ORIGINAL ARTICLE

Use of Multiple Biomarkers to Improve the Prediction of Death from Cardiovascular Causes

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ABSTRACT

BACKGROUND

The incremental usefulness of adding multiple biomarkers from different disease pathways for predicting the risk of death from cardiovascular causes has not, to our knowledge, been evaluated among the elderly.

METHODS

We used data from the Uppsala Longitudinal Study of Adult Men (ULSAM), a community-based cohort of elderly men, to investigate whether a combination of biomarkers that reflect myocardial cell damage, left ventricular dysfunction, renal failure, and inflammation (troponin I, N-terminal pro–brain natriuretic peptide, cystatin C, and C-reactive protein, respectively) improved the risk stratification of a person beyond an assessment that was based on the established risk factors for cardiovascular disease (age, systolic blood pressure, use or nonuse of antihypertensive treatment, total cholesterol, high-density lipoprotein cholesterol, use or nonuse of lipid-lowering treatment, presence or absence of diabetes, smoking status, and body-mass index).

RESULTS

During follow-up (median, 10.0 years), 315 of the 1135 participants in our study (mean age, 71 years at baseline) died; 136 deaths were the result of cardiovascular disease. In Cox proportional-hazards models adjusted for established risk factors, all of the biomarkers significantly predicted the risk of death from cardiovascular causes. The C statistic increased significantly when the four biomarkers were incorporated into a model with established risk factors, both in the whole cohort (C statistic with biomarkers vs. without biomarkers, 0.766 vs. 0.664; $P<0.001$) and in the group of 661 participants who did not have cardiovascular disease at baseline (0.748 vs. 0.688, $P=0.03$). The improvement in risk assessment remained strong when it was estimated by other statistical measures of model discrimination, calibration, and global fit.

CONCLUSIONS

Our data suggest that in elderly men with or without prevalent cardiovascular disease, the simultaneous addition of several biomarkers of cardiovascular and renal abnormalities substantially improves the risk stratification for death from cardiovascular causes beyond that of a model that is based only on established risk factors.

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The assessment of cardiovascular risk with the use of established risk factors does not fully explain the development of cardiovascular disease in the community.[1-3] Even though several new biomarkers have been reported to be associated with increased relative risks of cardiovascular events independently of established risk factors, these biomarkers have not been shown to contribute substantially to the risk stratification of the individual patient as evaluated by the area under the receiver-operating-characteristic curve (C statistic).[1-3] It has been suggested that the evaluation of whether a risk marker more accurately stratifies people into higher- or lower-risk categories (reclassification) is a valuable supplement to the C-statistic analyses.[4-6] An enhanced risk assessment would be of great clinical value if it could more accurately identify people who are at increased risk for cardiovascular disease and who could then be targeted for preventive measures.

Established risk factors do not directly reflect myocardial cell damage, left ventricular dysfunction, renal failure, and inflammation — clinical conditions that have been shown to be associated with an increased risk of cardiovascular disease and death.[7-10] We hypothesized that the addition of a combination of biomarkers from these pathophysiological pathways — a multimarker approach — could add substantial prognostic information with respect to the risk of death from cardiovascular causes. Accordingly, we investigated whether the incorporation of troponin I, N-terminal pro–brain natriuretic peptide, cystatin C, and high-sensitivity C-reactive protein in a model with established risk factors improved the prediction of death from cardiovascular causes in a community-based cohort of elderly men.

METHODS

STUDY POPULATION

We used data from the Uppsala Longitudinal Study of Adult Men (ULSAM), which was initiated in 1970. All 50-year-old men who were born between 1920 and 1924 and were living in Uppsala, Sweden, were invited to participate in a health survey that focused on identifying cardiovascular risk factors (described in detail at www.pubcare.uu.se/ULSAM). The present analyses are based on the third examination-cycle of the ULSAM cohort, when participants were approximately 71 years of age (1991 to 1995). Of the 1221 participants, 1135 had valid measurements of troponin I, N-terminal pro–brain natriuretic peptide, cystatin C, high-sensitivity C-reactive protein, and all covariates that we examined. We also examined a subgroup of 661 men who did not have prevalent cardiovascular disease at baseline. For this subgroup, the following exclusion criteria were used: prior myocardial infarction or angina pectoris, as noted in the medical history; Q or QS waves or left bundle-branch block (Minnesota codes 1.1 to 1.3 and 7.1, respectively) on the baseline electrocardiogram; a history of any cardiovascular disease, as noted in the Swedish Hospital Discharge Register (*International Classification of Diseases, 10th revision* [ICD-10] codes I00 to I99); or current treatment with nitroglycerin or cardiac glycosides. All participants gave written informed consent, and the ethics committee of Uppsala University approved the study.

