Novel and Conventional Biomarkers for Prediction of Incident Cardiovascular Events in the Community

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Context  Prior studies have demonstrated conflicting results regarding how much information novel biomarkers add to cardiovascular risk assessment.

Objective  To evaluate the utility of contemporary biomarkers for predicting cardiovascular risk when added to conventional risk factors.

Design, Setting, and Participants  Cohort study of 5067 participants (mean age, 58 years; 60% women) without cardiovascular disease from Malmö, Sweden, who attended a baseline examination between 1991 and 1994. Participants underwent measurement of C-reactive protein (CRP), cystatin C, lipoprotein-associated phospholipase 2, midregional proadrenomedullin (MR-proADM), midregional proatrial natriuretic peptide, and N-terminal pro-B-type natriuretic peptide (N-BNP) and underwent follow-up until 2006 using the Swedish national hospital discharge and cause-of-death registers and the Stroke in Malmö register for first cardiovascular events (myocardial infarction, stroke, coronary death).

Main Outcome Measures  Incident cardiovascular and coronary events.

Results  During median follow-up of 12.8 years, there were 418 cardiovascular and 230 coronary events. Models with conventional risk factors had C statistics of 0.758 (95% confidence interval [CI], 0.734 to 0.781) and 0.760 (0.730 to 0.789) for cardiovascular and coronary events, respectively. Biomarkers retained in backward-elimination models were CRP and N-BNP for cardiovascular events and MR-proADM and N-BNP for coronary events, which increased the C statistic by 0.007 (P = .04) and 0.009 (P = .08), respectively. The proportion of participants reclassified was modest (8% for cardiovascular risk, 5% for coronary risk). Net reclassification improvement was nonsignificant for cardiovascular events (0.0%; 95% CI, −0.3% to 4.3%) and coronary events (4.7%; 95% CI, −0.76% to 10.1%). Greater improvements were observed in analyses restricted to intermediate-risk individuals (cardiovascular events: 7.4%; 95% CI, 0.7% to 14.1%; P = .02; coronary events: 14.6%; 95% CI, 5.0% to 24.2%; P = .003). However, correct reclassification was almost entirely confined to down-classification of individuals without events rather than up-classification of those with events.

Conclusions  Selected biomarkers may be used to predict future cardiovascular events, but the gains over conventional risk factors are minimal. Risk classification improved in intermediate-risk individuals, mainly through the identification of those unlikely to develop events.


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density lipoprotein cholesterol levels less than 130 mg/dL (3.37 mmol/L) and C-reactive protein (CRP) levels of 2 mg/L (19 nmol/L) or greater.2

However, prior studies have reached differing conclusions regarding the utility of biomarkers for cardiovascular risk prediction. Some reports indicate that biomarkers such as CRP aid risk prediction,3,4 whereas other studies conclude that such biomarkers contribute relatively little incremental information.5,6 A number of factors influence how well biomarkers predict outcomes, including the population studied, the statistical methods for evaluating the biomarkers, and the specific biomarkers selected. Studies focusing on high-risk populations often yield favorable estimates of biomarker performance,3,7 but the greatest need for new risk markers exists in low- to intermediate-risk populations, for whom the data are most conflicting.8

With regard to the statistical approaches to evaluating new biomarkers, it is widely accepted that basic association measures such as hazard ratios or odds ratios alone are insufficient to assess prognostic utility.9 Newer metrics assess how well biomarkers assign patients to clinical risk categories,3,10 but studies are only beginning to incorporate such metrics.4 Another important consideration is the selection of biomarkers. Although several biomarkers consistently predict cardiovascular events after adjustment for conventional risk factors,11 few population-based studies have incorporated multiple informative biomarkers simultaneously, an approach that has the greatest prospect of providing incremental information.8

Although CRP and N-terminal pro-B-type natriuretic peptide (N-BNP) are relatively well studied in the primary prevention setting,6 a variety of newer biomarkers have generated interest as well.12-15 Cystatin C is a novel marker of renal function that predicts cardiovascular events better than serum creatinine.13 Lipoprotein- associated phospholipase 2 (Lp-PLA2) has been related to cardiovascular risk16,17 and has attracted interest because of the development of pharmacological agents inhibiting Lp-PLA2.18 Midregional proadrenomedullin (MR-proADM) and midregional proatrial natriuretic peptide (MR-proANP) are newer biomarkers that predict prognosis in patients following myocardial infarction.19,20

