ORIGINAL ARTICLE

Multiple Biomarkers for the Prediction of First Major Cardiovascular Events and Death

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ABSTRACT

BACKGROUND

Few investigations have evaluated the incremental usefulness of multiple biomarkers from distinct biologic pathways for predicting the risk of cardiovascular events.

METHODS

We measured 10 biomarkers in 3209 participants attending a routine examination cycle of the Framingham Heart Study: the levels of C-reactive protein, B-type natriuretic peptide, N-terminal pro–atrial natriuretic peptide, aldosterone, renin, fibrinogen, D-dimer, plasminogen-activator inhibitor type 1, and homocysteine; and the urinary albumin-to-creatinine ratio.

RESULTS

During follow-up (median, 7.4 years), 207 participants died and 169 had a first major cardiovascular event. In Cox proportional-hazards models adjusting for conventional risk factors, the following biomarkers most strongly predicted the risk of death (each biomarker is followed by the adjusted hazard ratio per 1 SD increment in the log values): B-type natriuretic peptide level (1.40), C-reactive protein level (1.39), the urinary albumin-to-creatinine ratio (1.22), homocysteine level (1.20), and renin level (1.17). The biomarkers that most strongly predicted major cardiovascular events were B-type natriuretic peptide level (adjusted hazard ratio, 1.25 per 1 SD increment in the log values) and the urinary albumin-to-creatinine ratio (1.20). Persons with "multimarker" scores (based on regression coefficients of significant biomarkers) in the highest quintile as compared with those with scores in the lowest two quintiles had elevated risks of death (adjusted hazard ratio, 4.08; $P<0.001$) and major cardiovascular events (adjusted hazard ratio, 1.84; $P=0.02$). However, the addition of multimarker scores to conventional risk factors resulted in only small increases in the ability to classify risk, as measured by the C statistic.

CONCLUSIONS

For assessing risk in individual persons, the use of the 10 contemporary biomarkers that we studied adds only moderately to standard risk factors.

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Established cardiovascular risk factors, including dyslipidemia, smoking, hypertension, and diabetes mellitus, have been incorporated into algorithms for risk assessment in the general population,[1,2] but these characteristics do not fully explain cardiovascular risk.[3-5] There is substantial interest in the use of newer biomarkers to identify persons who are at risk for the development of cardiovascular disease and who could be targeted for preventive measures.[6] Many individual biomarkers have been related to cardiovascular risk in ambulatory persons, including levels of C-reactive protein,[7,8] B-type natriuretic peptide,[9] fibrinogen,[10] D-dimer,[11] and homocysteine.[12] Measurement of several biomarkers simultaneously (the "multimarker" approach) could enhance risk stratification of ambulatory persons. We therefore evaluated the usefulness of 10 previously reported biomarkers for predicting death and major cardiovascular events in a large, community-based cohort.

Methods

Study Sample

Participants attending the sixth examination cycle (1995 through 1998) of the Framingham Offspring Study were eligible for inclusion in this study. The institutional review board of Boston University Medical Center approved the protocol, and participants provided written informed consent.

All participants provided a medical history and underwent a physical examination and laboratory assessment of cardiovascular risk factors. We assessed the participants for cigarette smoking and diabetes mellitus and measured blood pressure, body-mass index, total cholesterol levels, high-density lipoprotein (HDL) cholesterol levels, and serum creatinine levels. Medication use was recorded. For this study, we excluded persons who had serum creatinine levels greater than 2.0 mg per deciliter (176.8 μmol per liter) or missing covariates.

Biomarker Selection and Measurement

Ten biomarkers were selected because of reported associations with death or cardiovascular events,[7,9,10,12-16] biologic plausibility, and availability at the sixth examination cycle. We measured high-sensitivity C-reactive protein (a marker of inflammation); B-type natriuretic peptide, N-terminal pro–atrial natriuretic peptide, serum aldosterone, and plasma renin (markers of neurohormonal activity); fibrinogen (a marker of thrombosis and inflammation); plasminogen-activator inhibitor type 1 (a marker of fibrinolytic potential and endothelial function); D-dimer (a marker of thrombosis); homocysteine (a marker of endothelial function and oxidant stress); and the urinary albumin-to-creatinine ratio (a marker of glomerular endothelial function).