BASELINE EXAMINATIONS

Venous blood samples were drawn at baseline and were stored at –70°C for a mean (±SD) of 11±2 years until analysis.[10] Biomarker determinations were performed as previously described in detail.[10-15] In short, plasma troponin I was measured with the AccuTnI assay (Beckman Coulter).[10] Plasma N-terminal pro–brain natriuretic peptide was determined with a sandwich immunoassay on an Elecsys 2010 analyzer (Roche Diagnostics).[12] Cystatin C and high-sensitivity C-reactive protein were assayed with the use of latex-enhanced reagents (Siemens), on a BN ProSpec analyzer (Siemens).[11,13] Fasting plasma glucose and serum cholesterol levels were measured by routine laboratory analysis.[14,15] Weight, height, body-mass index, electrocardiogram, and systolic and diastolic blood pressures (measured with the participant in a supine position) were assessed under standardized conditions.[14,15] Diabetes mellitus was defined as a fasting plasma glucose level of 7.0 mmol per liter (126 mg per deciliter) or more or the use of oral hypoglycemic agents or insulin. Information with respect to smoking status (current smoker vs. nonsmoker) was obtained from a questionnaire.

OUTCOMES AND FOLLOW-UP

Death from all causes and death from cardiovascular disease (ICD-10 codes I00 to I99) were identified with the use of the Swedish Cause of Death

Register. The median follow-up was 10.0 years (range, 0.9 to 12.4).

STATISTICAL ANALYSES

Logarithmic transformation was performed to achieve a normal distribution for skewed variables (troponin I, N-terminal pro–brain natriuretic peptide, cystatin C, and C-reactive protein). The relations of biomarkers to death from all causes and from cardiovascular causes were investigated with the use of Cox proportional-hazards regression in two sets of models: crude models and models adjusted for established risk factors for cardiovascular disease (age at baseline, systolic blood pressure, use or nonuse of antihypertensive treatment, total cholesterol, high-density lipoprotein cholesterol, use or nonuse of lipid-lowering treatment, presence or absence of diabetes, smoking status, and body-mass index).

In these models, we evaluated the effects of the biomarkers according to three criteria: a 1-SD increase in the biomarker levels (continuous model); biomarker levels that were above or below cutoff points suggested in the literature (troponin I, >0.021 μg per liter[10]; N-terminal pro–brain natriuretic peptide, ≥386 ng per liter[16]; cystatin C, ≥1.29 mg per liter[8]; and C-reactive protein, >3 mg per liter[17]); and biomarker levels that were above or below cutoff points identified to achieve optimal discrimination as evaluated by the integrated discrimination improvement measure[5] (troponin I, >0.035 μg per liter; N-terminal pro–brain natriuretic peptide, >309 ng per liter; cystatin C, >1.50 mg per liter; and C-reactive protein, ≥4.60 mg per liter).

Proportional-hazards assumptions were confirmed by Schoenfeld's tests. Estimates of the C statistic for the Cox regression models were calculated according to the method of Pencina et al.[18] Differences in C statistics (with 95% confidence intervals) after the addition of the biomarkers to a model with established risk factors were estimated with the method described by Antolini et al.[19] We also investigated whether the addition of different combinations of two or three of the biomarkers improved the discrimination of the model.