The present investigation was undertaken to address the limitations of prior studies assessing biomarkers for primary cardiovascular prevention. We studied a large, middle-aged, population-based cohort without cardiovascular disease, using a variety of newer statistical measures designed specifically to evaluate risk-prediction models. We assessed both older (CRP, N-BNP) and newer (cystatin C, Lp-PLA2, MR-proADM, MR-proANP) cardiovascular biomarkers, individually and in combination, compared with a basic model including conventional risk factors.

METHODS

Study Population

The Malmö Diet and Cancer (MDC) study is a population-based, prospective epidemiologic cohort of 28,449 persons enrolled between 1991 and 1996. From this cohort, 6103 persons were randomly selected to participate in the MDC cardiovascular cohort, which was designed to investigate the epidemiology of carotid artery disease.16 We excluded participants with prior myocardial infarction or stroke at baseline (n=143). Of the remaining participants, fasting plasma samples were available for 5400; among these, complete data on conventional cardiovascular risk factors were available for 5067. The individual plasma biomarkers were successfully measured in 4713 to 4936 of the 5067 participants. Participants with measurement of biomarkers did not differ from eligible participants in the original MDC cardiovascular cohort with regard to mean age, sex, mean systolic and diastolic blood pressure, mean body mass index, and smoking prevalence.

All participants provided written informed consent, and the study was approved by the ethical committee at Lund University, Lund, Sweden.

Clinical Examination and Assays

Participants underwent a medical history, physical examination, and laboratory assessment. Blood pressure was measured using a mercury-column sphygmomanometer after 10 minutes of rest in the supine position. Hypertension was defined as systolic or diastolic blood pressure of 140/90 mm Hg or greater or use of antihypertensive medication. Diabetes mellitus was defined as a fasting whole blood glucose level greater than 109 mg/dL (6.0 mmol/L), a self-reported physician diagnosis of diabetes, or use of antidiabetic medication. Cigarette smoking was elicited by a self-administered questionnaire, with current cigarette smoking defined as any use within the past year. We measured fasting levels of total cholesterol, high-density lipoprotein cholesterol, and triglycerides according to standard procedures at the Department of Clinical Chemistry, University Hospital Malmö. Levels of low-density lipoprotein cholesterol were calculated according to the Friedewald formula.

Cardiovascular biomarkers were analyzed in fasting EDTA plasma specimens that had been frozen at −80°C immediately after collection. The selection of biomarkers was based on the results of prior population- and hospital-based studies.3,6,12-15

Levels of CRP were measured by high-sensitivity assay (Roche Diagnostics, Basel, Switzerland). Levels of cystatin C were measured using a particle-enhanced immunonephelometric assay (N Latex Cystatin C; Dade Behring, Deerfield, Illinois).13 Lp-PLA2 activity was measured in duplicate using [3H]-platelet activating factor as substrate.16 Levels of MR-proADM and MR-proANP were measured using immunoluminometric sandwich assays targeted against amino acids in the midregions of the respective peptide (BRAHMS AG, Hennigsdorf, Germany).20,21 Levels of N-BNP were determined using the Dimension RxL automated N-BNP method (Siemens Diagnostics, Nürnberg, Germany).22 The minimum
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detection limits for MR-proADM and MR-proANP were 0.08 nmol/L and 6 pmol/L, respectively. Censoring by the lower detection limit occurred in 7 individuals for MR-proADM and in 3 individuals for MR-proANP. The maximum detection limits for MR-proADM and MR-proANP were 25 nmol/L and 3000 pmol/L, respectively, and no individuals were censored at these thresholds.

Mean interassay coefficients of variation were 4.6% for CRP, 4.3% for cystatin C, 5.8% for Lp-PLA2, 10% or less for MR-proADM and MR-proANP, and 2.7% for N-BNP.