Fasting blood samples were collected in the morning, after participants had been supine for approximately 10 minutes. Specimens were immediately centrifuged and stored at –70°C. The albumin-to-creatinine ratio in morning urine specimens was assessed. Standard assays were used for all biomarkers (see the Supplementary Appendix, available with the full text of this article at www.nejm.org).

Outcomes

Two outcomes were assessed for inclusion in the prediction analysis — death from any cause and major cardiovascular events. Death from any cause was assessed for all study participants. Major cardiovascular events were assessed only for those participants who had not previously had such an event. Fatal and nonfatal myocardial infarction, coronary insufficiency (prolonged angina with documented electrocardiographic changes), heart failure, and stroke were classified as major cardiovascular events, whereas angina, intermittent claudication, and transient ischemic attack were classified as "nonmajor" cardiovascular events. All suspected major cardiovascular events were reviewed by a committee of three investigators, using previously described criteria.[17]

Statistical Analysis

We used multivariable proportional-hazards models to examine the association of biomarker levels with the risks of death and major cardiovascular events.[18] For each outcome, we performed two sets of prespecified analyses — one that included the urinary albumin-to-creatinine ratio and one that did not — because urine samples were available for only a subgroup of the participants. Logarithmic transformation was performed to normalize the distribution of the biomarkers.

To reduce the number of false positive results from multiple testing, we used a sequential approach. First, we fitted a multivariable Cox regres-

sion model, entering the biomarkers as a set, after confirming that the assumption of proportionality was met. A multivariable P value for the set was determined with the use of a likelihood-ratio test, obtained by subtracting –2 log likelihood for the larger model (clinical covariates and biomarkers) from that for the smaller model (clinical covariates only). Subsequent analyses were performed if the multivariable P value was less than 0.05. Second, a parsimonious set of biomarkers was selected with the use of backward elimination (retention threshold, $P<0.05$). Third, we used the following equation to construct a multimarker score (H) based on the biomarkers chosen from the previous step: $H=(\beta_1 \times \text{biomarker A})+(\beta_2 \times \text{biomarker B})+(\beta_3 \times \text{biomarker C})$, and so on, where β_1, β_2, and β_3 denote the estimates of beta coefficients for biomarkers A, B, and C and were obtained by fitting the multivariable Cox model for the outcome of interest. Participants were categorized according to quintiles of the multimarker score, with the lowest two quintiles labeled low risk, the third and fourth quintiles labeled intermediate risk, and the top quintile labeled high risk. Cumulative probability curves were constructed for subjects with low, intermediate, and high multimarker scores with the use of the Kaplan–Meier method.

We then calculated hazard ratios for death and major cardiovascular events for the low-, intermediate-, and high-risk strata of the multimarker score. The hazard ratios were adjusted for age, sex, and conventional risk factors, including cigarette smoking on a regular basis in the past year, blood-pressure categories (a systolic pressure below 120 mm Hg and a diastolic pressure below 80 mm Hg, a systolic pressure of 120 to 129 mm Hg or a diastolic pressure of 80 to 84 mm Hg, a systolic pressure of 130 to 139 mm Hg or a diastolic pressure of 85 to 89 mm Hg, a systolic pressure of 140 to 159 mm Hg or a diastolic pressure of 90 to 99 mm Hg, a systolic pressure of 160 mm Hg or higher or a diastolic pressure of 100 mm Hg or higher or use of antihypertensive therapy), total-cholesterol categories (less than 160 mg per deciliter [4.1 mmol per liter], 160 to 199 mg per deciliter [4.1 to 5.1 mmol per liter], 200 to 239 mg per deciliter [5.2 to 6.2 mmol per liter], 240 to 279 mg per deciliter [6.2 to 7.2 mmol per liter], and 280 mg per deciliter [7.2 mmol per liter] or higher), HDL categories (less than 35 mg per deciliter [0.9 mmol per liter], 35 to 44 mg per deciliter, 45 to 49 mg per deciliter, 50 to 59 mg per deciliter, and 60 mg per deciliter or higher), and diabetes (fasting glucose level of 126 mg per deciliter [7.0 mmol per liter] or higher or the use of antidiabetes medication). Analyses also adjusted for body-mass index and serum creatinine level. A previous major cardiovascular event was an exclusion factor in models for major cardiovascular events and a covariate in models for death.