The increased discriminative value of the biomarkers was further examined with the method described by Pencina et al.[5] This method is based on the difference between two models in the individual estimated probability that a case subject will be categorized as a case subject. An increased probability that case subjects will be categorized as case subjects and a decreased probability that control subjects will be categorized as case subjects imply better prediction ability, whereas the opposite implies worse prediction ability. There are two versions of the measure. The first version (net reclassification improvement) requires that there exist a priori meaningful risk categories (we have used 0 to 5%, 6 to 20%, and >20% for the risk of death from cardiovascular causes). In net reclassification improvement, only those changes in estimated prediction probabilities that imply a change from one category to another are considered. The second version (integrated discrimination improvement) considers the change in the estimated prediction probabilities as a continuous variable.

To assess the calibration of the Cox models, we used the Grønnesby and Borgan calibration test,[20] which compares the number of events that are observed with those that are expected on the basis of estimation from the models, within five risk-score groups. We also performed likelihood-ratio tests to evaluate whether the global model fit improved after the addition of the biomarkers.

All analyses were also performed among participants who did not have prevalent cardiovascular disease at baseline.

We examined effect modification by testing the statistical significance of the interaction among all of the biomarkers. None of the interaction terms reached statistical significance (P>0.08 for all tests).

P values of less than 0.05 from two-sided tests were considered to indicate statistical significance. The statistical software packages STATA (version 10.0) (StataCorp) and SAS (version 9.1 for Windows) (SAS Institute) were used.

RESULTS

Baseline characteristics of the entire sample and of the participants who did not have cardiovascular disease at baseline are shown in Table 1. The mean age at baseline was 71.0 years (range, 69.4 to 73.6). During the follow-up period (median, 10.0 years; range, 0.9 to 12.4), 315 participants died (rate, 3.0 per 100 person-years at risk); 136 deaths were from cardiovascular disease (rate, 1.3 per 100 person-years at risk). In the subgroup of participants who did not have prevalent cardio-

Table 1. Baseline Characteristics of the Study Cohort.*

Characteristic	Whole Sample	Participants without Cardiovascular Disease
No. of subjects	1135	661
Age — yr	71±0.6	71±0.6
Body-mass index†	26.3±3.4	26.0±3.2
Serum cholesterol — mmol/liter‡		
Total	5.81±0.99	5.77±0.98
HDL	1.28±0.35	1.31±0.35
Blood pressure — mm Hg		
Systolic	147±19	148±19
Diastolic	84±9	84±9
Plasma troponin I — μg/liter	0.021±0.17	0.022±0.22
Plasma NT-pro-BNP — ng/liter	232±397	145±213
Serum cystatin C — mg/liter	1.24±0.27	1.22±0.23
Serum C-reactive protein — mg/liter	3.37±4.77	3.27±4.55
Smoker — no. (%)	235 (20.7)	140 (21.2)
Diabetes — no. (%)	121 (10.7)	58 (8.8)
Hypertension — no. (%)	849 (74.8)	472 (71.4)
Antihypertensive treatment — no. (%)	378 (33.3)	146 (22.1)
ACE-inhibitor treatment — no. (%)	66 (5.8)	25 (3.8)
Dyslipidemia — no. (%)	994 (87.6)	575 (87.0)
Lipid-lowering treatment — no. (%)	106 (9.3)	44 (6.7)
Aspirin treatment — no. (%)	105 (9.3)	8 (1.2)
Previous cardiovascular disease — no. (%)	474 (41.8)	—

* Plus–minus values are means ±SD. ACE denotes angiotensin-converting enzyme, HDL high-density lipoprotein, and NT-pro-BNP N-terminal pro–brain natriuretic peptide.
† Body-mass index is the weight in kilograms divided by the square of the height in meters.
‡ To convert the values for serum cholesterol to milligrams per deciliter, multiply by 38.67.

vascular disease at baseline, 149 died (rate, 2.4 per 100 person-years at risk); 54 deaths were from cardiovascular disease (rate, 0.9 per 100 person-years at risk). No subjects were lost to follow-up.