Clinical End Points
We examined 2 primary outcomes: coronary events and cardiovascular events. The procedure for ascertaining outcome events has been detailed previously.23,24 Coronary events were defined as fatal or nonfatal myocardial infarction or death due to ischemic heart disease. Cardiovascular events were defined as coronary events or fatal or nonfatal stroke. Events were identified through linkage of the 10-digit personal identification number of each Swedish citizen with 3 registries: the Swedish Hospital Discharge Register, the Swedish Cause of Death Register, and the Stroke in Malmö register. Myocardial infarction was defined on the basis of International Classification of Diseases, 9th and 10th revisions (ICD-9 and ICD-10) codes 410 and 121, respectively. Death attributable to ischemic heart disease was defined on the basis of codes 412 and 414 (ICD-9) or 122-123 and 125 (ICD-10). Fatal or nonfatal stroke was defined using codes 430, 431, 434, and 436 (ICD-9) and 160, 161, 163, and 164 (ICD-10). Classification of outcomes using these registries has been previously validated.25,26 and the sensitivity of the registry for detecting events such as myocardial infarction has been shown to exceed 90%.27 Follow-up for outcomes extended to January 1, 2006.

We also analyzed 2 secondary outcomes: total mortality and total cardiovascular events (including heart failure). Heart failure was defined from the Swedish Hospital Discharge Register using codes 429 (ICD-9) and 150 (ICD-10). The primary diagnosis of heart failure in the Swedish Hospital Discharge Register has been shown to have an accuracy of 95%.28

Statistical Analyses
Continuous biomarker variables with right-skewed distributions (CRP, MR-proANP, and N-BNP) were log-transformed before analysis. We performed multivariable Cox proportional hazards models to examine the association between biomarkers and incident events. All models were adjusted for age, sex, systolic blood pressure, diastolic blood pressure, use of antihypertensive therapy, current smoking, diabetes, levels of low-density and high-density lipoprotein cholesterol, and body mass index. We confirmed that the proportionality of hazards assumption was met. Hazard ratios (HRs) were expressed per 1-SD increment in the respective biomarker.

Each biomarker was individually tested in models for cardiovascular and coronary events, with adjustment for conventional risk factors. The initial analyses used all participants with available data for the biomarker being studied. Thus, sample sizes for these analyses ranged from 4713 (for cystatin C and N-BNP) to 4936 (for Lp-PLA2), corresponding to the number of participants in whom the biomarker was measured.

We then examined the joint and comparative value of biomarkers for predicting cardiovascular and coronary events. These analyses included only the biomarkers with a statistically significant association with the end point in the initial stage (5 biomarkers for cardiovascular events and 3 biomarkers for coronary events). To standardize the number of participants for the biomarker–risk factor comparisons and the biomarker-biomarker comparisons, we restricted subsequent analyses to participants with complete data on all biomarkers being studied for the respective end point (n=4483 for cardiovascular events and n=4600 for coronary events). In this common sample, we examined models with no biomarkers, models with individual biomarkers, and models with multiple biomarkers. For models with multiple biomarkers, we entered all biomarkers into a backward elimination model, with the conventional risk factors forced in and a retention elimination model, with the conventional risk factors forced in and a retention P value less than .05. With the observed incidence rates, we had 80% power at α=.05 to detect HRs for a 1-SD increase in any biomarker of 1.17 for cardiovascular events and 1.22 for coronary events.

To assess model discrimination, we calculated the C statistic for models with conventional risk factors with and without biomarkers.29 To assess global calibration of the risk models, we calculated modified Hosmer-Lemeshow statistics for models with and without biomarkers.30 We also evaluated the ability of biomarkers to reclassify risk, following methods suggested previously.3,10 Using multivariable risk models with the clinical covariates noted above, participants were initially classified as at low, intermediate, or high risk if their predicted 10-year risk of a coronary event was less than 6%, 6% to less than 20%, or 20% or greater, respectively. Clinical covariates were entered into the model as continuous variables, with the exception of sex, cigarette smoking, use of antihypertensive therapy, and diabetes, which were entered as dichotomous variables. Participants could then be reclassified into different categories with the addition of the biomarker data. We assessed the number of participants reclassified and also calculated the net reclassification improvement (NRI) and integrated discrimination improvement (IDI).10

In secondary analyses, we repeated the reclassification analysis using the National Cholesterol Education Program Adult Treatment Panel III (ATP III) algorithm as the base clinical model.31 Under ATP III, individuals with diabetes are automatically assigned to the highest risk category.