The ability to classify risk was assessed with the use of the C statistic.[19] The overall C statistic is defined as the probability of concordance among persons who can be compared. Two subjects can be compared if it can be determined who had a longer time to event (time to event vs. time to event, or time to event vs. time to censoring, if time to censoring was longer than time to event). Subjects are considered concordant if their predicted event probabilities and their actual survival times go in the same direction; if their predicted probabilities are tied, they are considered 0.5 concordant. The C statistic is estimated as the sum of concordance values divided by the number of comparable pairs. Also, receiver-operating-characteristic (ROC) curves were plotted for models with biomarkers and for those without biomarkers. Because standard methods do not exist for deriving ROC curves for time-to-event data, we used occurrence as compared with nonoccurrence of events within 5 years as the outcome for these analyses.

In secondary analyses, we adjusted for medication use, evaluated whether the association of biomarkers with outcomes varied according to age or sex, and replaced total-cholesterol categories with low-density lipoprotein (LDL) cholesterol categories (less than 100 mg per deciliter [2.6 mmol per liter], 100 to 129 mg per deciliter [2.6 to 3.3 mmol per liter], 130 to 159 mg per deciliter [3.4 to 4.1 mmol per liter], 160 to 189 mg per deciliter [4.1 to 4.9 mmol per liter], and 190 mg per deciliter [4.9 mmol per liter] or higher).[1] The Friedewald equation[20] was used to estimate LDL cholesterol levels, excluding participants with triglyceride levels of 400 mg per deciliter (4.5 mmol per liter) or higher. We also repeated a Cox proportional-hazards model for major cardiovascular events, adjusting for previous "nonmajor" cardiovascular events (angina, intermittent claudication, or transient ischemic attack). Analyses were performed with the use of SAS software, version 8 (SAS Institute).

RESULTS

A total of 3532 persons attended the sixth examination cycle of the Framingham Offspring Study. Of these, 21 were excluded for serum creatinine levels above 2.0 mg per deciliter and 302 were excluded for missing covariates. Characteristics of the remaining 3209 persons who constituted the study sample are shown in Table 1. The mean age of participants at the time of study enrollment was 59±10 years. Fifty-three percent of the participants were women, and 6% had prevalent major cardiovascular disease. Median levels of the biomarkers are noted in Table 1; all biomarkers were available for all participants except the urinary albumin-to-creatinine ratio, which was available for 2750 of the participants (86%).

Table 1. Baseline Characteristics of the Study Participants.*

Characteristic	Men (N=1497)	Women (N=1712)
Mean age — yr	59±10	59±10
Body-mass index	28.6±4.4	27.4±5.7
Total cholesterol — mg/dl	198±37	211±38
High-density lipoprotein cholesterol — mg/dl	43±12	58±16
Current smoker — no. (%)	213 (14)	267 (16)
Hypertension — no. (%)	675 (45)	657 (38)
Diabetes mellitus — no. (%)	180 (12)	134 (8)
Serum creatinine — mg/dl	1.2±0.2	1.1±0.2
Use of statin medications — no. (%)	197 (13)	151 (9)
Use of antihypertensive therapy — no. (%)	468 (31)	437 (26)
Daily use of aspirin — no. (%)†	416 (28)	251 (15)
Prevalent cardiovascular disease — no. (%)‡	132 (9)	49 (3)
Biomarker levels	*median (interquartile range)*	
C-reactive protein — mg/liter	1.8 (0.9–3.8)	2.4 (1.0–5.7)
B-type natriuretic peptide — pg/ml	6.6 (4.0–16.4)	10.2 (4.1–20.4)
N-terminal pro–atrial natriuretic peptide — pmol/liter	290 (196–438)	352 (254–499)
Aldosterone — ng/dl	9.0 (7.0–13.0)	11.0 (7.0–15.0)
Renin — mU/liter	14.0 (8.0–25.0)	11.0 (6.0–19.0)
Fibrinogen — mg/dl	323 (288–375)	336 (295–381)
D-dimer — ng/ml	297 (181–466)	336 (232–483)
Plasminogen-activator inhibitor type 1 — ng/ml	25.5 (17.1–35.9)	20.3 (12.2–31.8)
Homocysteine — mmol/liter	9.8 (8.3–11.8)	8.4 (7.0–10.3)
Urinary albumin-to-creatinine ratio§	4.8 (2.1–10.8)	8.6 (3.6–17.4)

