- and sex-adjusted model	1.01 (0.86–1.15)		0.891	1.07 (0.91–1.25)		0.410
Basic multivariable model [†]	1.01 (0.87–1.17)		0.942	1.00 (0.84–1.20)		0.999
Basic model [‡] + other biomarkers [‡]	1.00 (0.86–1.16)		0.966	1.00 (0.83–1.19)		0.961
Hemoglobin A1c						
<i>Diabetes Mellitus</i> [§]	Yes	No	Yes and No Combined			
Age- and sex-adjusted model	1.64 (1.32–2.03)	<0.001	0.72 (0.48–1.07)	0.099	1.43 (1.24–1.66)	<0.001
Basic multivariable model [†]	1.63 (1.32–2.02)	<0.001	0.74 (0.49–1.13)	0.165	1.30 (1.06–1.60)	0.012
Basic model [‡] + other biomarkers [‡]	1.46 (1.15–1.84)	0.002	0.70 (0.46–1.07)	0.096	1.17 (0.94–1.45)	0.169
Urinary albumin/creatinine ratio						
<i>Diabetes Mellitus</i> [§]	Yes	No	Yes			
Age- and sex-adjusted model	1.69 (1.44–1.98)	<0.001	1.13 (0.88–1.45)	0.323	1.70 (1.42–2.02)	0.94 (0.69–1.29)
					<0.001	0.711

Biomarker	All-Cause Mortality		Major Cardiovascular Events	
	Hazard Ratio (95% Confidence Interval) per Standard Deviation Log Increase*	p Value	Hazard Ratio (95% Confidence Interval) per Standard Deviation Log Increase*	P Value
Basic multivariable model [†]	1.79 (1.50–2.13)	<0.001	1.64 (1.35–1.99)	<0.001
Basic model + other biomarkers [‡]	1.64 (1.35–2.00)	<0.001	1.57 (1.25–1.96)	<0.001

* Standard deviations: B-type natriuretic peptide = 1.428; C-reactive protein = 0.973; fibrinogen = 0.186; plasminogen activator inhibitor 1 = 0.679; hemoglobin A1c = 0.294; urine albumin/creatinine ratio = 1.535.

[†] Adjusted for age (continuous), sex, waist-hip ratio (continuous), hypertension (yes/no), diabetes (yes/no), smoking status (current vs. ever/never), total/HDL cholesterol (continuous), serum creatinine (continuous).

[‡] Including only biomarkers significantly associated with outcome after adjustment for standard clinical and laboratory covariates.

[§] Because of a significant interaction, effect estimates are stratified by presence or absence of diabetes mellitus.

Table 3
Discriminative Utility of Biomarkers for All-Cause Mortality and Cardiovascular Events

	c-statistic	95% Confidence Interval	p Value (vs. Basic Model)
<i>All-Cause Mortality</i>			
Basic Model *	0.678	0.635–0.721	--
Basic Model + logFibrinogen	0.687	0.644–0.729	0.281
Basic Model + logBNP	0.687	0.645–0.729	0.206
Basic Model + logHbA1c + logHbA1c×diabetes	0.702	0.659–0.745	0.034
Basic Model + logUACR + logUACR×diabetes	0.710	0.668–0.753	0.010
Basic Model + logHbA1c + logUACR + logHbA1c×diabetes + logUACR×diabetes	0.722	0.688–0.772	0.003
Basic Model + logBNP + logHbA1c + logUACR	0.729	0.687–0.771	0.001
logHbA1c×diabetes + logUACR×diabetes			0.231 (vs. UACR)
Basic Model + logFibrinogen + logHbA1c + logUACR + logHbA1c×diabetes + logUACR×diabetes	0.724	0.682–0.767	0.003
Basic Model + logBNP + logFibrinogen + logHbA1c + logUACR + logHbA1c×diabetes + logUACR×diabetes	0.730	0.688–0.772	0.001
			0.054 (vs. UACR)
<i>Major Cardiovascular Events</i>			
Basic Model *	0.674	0.627–0.720	--
Basic Model + logHbA1c	0.675	0.628–0.721	0.337
Basic Model + logBNP	0.678	0.632–0.725	0.312
Basic Model + logFibrinogen	0.687	0.641–0.733	0.100
Basic Model + logUACR + logUACR×diabetes	0.693	0.646–0.739	0.089
Basic Model + logFibrinogen + logUACR + logUACR×diabetes	0.701	0.654–0.747	0.041

* Age, sex, waist-hip ratio, hypertension, diabetes, current smoking, total cholesterol/HDL ratio, serum creatinine.

Table 4
Reclassification of Participants Dying or Alive at Follow-up

Basic Model [*]	Model with Multiple Biomarkers [†]			
	<10% Risk	10–19% Risk	≥20% Risk	Total Number
Participants Dying				
<10% Risk	2 (66.7%)	1 (33.3%)	0	3
10–19% Risk	3 (9.7%)	15 (48.4%)	13 (41.9%)	31
≥20% Risk	0	13 (7.6%)	158 (92.4%)	171
Total Number	5	29	171	205
Participants Alive				
<10% Risk	3 (75.0%)	1 (25.0%)	0	4
10–19% Risk	26 (14.3%)	130 (71.4%)	26 (14.3%)	182
≥20% Risk	10 (2.6%)	69 (18.3%)	299 (79.1%)	378
Total Number	39	200	325	564

^{*} Age, sex, waist-hip ratio, hypertension, diabetes, current smoking, total cholesterol/HDL ratio, serum creatinine.

[†] Basic Model + logBNP + logFibrinogen + logUACR + logHbA1c + logUACR×diabetes + logHbA1c×diabetes.