All analyses were performed using Stata version 8.0 (StataCorp, College Station, Texas).
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Station, Texas), except for the tests for the proportionality of hazards assumption, which were performed using the survival package for R, and the C statistics, which were generated using the ROCR package for R (available at http://www.r-project.org). Tests were considered significant at a 2-sided \( P < .05 \).

The principal investigators (O.M., C.N.-C., T.J.W.) initiated and designed the study, selected the biomarkers, and performed all analyses. Industry sponsors provided support for the biomarker measurements but had no access to the primary study data.

RESULTS

Characteristics of the study sample are shown in Table 1. The mean age was 58 (SD, 6) years. Hypertension was common, with 3204 participants (63%) taking antihypertensive therapy or having a blood pressure of 140/90 mm Hg or higher. Diabetes mellitus was present in 391 participants (8%). Median follow-up was 12.8 (interquartile range, 12.1-13.5) years; during that time, there were 418 cardiovascular and 230 cardiac events.

The highest age- and sex-adjusted correlations between biomarkers were observed between MR-proANP and N-BNP \( (r=0.47; 95\% \text{ confidence interval [CI], 0.43 to 0.49}) \) and between cystatin C and MR-proADM \( (r=0.47; 95\% \text{ CI, 0.45 to 0.49}) \).

Prediction of Cardiovascular Events Using Single Biomarkers

The proportionality of hazards criterion was met in all analyses of biomarkers in relation to cardiovascular and coronary events. The 10-year incidence of cardiovascular events was 7.8%. After adjustment for conventional risk factors, 5 of 6 biomarkers examined individually showed a significant relationship with incident cardiovascular events. The comparative performance of the biomarkers was assessed in the 4483 participants with data on all 5 biomarkers; of these, 364 experienced a first incident cardiovascular event during follow-up. Multivariable-adjusted HRs for each biomarker are shown in Table 2. The strongest associations were observed for CRP (multivariable-adjusted HR per 1-SD increment in CRP, 1.19; 95% CI, 1.07 to 1.32) and N-BNP (1.22; 95% CI, 1.10 to 1.36).

Several metrics were used to summarize the prognostic utility of adding individual biomarkers to conventional risk factors (Table 2). A model based on conventional risk factors had a C statistic of 0.758 (95% CI, 0.734 to 0.781), and the addition of individual biomarkers resulted in small increases in the C statistic (all changes less than 0.005) (Table 2). Models with or without biomarkers were well calibrated, with modified Hosmer-Lemeshow \( P \) values greater than .05. The NRI and IDI were nonsignificant for all biomarkers.

Prediction of Coronary Events Using Single Biomarkers

The 10-year incidence of coronary events was 4.4%. Three biomarkers (cystatin C, MR-proADM, and N-BNP) were significant predictors of first incident coronary events after multivariable adjustment. The adjusted HR associated with CRP had borderline significance (\( P = .05 \)).

Results based on the 4600 participants with data on the 3 significant biomarkers, in whom there were 216 first incident coronary events, are shown in Table 2. Increased levels of MR-proADM and N-BNP were associated with the highest hazards for coronary events, with adjusted HRs per 1-SD increment of 1.21 (95% CI, 1.07 to 1.37) and 1.28 (95% CI, 1.12 to 1.47), respectively. The C statistic associated with conventional risk factors for predicting coronary events was 0.760 (95% CI, 0.730 to 0.789). As with cardiovascular factors, 5 of 6 biomarkers examined individually showed a significant relationship with incident cardiovascular events. The comparative performance of the biomarkers was assessed in the 4483 participants with data on all 5 biomarkers; of these, 364 experienced a first incident cardiovascular event during follow-up. Multivariable-adjusted HRs for each biomarker are shown in Table 2. The strongest associations were observed for CRP (multivariable-adjusted HR per 1-SD increment in CRP, 1.19; 95% CI, 1.07 to 1.32) and N-BNP (1.22; 95% CI, 1.10 to 1.36).

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cular events, addition of individual biomarkers did not increase the C statistic appreciably (Table 2). Model calibration was good ( Hosmer-Lemeshow \( P > .05 \)) with or without biomarkers, and the NRI was nonsignificant. The IDI was significant for MR-proADM \( (P = .02) \) and borderline significant for N-BNP \( (P = .08) \).