* Plus–minus values are means ±SD. Body-mass index is the weight in kilograms divided by the square of the height in meters. To convert values for cholesterol to millimoles per liter, multiply by 0.02586. To convert values for aldosterone to picomoles per liter, multiply by 27.74.
† Thirteen participants were not assessed for daily aspirin use.
‡ Prevalent cardiovascular disease includes previous myocardial infarction, coronary insufficiency, heart failure, and stroke.
§ The ratio, with both substances measured in milligrams per gram, is based on 2750 participants (86%) for whom urine samples were available.

During up to 10 years of follow-up (median, 7.4 years), 207 of 3209 participants (6%) died, of whom 72 were women, and 169 of 3028 participants (6%, excluding 181 with prevalent cardiovascular disease at baseline) had a major cardiovascular event, of whom 68 were women. The biomarker panel was associated with both outcomes in models that adjusted for conventional risk factors. In analyses restricted to the nine biomarkers in blood, multivariable P values for the biomarker panel were as follows: P<0.001 for death and P=0.005 for major cardiovascular events. For all 10 biomarkers (including the urinary albumin-to-creatinine ratio), multivariable P values were as follows: P<0.001 for death and P=0.04 for major cardiovascular events.

In backward-elimination models, the following five biomarkers were retained as predictors of death in analyses restricted to blood biomarkers: levels of C-reactive protein, N-terminal pro–atrial natriuretic peptide, homocysteine, plasma renin, and D-dimer. When the urinary albumin-to creatinine ratio was included, it replaced D-dimer, and B-type natriuretic peptide replaced N-terminal pro–atrial natriuretic peptide. Thus, the final model contained the following biomarkers: B-type natriuretic peptide level (adjusted hazard ratio, 1.40 per 1 SD increment in the log value), C-reactive protein level (1.39), urinary albumin-to-creatinine ratio (1.22), homocysteine level (1.20), and renin level (1.17) (see the Supplementary Appendix).

For major cardiovascular events, two biomarkers were retained in analyses excluding the urinary albumin-to-creatinine ratio — B-type natriuretic peptide and plasminogen-activator inhibitor type 1. When the urinary albumin-to-creatinine ratio was included, it entered the model, and plasminogen-activator inhibitor type 1 became marginally significant (P=0.05). The final model therefore included B-type natriuretic peptide (adjusted hazard ratio, 1.25) and the urinary albumin-to-creatinine ratio (1.20). For the remaining

analyses, we used models that included the urinary albumin-to-creatinine ratio, because it was a significant predictor of both outcomes.

USEFULNESS OF MULTIMARKER SCORES

Biomarkers selected with the use of backward elimination were incorporated into multimarker scores, according to the formulas in Table 2. Because the multimarker scores included the urinary albumin-to-creatinine ratio, the models on which the scores are based were restricted to participants with a urine sample. Thus, for death from any cause, the number of events and the number at risk were 172 and 2750, respectively, whereas for major cardiovascular events, the number of events and the number at risk were 133 and 2598, respectively. Figures 1A and 1B show the Kaplan–Meier curves depicting the cumulative probability of death and major cardiovascular events for persons with low, intermediate, and high multimarker scores. Multivariable-adjusted hazard ratios for death and major cardiovascular events for persons with low, intermediate, and high multimarker scores are shown in Table 3. Persons with high multimarker scores had a risk of death four times as great and a risk of major cardiovascular events almost two times as great as persons with low multimarker scores ($P<0.001$ and $P=0.02$, respectively).