COX REGRESSION

All of the biomarkers (troponin I, N-terminal pro–brain natriuretic peptide, cystatin C, and C-reactive protein) predicted death from cardiovascular causes and from all causes in crude and multivariable models in the whole cohort and in the group of participants who did not have cardiovascular disease at baseline (Table 2). The crude estimates of hazard ratios, C statistics, and calibration chi-square values for the individual established risk factors and for the biomarkers for the prediction of death from cardiovascular causes and from all causes are shown in Table 1 of the Supplementary Appendix (available with the full text of this article at www.nejm.org).

Hazard ratios for different combinations of elevated biomarkers in the whole cohort are shown in Table 2 of the Supplementary Appendix. Participants who had elevated levels of any two of the biomarkers had a risk of death from cardiovascular causes that was increased by a factor of more than 3 ($P<0.001$ for all comparisons). The risk was increased by a factor of more than 7 among participants with elevated levels of any three of the biomarkers, and by more than 16

Table 2. Hazard Ratios for Death from All Causes and from Cardiovascular Causes, According to Biomarker Levels.*

Biomarker and Criterion	Death from Cardiovascular Causes		Death from All Causes	
	Hazard Ratio (95% CI)	P Value	Hazard Ratio (95% CI)	P Value
Whole sample				
Troponin I				
1-SD increase	1.60 (1.41–1.82)	<0.001	1.36 (1.24–1.50)	<0.001
>0.021 μg/liter†	3.40 (2.31–5.00)	<0.001	2.28 (1.71–3.03)	<0.001
>0.035 μg/liter‡	4.08 (2.55–6.55)	<0.001	2.78 (1.92–4.02)	<0.001
NT-pro-BNP				
1-SD increase	2.03 (1.72–2.39)	<0.001	1.58 (1.41–1.76)	<0.001
≥386 ng/liter†	3.77 (2.60–5.46)	<0.001	2.53 (1.94–3.29)	<0.001
>309 ng/liter‡	4.10 (2.86–5.88)	<0.001	2.55 (1.98–3.28)	<0.001
Cystatin C				
1-SD increase	1.43 (1.22–1.66)	<0.001	1.31 (1.18–1.46)	<0.001
≥1.29 mg/liter†	2.01 (1.42–2.85)	<0.001	1.46 (1.16–1.85)	<0.001
>1.50 mg/liter‡	2.04 (1.34–3.12)	<0.001	1.90 (1.41–2.55)	<0.001
C-reactive protein				
1-SD increase	1.49 (1.24–1.78)	<0.001	1.41 (1.26–1.58)	<0.001
>3.0 mg/liter†	1.97 (1.39–2.78)	<0.001	1.64 (1.30–2.06)	<0.001
≥4.6 mg/liter‡	2.19 (1.53–3.12)	<0.001	1.92 (1.51–2.45)	<0.001
Participants without CVD at baseline				
Troponin I				
1-SD increase	1.77 (1.43–2.18)	<0.001	1.32 (1.13–1.56)	<0.001
>0.021 μg/liter†	2.85 (1.41–5.75)	0.003	1.60 (0.96–2.67)	0.07
>0.035 μg/liter‡	4.46 (1.93–10.28)	<0.001	1.82 (0.87–3.78)	0.11
NT-proBNP				
1-SD increase	2.16 (1.55–3.00)	<0.001	1.46 (1.18–1.80)	<0.001
≥386 ng/liter†	4.96 (2.48–9.92)	<0.001	2.60 (1.56–4.31)	<0.001
>309 ng/liter‡	4.69 (2.53–8.72)	<0.001	2.50 (1.60–3.89)	<0.001
Cystatin C				
1-SD increase	1.36 (1.05–1.77)	0.02	1.27 (1.08–1.50)	0.004
≥1.29 mg/liter†	2.02 (1.15–3.51)	0.01	1.59 (1.13–2.24)	0.008
>1.50 mg/liter‡	2.79 (1.44–5.41)	0.002	2.34 (1.50–3.65)	<0.001
C-reactive protein				
1-SD increase	1.49 (1.12–1.98)	0.006	1.35 (1.15–1.60)	<0.001
>3.0 mg/liter†	2.00 (1.16–3.44)	0.01	1.39 (0.99–1.94)	0.06
≥4.6 mg/liter‡	2.72 (1.56–4.73)	<0.001	1.79 (1.26–2.57)	<0.001