**Multiple Biomarkers for Cardiovascular and Coronary Events**

In backward elimination models, 2 biomarkers were retained for prediction of cardiovascular events (CRP and N-BNP), and 2 biomarkers were retained for prediction of coronary events (MR-proADM and N-BNP). Results of multivariable Cox proportional hazards models for both outcomes are shown in Table 3. Incorporation of the set of significant biomarkers into prediction models for cardiovascular and coronary events led to small increments (approximately 0.01) in the C statistics. The NRI was nonsignificant for cardiovascular events \( (0.8\% \); 95% CI, \(-4.3\% \) to 4.3%; \( P = .99 \)) and coronary events \( (4.7\% \); 95% CI, \(-0.76\% \) to 10.1%; \( P = .09 \)). The IDI had \( P \) values of .08 for cardiovascular events and .03 for coronary events. Results for C statistics, NRI, and IDI were unchanged when models were modified to include either all biomarkers retained at \( P < .10 \) or all biomarkers regardless of \( P \) value.

**Table 2. Individual Biomarkers and Incident Cardiovascular and Coronary Events**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Multivariable-Adjusted HR (95% CI)</th>
<th>( P ) Value</th>
<th>C Statistic ( P ) Value</th>
<th>NRI ( P ) Value</th>
<th>( P ) Value for IDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>First cardiovascular events</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>1.19 (1.07-1.32)</td>
<td>.002</td>
<td>.003</td>
<td>.14</td>
<td>1.1</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>1.13 (1.03-1.23)</td>
<td>.006</td>
<td>.004</td>
<td>.01</td>
<td>0.6</td>
</tr>
<tr>
<td>MR-proADM</td>
<td>1.12 (1.01-1.24)</td>
<td>.04</td>
<td>0.003</td>
<td>.03</td>
<td>−1.4</td>
</tr>
<tr>
<td>MR-proANP</td>
<td>1.12 (1.00-1.25)</td>
<td>.04</td>
<td>0.001</td>
<td>.49</td>
<td>−0.2</td>
</tr>
<tr>
<td>N-BNP</td>
<td>1.22 (1.10-1.36)</td>
<td>&lt;.001</td>
<td>0.004</td>
<td>.12</td>
<td>0.4</td>
</tr>
<tr>
<td>First coronary events</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystatin C</td>
<td>1.15 (1.04-1.27)</td>
<td>.006</td>
<td>.004</td>
<td>.09</td>
<td>0.9</td>
</tr>
<tr>
<td>MR-proADM</td>
<td>1.21 (1.07-1.37)</td>
<td>.002</td>
<td>0.004</td>
<td>.14</td>
<td>2.4</td>
</tr>
<tr>
<td>N-BNP</td>
<td>1.28 (1.12-1.47)</td>
<td>&lt;.001</td>
<td>0.006</td>
<td>.21</td>
<td>1.2</td>
</tr>
</tbody>
</table>

**Table 3. Multiple Biomarkers and Incident Cardiovascular and Coronary Events**

<table>
<thead>
<tr>
<th>Biomarkers Retained by Backward Elimination</th>
<th>Multivariable-Adjusted HR (95% CI)</th>
<th>( P ) Value</th>
<th>C Statistic ( P ) Value</th>
<th>NRI ( P ) Value</th>
<th>( P ) Value for IDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>First cardiovascular events</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>1.17 (1.05-1.30)</td>
<td>.005</td>
<td>.005</td>
<td>.04</td>
<td>0.0</td>
</tr>
<tr>
<td>N-BNP</td>
<td>1.21 (1.08-1.34)</td>
<td>.001</td>
<td>0.007</td>
<td>.04</td>
<td>0.0</td>
</tr>
<tr>
<td>First coronary events</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MR-proADM</td>
<td>1.16 (1.02-1.32)</td>
<td>.02</td>
<td>.000</td>
<td>.08</td>
<td>4.7</td>
</tr>
<tr>
<td>N-BNP</td>
<td>1.24 (1.08-1.42)</td>
<td>.002</td>
<td>0.009</td>
<td>.08</td>
<td>4.7</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; HR, hazard ratio; IDI, integrated discrimination index; MR-proADM, midregional proadrenomedullin; MR-proANP, midregional proatrial natriuretic peptide; N-BNP, N-terminal pro-B-type natriuretic peptide; NRI, net reclassification improvement.