Table 2. Multimarker Scores for the Prediction of Death and Major Cardiovascular Events, with Cutoff Points Distinguishing Low, Intermediate, and High Risk.*

Risk Level	Multimarker Score for Death†	Multimarker Score for Cardiovascular Events‡
Low	<2.79	<0.67
Intermediate	2.79 to <3.45	0.67 to <1.03
High	≥3.45	≥1.03

* The lowest two quintiles are labeled low risk, the third and fourth quintiles are labeled intermediate risk, and the top quintile is labeled high risk.

† The score is calculated as 0.367 × (ln B-type natriuretic peptide, in picograms per milliliter) + 0.595 × (ln homocysteine, in millimoles per liter) + 0.153 × (ln renin, in milliunits per liter) + 0.284 × (ln C-reactive protein, in milligrams per liter) + 0.137 × (ln urinary albumin-to-creatinine ratio, with both substances measured in milligrams per gram), where ln denotes natural logarithm.

‡ The score is calculated as 0.257 × (ln B-type natriuretic peptide, in picograms per milliliter) + 0.128 × (ln urinary albumin-to-creatinine ratio, with both substances measured in milligrams per gram), where ln denotes natural logarithm.

C statistics for models of death were 0.75 (with age and sex as predictors), 0.79 (with age, sex, and multimarker score as predictors), 0.80 (with age, sex, and conventional risk factors as predictors), and 0.82 (with all predictors). C statistics for major cardiovascular events were 0.68 (with age and sex as predictors), 0.70 (with age, sex, and multimarker score as predictors), 0.76 (with age, sex, and conventional risk factors as predictors), and 0.77 (with all predictors). As shown in Figure 2, ROC curves overlapped for models with conventional risk factors with biomarkers and for models with conventional risk factors without biomarkers.

SECONDARY ANALYSES

Because plasminogen-activator inhibitor type 1 was marginally significant ($P=0.05$) in the backward-elimination model for major cardiovascular events, a secondary analysis was performed with this variable included in the model. This analysis resulted in an adjusted hazard ratio of 1.86 ($P=0.02$) for high multimarker scores and a C statistic of 0.77. Adjustment for the use of statins, aspirin, or antihypertensive medications or for previous "nonmajor" cardiovascular events did not alter our findings significantly. In addition, substituting LDL cholesterol for total cholesterol yielded results that were similar to those of the primary analyses. Interactions of age and sex with biomarkers for death and major cardiovascular events were not statistically significant.

DISCUSSION

We investigated the usefulness of 10 biomarkers for predicting death and major cardiovascular events in approximately 3000 persons followed for up to 10 years. We observed that the most informative biomarkers for predicting death were blood levels of B-type natriuretic peptide, C-reactive protein, homocysteine, and renin, and the urinary albumin-to-creatinine ratio, whereas the most informative biomarkers for predicting major cardiovascular events were B-type natriuretic peptide and the urinary albumin-to-creatinine ratio. Persons with high multimarker scores had a risk of death four times as great and a risk of major cardiovascular events almost two times as great as persons with low multimarker scores. Nonetheless, the use of multiple biomarkers added only moderately to the overall prediction of risk based