* Values were calculated with the use of multivariable Cox regression analysis. Data were adjusted for the following variables: age at baseline (continuous), systolic blood pressure (continuous), use or nonuse of antihypertensive treatment (binary), total cholesterol (continuous), high-density lipoprotein cholesterol (continuous), use or nonuse of lipid-lowering treatment (binary), presence or absence of diabetes (binary), smoking status (binary), and body-mass index (continuous). CVD denotes cardiovascular disease, and NT-pro-BNP N-terminal pro–brain natriuretic peptide.
† This is the cutoff point that has been suggested in the literature.
‡ This cutoff point has been identified for optimized discrimination in the present study.

among participants with elevated levels of all four biomarkers (P<0.001 for all comparisons).

DISCRIMINATION

In the whole cohort, as well as in the group of participants who did not have cardiovascular disease at baseline, the C statistic increased significantly for the prediction of death from cardiovascular causes when all the biomarkers we measured were incorporated into a model with the established risk factors (Table 3). This was also the case when we used the cutoff points suggested in the literature (C statistic for the whole cohort, 0.756; P<0.001; for participants without cardiovascular disease at baseline, 0.742; P=0.05) and when we used the cutoff points that were identified to achieve optimal discrimination (C statistic for the whole cohort, 0.755; P<0.001; for participants without cardiovascular disease at baseline, 0.751; P=0.02). The separate addition of troponin I and N-terminal pro–brain natriuretic peptide to the model with established risk factors also significantly improved the C statistic for predicting death from cardiovascular causes in the whole cohort, but not in the group of participants who did not have cardiovascular disease at baseline. The C statistic for the prediction of death from all causes followed a pattern that was similar to that for the prediction of death from cardiovascular causes, but with weaker associations (Table 3).

In the group of participants without cardiovascular disease at baseline, the C-statistic estimate for the addition of any combination of two biomarkers to the established risk factors was highest for the combination of N-terminal pro–brain natriuretic peptide and troponin I (0.737; P=0.06) (Table 3 of the Supplementary Appendix). The C-statistic estimate for the addition of any combination of three biomarkers was highest for the combination of N-terminal pro–brain natri-

Table 3. C Statistic for Cox Regression Models Predicting Death from Cardiovascular Causes and from All Causes in the Whole Sample and in the Subsample without Cardiovascular Disease at Baseline.*

Risk Factors and Biomarkers	C Statistic for Death from Cardiovascular Causes	P Value†	C Statistic for Death from All Causes	P Value†
Whole sample				
Established risk factors	0.664	Referent	0.604	Referent
Established risk factors plus troponin I	0.715	0.002	0.634	0.009
Established risk factors plus NT-pro-BNP	0.749	<0.001	0.657	<0.001
Established risk factors plus cystatin C	0.691	0.07	0.626	0.03
Established risk factors plus C-reactive protein	0.689	0.07	0.636	0.008
Established risk factors plus all biomarkers	0.766	<0.001	0.676	<0.001
Estimated difference with the addition of all biomarkers (95% CI)	0.102 (0.056 to 0.147)	<0.001	0.072 (0.041 to 0.104)	<0.001
Participants without CVD at baseline				
Established risk factors	0.688	Referent	0.638	Referent
Established risk factors plus troponin I	0.716	0.15	0.640	0.90
Established risk factors plus NT-pro-BNP	0.722	0.20	0.653	0.32
Established risk factors plus cystatin C	0.700	0.45	0.649	0.38
Established risk factors plus C-reactive protein	0.715	0.20	0.663	0.11
Established risk factors plus all biomarkers	0.748	0.03	0.668	0.09
Estimated difference with the addition of all biomarkers (95% CI)	0.059 (0.007 to 0.112)	0.03	0.030 (–0.005 to 0.064)	0.09

* Established risk factors included age at baseline (continuous variable), systolic blood pressure (continuous variable), use or nonuse of antihypertensive treatment (binary variable), total cholesterol (continuous variable), high-density lipoprotein cholesterol (continuous variable), use or nonuse of lipid-lowering treatment (binary variable), presence or absence of diabetes (binary variable), smoking status (binary variable), and body-mass index (continuous variable). The biomarkers were modeled as continuous variables. CVD denotes cardiovascular disease, and NT-pro-BNP N-terminal pro–brain natriuretic peptide.