Analyses based on a sample restricted to individuals with biomarkers shown in analyses of first cardiovascular events \( (n=4483 [364 events]) \) and first coronary events \( (n=4600 [216 events]) \). Multivariable Cox proportional hazards models were adjusted for age, sex, antihypertensive treatment, systolic blood pressure, diastolic blood pressure, body mass index, diabetes, levels of low-density and high-density lipoprotein cholesterol, and current smoking. For each end point, all biomarkers shown were entered together into the Cox model.

Hazard ratios expressed per 1-SD increment in biomarker concentration.

Increment in C statistic in a model with conventional risk factors and the biomarker set, compared with conventional risk factors alone \( (C=0.758 \) for cardiovascular events; \( C=0.760 \) for coronary events).

\( P \) value for increase in NRI or IDI in a model with conventional risk factors and the biomarker set, compared with conventional risk factors alone.
moved into the high-risk category. When high risk was redefined as a 10-year predicted risk of 10% or greater rather than 20%, the proportion of individuals reclassified as at high risk using biomarkers remained small (2.3% for cardiovascular events and 1.2% for coronary events).

Calibration was essentially the same in models with and without biomarkers. For cardiovascular events, actual event rates in the low-, intermediate-, and high-risk groups were 2%, 9%, and 22%, respectively, with conventional risk factors and 2%, 11%, and 24%, respectively, with conventional risk factors and biomarkers. Corresponding event rates for coronary disease were 2%, 9%, and 27%, respectively, with conventional risk factors and 2%, 10%, and 23% with risk factors and biomarkers.

We also assessed reclassification using the ATP III algorithm as the base clinical model rather than a model fitted using the Malmö data. With the addition of biomarkers to the ATP III algorithm, the NRI was significant for cardiovascular events, although the net proportion correctly reclassified was still modest (6.2%, P = .004). The NRI was nonsignificant for coronary events (P = .89).

### Analyses in Intermediate-Risk Participants

We performed additional analyses restricted to intermediate-risk participants (10-year predicted risk, 6% to 20%). Most intermediate-risk participants (57% for cardiovascular events and 59% for coronary events) had 10-year predicted risks less than 10%. Women comprised 44% of the intermediate-risk group for cardiovascular events and 26% of the intermediate-risk group for coronary events.

For cardiovascular disease, 13% of the overall number of intermediate-risk individuals were down-classified, and only 3% were up-classified. The NRI for this subgroup was significant (7.4%; 95% CI, 0.7% to 14.1%; P = .03), although this was driven solely by individuals without events who were correctly down-classified (133/973 [14%]). Among those with events, a greater number (n = 10 [8%]) were inappropriately down-classified than appropriately up-classified (n = 6 [4%]). Similarly, for coronary disease events, 19% were down-classified and only 4% were up-classified. The NRI was 14.6% (95% CI, 5.0% to 24.2%; P = .003), owing to the high proportion of individuals without events who were down-classified (107/525 [20%]). Among intermediate-risk individuals with coronary events, 3 (6%) were inappropriately down-classified and 2 (4%) were appropriately up-classified.

### Multimarker Scores

Simple “multimarker” risk scores were constructed for each end point. For each participant, standardized values of each biomarker (expressed in SD units from the mean) were summed to produce a score. Score values were then divided into quartiles (with the lowest scores in quartile 1). The median (range) multimarker scores in each quartile for cardiovascular events were −1.66 (−5.47 to −1.01), −0.52 (−1.01 to −0.04), 0.37 (−0.04 to 0.90), and 1.65 (0.90 to 5.62). The median (range) multimarker scores in each quartile for coronary events were −1.62 (−5.14 to −1.02), −0.50 (−1.02 to −0.06), 0.38 (−0.06 to 0.88), and 1.60 (0.88 to 11.65).