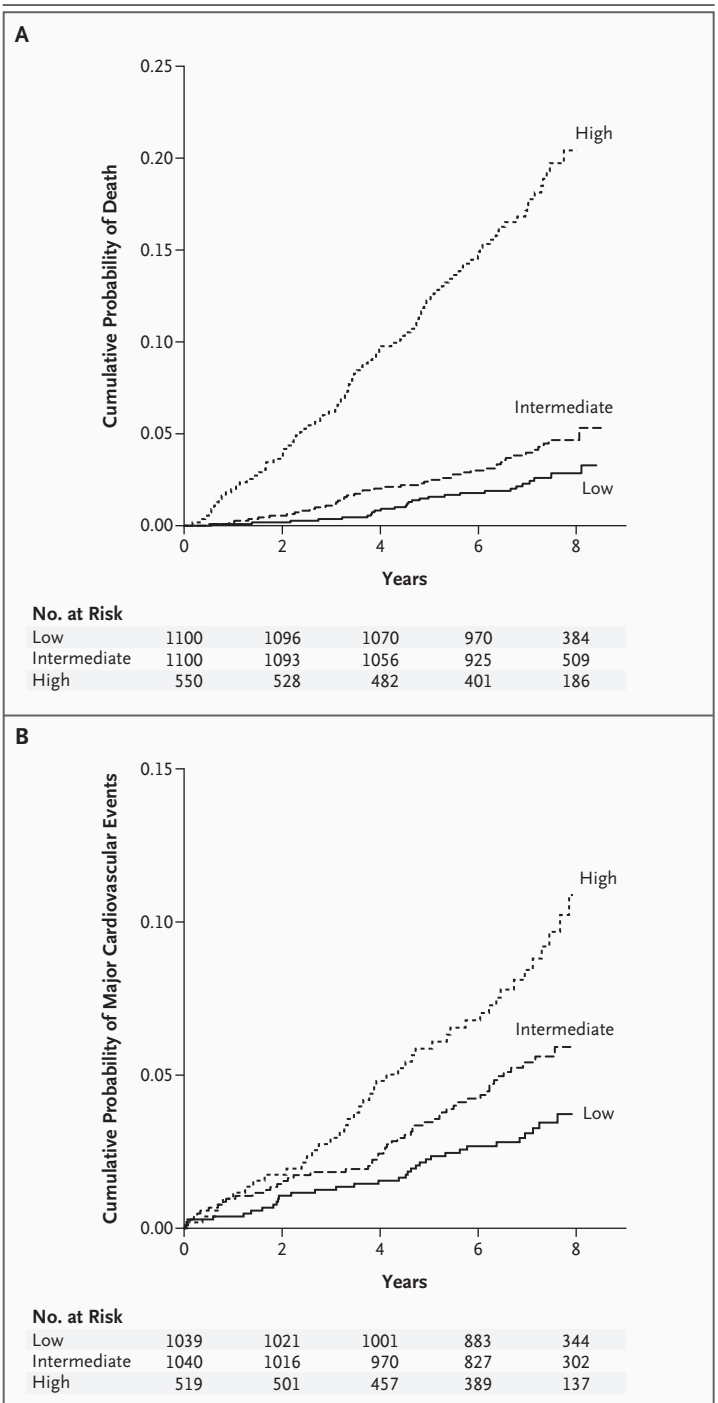

Figure 1. Kaplan–Meier Curves of the Cumulative Probability of Death (Panel A) and Major Cardiovascular Events (Panel B), According to Category of Multimarker Score.

Multimarker scores were classified as low, intermediate, or high, as described in Table 2.

on conventional cardiovascular risk factors, as evidenced by small changes in the C statistic.

These findings highlight the strengths and limitations of the use of current biomarkers for the prediction of cardiovascular risk in ambulatory persons. Although multiple biomarkers are associated with a high relative risk of adverse events, even in combination they add only moderately to the prediction of risk in an individual person. We used the C statistic for assessing the clinical usefulness of biomarkers, because it measures discrimination ability better than relative risk does.[21,22] One reason is that distributions of biomarker levels in persons with and in persons without cardiovascular events may overlap, even when large relative differences are present.[21] In addition, relative risk ratios may not reflect the fact that most persons can be effectively risk stratified with conventional risk factors.[22]

Our findings regarding the associations of biomarkers with the risks of death and incident major cardiovascular events are consistent with results of studies of single biomarkers involving B-type natriuretic peptide,[9,23] urinary albumin-to-creatinine ratio,[16] C-reactive protein,[24,25] homocysteine,[26-28] or renin.[29] Although higher plasminogen-activator inhibitor type 1 levels have been observed in persons with known cardiovascular disease,[30] previous studies relating this biomarker to incident cardiovascular disease have been inconclusive.[14,31]

Few community-based data compare cardiovascular biomarkers from different pathways or assess the incremental performance of a multimarker panel for risk prediction. A recent study reported that N-terminal pro–B-type natriuretic peptide and the urinary albumin-to-creatinine ratio, but not C-reactive protein, predicted the risk of death and cardiovascular events in 764 elderly persons.[32] Our data extend these findings to a younger and substantially larger cohort, with a larger panel of biomarkers and prospective assessments of clinical usefulness.