† P values are for the comparison with the model with established risk factors.

uretic peptide, troponin I, and C-reactive protein (0.748; P<0.03) (Table 3 of the Supplementary Appendix).

The replacement of body-mass index with waist-to-hip ratio in the model with established risk factors did not alter the results (data not shown).

Reclassification for participants who died from cardiovascular causes and for those who did not die in the subgroup of participants who did not have cardiovascular disease at baseline is summarized in Table 4. For 16 participants who died from cardiovascular causes, reclassification was more accurate when the model with all of the biomarkers was used, and for 7 participants, it became less accurate. Among the subjects who did not die, 115 were reclassified in a lower risk category and 60 were reclassified in a higher risk category. The net improvement in reclassification was estimated at 0.26 (P=0.005) after the addition of all of the biomarkers, and the integrated discrimination improvement was estimated as 0.074 (P=0.002). The estimates of integrated discrimination improvement suggest that the separate addition of troponin I, N-terminal pro–brain natriuretic peptide, and C-reactive protein or the addition of any combination of two or three biomarkers to the model with established risk factors improved the discriminatory property of the model for the prediction of risk among participants without cardiovascular disease at baseline (P<0.05 for all comparisons) (Table 3 of the Supplementary Appendix).

CALIBRATION

The P values for the Grønnesby and Borgan statistics indicated good calibration for the model with and without all four biomarkers, both in the whole cohort and in the group of participants who did not have cardiovascular disease at baseline (P>0.11 for all comparisons).

GLOBAL MODEL FIT

Models that included all four biomarkers showed better global fit than models with only the established risk factors, as evaluated by likelihood-ratio tests (P<0.001).

DISCUSSION

In this community-based sample of elderly men, the incorporation of a combination of biomarkers that reflect myocardial cell damage, ventricular function, renal function, and inflammation to a model with established risk factors improved the risk stratification for death from cardiovascular causes, as evidenced by a substantial increase in the C statistic. The results were consistent when

Table 4. Reclassification of Participants without Cardiovascular Disease at Baseline Who Died from Cardiovascular Causes or Who Did Not Die.*

Model with Established Risk Factors	Model with Established Risk Factors and Biomarkers			
	<6% Risk	6–20% Risk	>20% Risk	Total No.
		number (percent)		
Participants who died from cardiovascular causes				
<6% risk	9 (64.3)	5 (35.7)	0 (0)	14
6–20% risk	4 (14.8)	12 (44.4)	11 (40.7)	27
>20% risk	0 (0)	3 (23.1)	10 (76.9)	13
Total no.	13	20	21	54
Participants who did not die				
<6% risk	279 (86.6)	41 (12.7)	2 (0.6)	322
6–20% risk	102 (39.5)	137 (53.1)	19 (7.4)	258
>20% risk	2 (7.4)	13 (48.1)	12 (44.4)	27
Total no.	383	191	33	607

* Established risk factors included age at baseline (continuous variable), systolic blood pressure (continuous variable), use or nonuse of antihypertensive treatment (binary variable), total cholesterol (continuous variable), high-density lipoprotein cholesterol (continuous variable), use or nonuse of lipid-lowering treatment (binary variable), presence or absence of diabetes (binary variable), smoking status (binary variable), and body-mass index (continuous variable). The biomarkers were modeled as continuous variables. The net reclassification improvement was estimated at 0.26 (P=0.005).

we applied the model to participants who did not have apparent cardiovascular disease at baseline and when we used either the cutoff points for the biomarkers that have been suggested in the literature or those that were identified in the present study in order to achieve optimal discrimination. The improvement in risk assessment remained strong when it was estimated by means of statistical measures that evaluate model discrimination, model calibration, and global model fit.

In previous community-based studies that have evaluated a multimarker approach to the prediction of cardiovascular events,[1,3,21] the addition of multiple biomarkers to a model with established risk factors resulted in minor increases in the C statistic that did not reach statistical significance. The discrepancy between the results of the present study and those of previous studies might be explained, in part, by two key differences.