The Figure depicts the cumulative incidence of cardiovascular or coronary events, according to quartiles of the biomarker risk scores. In multivariable-adjusted models, HRs associated with the second through fourth quartiles of the score for cardiovascular events were 1.07 (95% CI, 0.75-1.52), 1.36 (0.98-1.89), and 1.61 (1.17-2.23) (P = .001 for trend). Adding this cardiovascular disease biomarker score to conventional risk factors resulted in small improvements in the C statistic (0.007), the NRI (0.0%, P = .88), and the IDI (P = .09). Adjusted HRs associated with the second through fourth quartiles of the score for coronary events were 1.01 (95% CI, 0.64-1.59), 1.11 (0.71-1.73), and 1.86 (1.22-2.83) (P = .001 for trend). Adding the score for coronary events to conventional risk factors increased the C statistic by 0.009, and the IDI was insignificant (P = .02). The NRI remained nonsignificant (5.5%, P = .06).

### Secondary End Points

There were 392 all-cause deaths in the follow-up period. In the stepwise prediction model for mortality, 3 biomarkers were retained: CRP (multivariable-adjusted HR per 1-SD increment in CRP, 1.16; 95% CI, 1.03-1.28; P = .007), MR-proADM (1.14; 95% CI, 1.03-1.26; P = .01), and N-BNP (1.13; 95% CI, 1.02-1.26; P = .02). The addition of biomarkers increased the C statistic for predicting total mortality from 0.700 to 0.711. The IDI was significant (P < .001). The NRI was not calculated, owing to the absence of clinical risk categories for mortality.
The addition of heart failure to the cardiovascular end point (481 events overall) did not change the biomarkers retained in the stepwise model (CRP [multivariable-adjusted HR, 1.22; 95% CI, 1.10-1.35; \(P < .001\)] and N-BNP [1.29; 95% CI, 1.17-1.43; \(P < .001\)]). The C statistic increased from 0.759 to 0.770, and the IDI was significant \((P = .003)\). The NRI remained nonsignificant \((1.5\%, P = .52)\).

**COMMENT**

We investigated a panel of contemporary biomarkers for predicting cardiovascular risk above and beyond conventional risk factors, in a population-based cohort with more than 50 000 person-years of longitudinal follow-up. When considered individually, 5 biomarkers predicted future cardiovascular events, and 3 predicted future coronary events in models adjusting for conventional risk factors. The best combinations of biomarkers were CRP and N-BNP for predicting cardiovascular events and MR-proADM and N-BNP for predicting coronary events. The use of multiple biomarkers minimally improved the accuracy of risk prediction models over and above conventional cardiovascular risk factors and did not reclassify a substantial proportion of individuals to higher or lower risk categories.

Whether novel biomarkers add useful information for risk prediction has been the focus of intense scrutiny in the cardiovascular literature. Conflicting findings have been attributed to a variety of factors. Inadequate statistical power, use of older biomarkers, and lack of consideration of measures such as calibration and reclassification have been invoked to explain the poor performance of biomarkers in some studies. Conversely, it has been argued that other studies overestimate the relative utility of biomarkers by examining homogeneous or highly selected samples or by using end points such as mortality that are poorly predicted by conventional cardiovascular risk factors.

The present study was undertaken to address these shortcomings. As one of the largest population-based studies of multiple biomarkers, it provides a clearer picture of the strengths and limitations of potential biomarker strategies in primary prevention. With use of biomarkers, it is possible to define groups with 2-fold differences in cardiovascular risk. Nonetheless, the translation of this benefit to individual risk prediction appears minimal.

Adding biomarkers to conventional risk factors improves the C statistic only minimally, a finding that confirms observations from several prior studies. Because the C statistic has been criticized as insensitive to small changes in predictive accuracy, we also calculated a newer measure called the IDI. This metric improves when novel markers correctly assign individuals to higher or lower probabilities of having events. The multimarker approach led to a near-significant change in the IDI for cardiovascular events (driven mainly by N-BNP) and to significant changes for coronary events (driven by MR-proADM and N-BNP) and total mortality (driven by CRP and N-BNP).