In our study, C-reactive protein predicted the risk of death but not of major cardiovascular events, after accounting for other biomarkers. Several studies of single markers, including a study based on an earlier examination cycle of the Framingham Heart Study, have shown little improvement in the prediction of risk with the addition

Table 3. Relation of Multimarker Risk Score to Outcomes.*

Multimarker Score	Death	Major Cardiovascular Events
	adjusted hazard ratios (95% CI)	
Low	1.0 (reference group)	1.0 (reference group)
Intermediate	1.34 (0.83–2.18)	1.54 (0.98–2.40)
High	4.08 (2.51–6.62)	1.84 (1.11–3.05)
P value for trend	<0.001	0.02

* Hazard ratios were adjusted for age; sex; body-mass index; categories of blood pressure, total cholesterol, and high-density lipoprotein cholesterol; smoking status; presence or absence of diabetes; serum creatinine level; and presence or absence of prevalent cardiovascular disease (for the model with death).

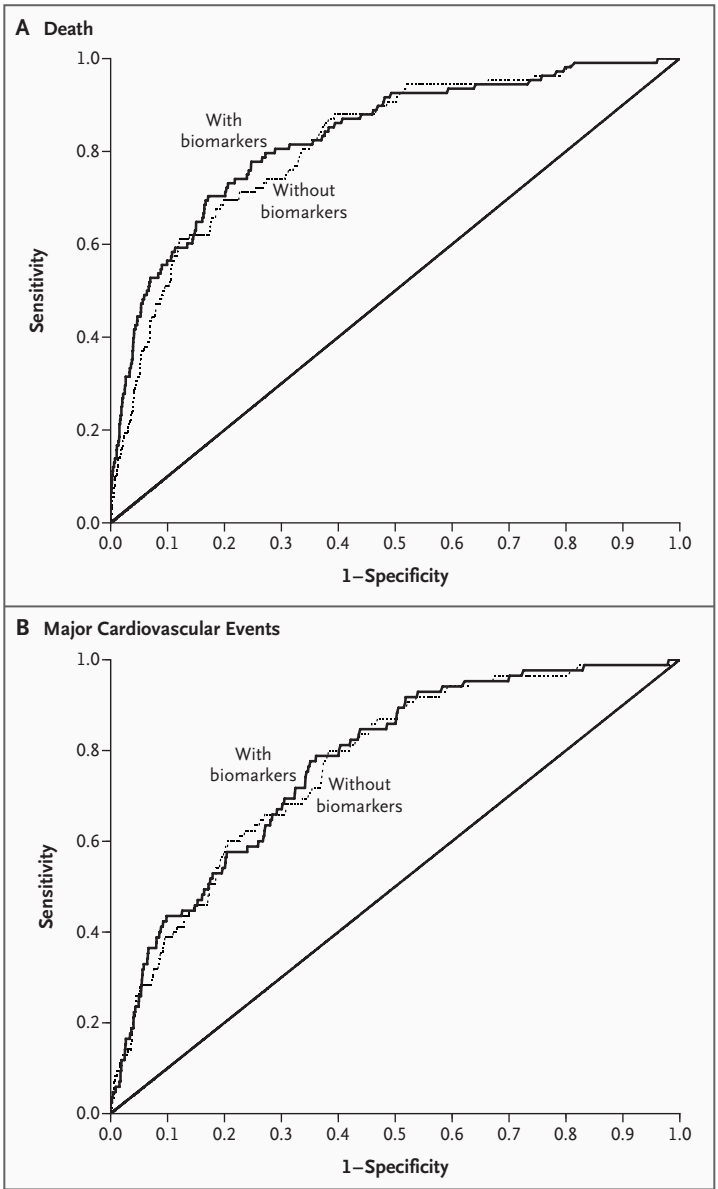

Figure 2. Receiver-Operating-Characteristic Curves for Death (Panel A) and for Major Cardiovascular Events (Panel B) during 5-Year Follow-up.