First, the participants in the present study were, on average, more than 10 years older than participants in previous studies. The fact that we evaluated the addition of the biomarkers in an elderly cohort may be of particular importance because the relative risk that is associated with the established risk factors has been shown to diminish with advancing age.[22-24] This observation is supported by the fact that the C statistic for the established risk factors in the present study appears to be lower than that in previous studies of cohorts with younger participants.[1-3,21] It is possible that the established risk factors would have performed better in a sample of younger people who had a lower absolute risk and a lower prevalence of subclinical cardiovascular damage.

Second, the predictive ability of the combination of N-terminal pro–brain natriuretic peptide, troponin I, cystatin C, and C-reactive protein was not evaluated in previous studies. These biomarkers have been suggested to be closely associated with cardiovascular and renal damage.[25-28] A recent study showed that 20% of 70-year-old Swedish men and women who did not have apparent cardiovascular disease had myocardial scarring, possibly as a result of previous, unrecognized myocardial infarctions.[29] Thus, one reason that the addition of these biomarkers contributed to the prediction of death from cardiovascular causes in people who did not have apparent cardiovascular disease may be that they better identify those who have structural and functional abnormalities in the cardiovascular system that have not yet led to overt cardiovascular disease.

In our group of subjects without cardiovascular disease at baseline, none of the biomarkers significantly increased the C statistic when they were added separately to a model with established risk (Table 3), a finding that is consistent with the results of previous studies.[1-3] However, the combination of all of the biomarkers increased the C statistic. This finding supports the rationale of adding multiple biomarkers that reflect different disease pathways in assessing the risk of death from cardiovascular causes.

The C statistic is the most commonly used method of determining model discrimination — how well the model can discriminate between persons in whom the disease of interest will develop and those who will remain free of the disease. In the present study, we also evaluated a recently described measure of model discrimination (integrated discrimination improvement),[5] which appears to be a more sensitive test of improvement in model discrimination than the C-statistic analyses (Table 3 of the Supplementary Appendix). Our findings suggest that it may be worthwhile to reevaluate previous studies in which biomarkers were discarded as potentially important risk factors because of a nonsignificant increase in the C statistic.

The sole reliance on the C statistic for the evaluation of biomarkers as risk predictors has been questioned, because very large independent associations of a new marker with the outcome are required to result in a significant increase in the C statistic.[4-6,30] Consequently, the significant increments in the C statistic in the present study indicate that our multimarker approach represents a substantial improvement in the performance of the model. The consistency of results across all models, subgroups, and statistical methods of determining model improvement provides further support for the validity of our findings.

Because we examined only men of the same age with a similar ethnic background, the generalizability of our findings to women or to other age and ethnic groups is unknown. Further studies are needed to determine whether the combination of biomarkers improves the risk stratification in women, in younger persons, and in other ethnic groups.

All of the biomarkers were analyzed in sam-

ples that had been frozen for more than 10 years. Consequently, there is a risk that the absolute levels of the biomarkers could have been affected by having been taken from frozen, rather than fresh, samples. Ideally, future studies should validate the suggested optimal cutoff points for the biomarkers in fresh samples.

Whereas it has been shown that reducing the levels of established risk factors decreases the risk of cardiovascular disease in the general population, there is currently little evidence that reducing the levels of the biomarkers will reduce the risk. Thus, our data should not be construed as implying a direct benefit of a reduction in the biomarkers. We selected biomarkers that have previously been shown to be promising predictors of cardiovascular events in the general population[7-10]; it is possible that other biomarkers that were not tested would have provided additional prognostic information.

Our data suggest that the approach of simultaneously adding several biomarkers of cardiovascular disease to the model that includes the established risk factors substantially improves the risk stratification for death from cardiovascular causes among elderly men, both those with and those without prevalent cardiovascular disease. If these results are validated, the incorporation of these factors in clinical practice for the prediction of death from cardiovascular causes could be accomplished quickly, since the measurement of these biomarkers is already well established for diagnostic use.

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