What may be relevant to clinical care, however, is not whether changes in predicted probabilities are statistically significant but whether they result in reclassification of individuals to new, clinically meaningful risk categories. Our data indicate that a relatively small proportion of individuals are moved to new risk categories by the addition of biomarkers—8% or fewer when both upward and downward risk category movement are included and fewer than

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**Figure.** One Minus Cumulative Cardiovascular and Coronary Event-Free Survival During Follow-up in Quartiles of a Multimarker Score Based on Summed Standardized Values of Biomarkers

<table>
<thead>
<tr>
<th>No. at risk</th>
<th>Quartile 1</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
</tr>
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<td>1094</td>
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</tr>
<tr>
<td>4</td>
<td>1106</td>
<td>1094</td>
<td>1073</td>
<td>1040</td>
</tr>
</tbody>
</table>

CRP indicates C-reactive protein; MR-proADM, midregional proadrenomedullin; N-BNP, N-terminal pro-B-type natriuretic peptide.
1% when only the movements likely to lead to changes in therapy according to the ATP III guidelines are included. Furthermore, these reclassifications result in only modest improvements in the overall concordance between risk categories and actual event rates, as measured by the NRI.10

Rather than screening the entire adult population using biomarkers, an alternate strategy would be to focus on those individuals deemed to be at intermediate risk, often defined as having a 10-year predicted event rate of 6% to 19%.3 Our estimates of NRI are higher in this group (7.5% for cardiovascular events, P = .03; 14.6% for coronary events, P = .003). The NRI in intermediate-risk individuals has been described as the “clinical” NRI, emphasizing the potential application to clinical screening.36 However, it is notable that the significance of the NRI in this setting is driven primarily by the down-classification of individuals who do not have events. Although informative, such shifts are much less likely to lead to changes in therapy than upward shifts, at least under current guidelines. Another shortcoming of the clinical NRI is that it does not account for inaccurate reclassification from other categories into the intermediate-risk group.37 For instance, imagine a marker that reclassifies every person with an event from intermediate risk to high risk, and every person without an event from intermediate risk to low risk, but at the same time moves the same number of events from high to intermediate risk and nonevents from low to intermediate risk. Such a marker would have a perfect clinical NRI (100%) but a true NRI (considering the whole sample) of 0%.

It is possible that the performance of the biomarkers would have been superior in a cohort at higher risk. Some of the biomarkers studied, including N-BNP, have shown better discriminative ability in elderly4 or high-risk7 populations. However, individuals at low to intermediate risk comprise the group in whom novel risk markers are most needed, because a large number of cardiovascular events derive from this group, and individuals in this group are the least likely to be targeted for proven, preventive therapies.

Statins for primary prevention confer benefit in individuals across a broad range of baseline cardiovascular risk.2,38,39 However, treating unselected individuals with statins may not be practical if absolute event rates are low or therapies are expensive. Thus, reclassifying individuals as being at low or high risk could have immediate clinical relevance with regard to identifying candidates for statin therapy. Our findings support the premise that biomarkers could be used to refine these classifications but also highlight the relatively low proportion of individuals meaningfully reclassified with existing biomarkers.

These data do not exclude a future role for circulating biomarkers as adjuncts to conventional risk factors, nor do they minimize the potential for biomarkers to provide insight into underlying mechanisms of disease. Several biomarkers studied did lead to shifts in predictive accuracy that were at least statistically significant. The challenge will be to find new cardiovascular biomarkers that alone or in combination with existing biomarkers can bring about improvements in risk assessment that are not just statistically significant but clinically significant as well.40

Author Contributions: Dr Melander had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Melander, Newton-Cheh, Wang.

Acquisition of data: Melander, Hedblad, Berglund, Persson, Magnusson, Christensson, Struck, Morgenthaler, Bergman.

Analysis and interpretation of data: Melander, Newton-Cheh, Almgren, Hedblad, Engström, Smith, Magnusson, Christensson, Struck, Morgenthaler, Bergmann, Pencina, Wang.

Drafting of the manuscript: Melander, Newton-Cheh, Wang.

Critical revision of the manuscript for important intellectual content: Melander, Newton-Cheh, Almgren, Hedblad, Berglund, Engström, Persson, Smith, Magnusson, Christensson, Struck, Morgenthaler, Bergmann, Pencina, Wang.


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Study supervision: Melander, Newton-Cheh, Wang.

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BIOMARKERS AND INCIDENT CARDIOVASCULAR EVENTS


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