For each end point, curves are based on models of the prediction of risk with the use of conventional risk factors with or without biomarkers (multimarker score). Biomarkers for death were B-type natriuretic peptide, C-reactive protein, the urinary albumin-to-creatinine ratio, homocysteine, and renin. Biomarkers for major cardiovascular events were B-type natriuretic peptide and the urinary albumin-to-creatinine ratio.

of C-reactive protein to conventional risk factors.[33,34] Recent data indicate only a moderate association between high-sensitivity C-reactive protein and cardiovascular events, with relative risks of 1.3 to 1.5 associated with levels in the highest third as compared with the lowest third.[8,35] We did not have statistical power to exclude a similarly limited association between C-reactive protein and major cardiovascular events. Nonetheless, our data suggest that B-type natriuretic peptide and the urinary albumin-to-creatinine ratio have stronger relations with global cardiovascular risk than does C-reactive protein, an observation consistent with other studies assessing these biomarkers simultaneously in high-risk populations.[32,36,37]

There has been interest in refining risk-stratification algorithms by adding information from biomarkers representing pathways involved in atherogenesis or vascular function.[6] Practice guidelines, such as those relating to C-reactive protein,[38] have begun to address the use of biomarker screening for primary prevention. Our data indicate that contemporary biomarkers contribute only moderately to the prediction of risk once conventional risk factors are considered.

The assessment of biomarkers may still be useful for identifying subgroups that would benefit most from additional testing. Such a group may consist of persons who are at intermediate risk for a cardiovascular event and in whom adjustments in predicted risk may alter the aggressiveness of the modification of risk factors such as the lowering of serum cholesterol levels or blood pressure.[22,38] Furthermore, this approach may permit more efficient targeting of populations that would be suitable for testing new strategies of prevention.[21]

Cost-effectiveness also influences the clinical

decision to measure new markers. Relatively small improvements in the ability to predict risk may be tolerated for screening tests that are simple and inexpensive, whereas large increments in such predictive usefulness may be necessary to justify costlier tests. Data regarding the costs and benefits of biomarkers in the preventive setting are needed.

Several limitations of our analysis deserve comment. We selected biomarkers on the basis of previous experimental and clinical studies; we acknowledge that other biomarkers not tested, such as lipoprotein-associated phospholipase A_2,[39] might have provided additional information. Because of the concern regarding multiple testing, we did not test the association of each individual marker with outcomes. Instead, we used a global test of the biomarker panel, followed by backward elimination to select the most predictive biomarkers. The failure of a specific biomarker to be retained in the final model does not imply that it is not related to outcomes.

We did not include "nonmajor" cardiovascular events (angina, intermittent claudication, or transient ischemic attack) in the cardiovascular end point or baseline exclusions. Thus, our participants cannot be viewed strictly as a cohort for studying primary prevention. We intended for the study sample to reflect a general, unselected population with varying baseline risks.

It is possible that the association between biomarker levels and outcomes was partly mediated by visceral adiposity or insulin resistance. Although we adjusted for body-mass index in our analyses, measures of insulin resistance were not available at the baseline examination. This limitation may be particularly relevant for biomarkers that correlate with insulin resistance, such as C-reactive protein and plasminogen-activator inhibitor type 1.[40]

In summary, biomarkers from multiple, biologically distinct pathways are associated with the risks of death and major cardiovascular events. Nonetheless, the use of contemporary biomarkers adds only moderately to standard risk factors for risk assessment of individual persons. These results highlight the importance of evaluating putative biomarkers with the use of prospective data and explicit assessments of the ability to classify risk. The future success of biomarker strategies may depend on the discovery of new biomarkers to complement the best existing ones, perhaps with the help of new, unbiased approaches.

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