Comparative Impact of Multiple Biomarkers and N-Terminal Pro-Brain Natriuretic Peptide in the Context of Conventional Risk Factors for the Prediction of Recurrent Cardiovascular Events in the Heart Outcomes Prevention Evaluation (HOPE) Study

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Background—Individual markers of inflammation may add incremental predictive value in the context of conventionally available risk factors. We evaluated the ability of 9 inflammatory biomarkers, microalbuminuria, and N-terminal pro-brain natriuretic peptide (Nt-proBNP) to improve cardiovascular risk prediction beyond that obtained from traditional risk factors in a secondary-prevention population.

Methods and Results—We measured biomarkers representing the acute-phase reaction (C-reactive protein, fibrinogen, and interleukin-6), proinflammatory pathways (soluble tumor necrosis factor receptor-1 and -2, soluble interleukin-1 receptor antagonist, and interleukin-18), endothelial activation (soluble vascular adhesion molecule-1 and soluble intercellular adhesion molecule-1), Nt-proBNP, and microalbuminuria in 3199 study individuals of the Heart Outcomes Prevention Evaluation (HOPE) Study and assessed their association with risk of myocardial infarction, stroke, or cardiovascular death (primary outcome, n=501) over 4.5 years of follow-up. In a backward Cox regression procedure that included risk factors and biomarkers, Nt-proBNP (hazard ratio [HR] 1.72 per increment SD, 95% CI 1.39 to 2.12; P<0.0001), soluble intercellular adhesion molecule-1 (HR 1.46, 95% CI 1.19 to 1.80; P=0.0003), microalbuminuria (HR 1.55, 95% CI 1.22 to 1.98; P=0.0004), soluble interleukin-1 receptor antagonist (HR 1.30, 95% CI 1.05 to 1.61; P=0.02), and fibrinogen (HR 1.31, 95% CI 1.05 to 1.62; P=0.02) remained significantly related to the primary outcome. Only inclusion of Nt-proBNP provided incremental information above that obtained by models of traditional risk factors.

Conclusions—Although levels of various inflammatory biomarkers are significantly related to future cardiovascular risk, their incremental predictive value is modest. A model consisting of simple traditional risk factors and Nt-proBNP provided the best clinical prediction in the secondary-prevention population. (Circulation. 2006;114:201-208.)

Key Words: prognosis ■ cardiovascular disease ■ inflammation ■ risk factors

Recent data indicate that 9 simple risk factors, including abnormal apolipoprotein levels, smoking, diabetes mellitus, and hypertension, account for ≈90% of the risk of acute myocardial infarction globally.1 Importantly, most of these traditional risk factors are modifiable, and intervention is likely to reduce the risk of cardiovascular disease (CVD).2 A number of “novel” risk markers have been proposed as providing additional prognostic information; however, few studies had a large range of markers, and therefore, it is not clear whether each of the proposed markers provide incremental predictive information. Elevations in plasma of inflammatory biomarkers have been related to future cardiovascular events in individuals without3–6 or with7–10 prior CVD.

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Levels of brain natriuretic peptide (BNP) and its inactive N-terminal fragment (Nt-proBNP) are used for diagnosis and...
prognosis of heart failure,11 risk stratification after acute coronary syndrome,12 and to predict events both in patients with stable angina13 and among apparently healthy individuals.14 Progressive increases in microalbuminuria also predict future vascular events.15 However, it is unclear whether simultaneous testing of multiple biomarkers compared with each alone adds clinically useful incremental information. Alternatively, a few of these markers may provide most of the incremental predictive information once easily available clinical information is known.

To directly address this issue, we evaluated the incremental value of markers that reflect the acute-phase reaction (C-reactive protein [CRP], fibrinogen, and interleukin [IL]-6), the proinflammatory pathways (soluble tumor necrosis factor receptor [sTNF-R]-1 and -2, soluble IL-1 receptor antagonist [sIL-1Ra], and IL-18), endothelial cell activation (soluble vascular cell adhesion molecule [sVCAM]-1 and soluble intercellular adhesion molecule [sICAM]-1), microalbuminuria, and Nt-proBNP compared with more readily available, simple risk factors for the prediction of cardiovascular events in 3199 study individuals enrolled in the Heart Outcomes Prevention Evaluation (HOPE) Study. Of particular importance, the present study evaluates these biomarkers in a population already known to have atherosclerosis, and the results illustrate their impact in a setting of secondary prevention.

Methods

HOPE Study Population

The HOPE study was a multicenter, randomized, clinical trial of ramipril, vitamin E, both, or neither for the prevention of cardiovascular endpoints among 9541 patients with previous coronary artery disease (previous myocardial infarction, stable angina, or unstable angina, each with documented coronary artery disease or positive stress test, multivessel percutaneous transluminal coronary angioplasty, or coronary artery bypass grafting), stroke, peripheral vascular disease (PVD; claudication, a history of peripheral arterial disease, or a ratio of blood pressure in the ankle to blood pressure in at least 1 arm of <0.90), or a history of diabetes mellitus.

Patients were excluded if they had heart failure or if they were known to have a low ejection fraction (<40%), uncontrolled hypertension, or overt nephropathy or to have had a myocardial infarction or stroke in the 4 weeks before enrolment. A detailed study description has been published elsewhere.16 Baseline blood samples, taken between 1993 and 1995, have only been drawn from the 3199 Canadian HOPE study patients, who have similar baseline data compared with the overall study cohort. Blood samples were processed immediately, separated into multiple aliquots, and shipped on dry ice to the core laboratory where they were stored at −80°C. The HOPE central laboratory was located at the Hamilton Health Sciences in Hamilton, Ontario, Canada.

Clinical Events

The primary HOPE study end points were myocardial infarction, stroke, or cardiovascular death, which occurred in 501 of 3199 patients (15.7%) during a mean follow-up of 4.5 years. These end points are considered as the primary outcome. All events were centrally adjudicated by a blinded committee unaware of the patients’ status, biochemical values, or treatment allocation. Stroke did not include transient ischemic attack, and symptoms had to be present for 24 hours. Detailed information about the follow-up procedure is published elsewhere.16 We further evaluated the coronary end points (fatal [n=134] and nonfatal [n=237] myocardial infarction) separately from the stroke end points (n=108). A total of 22 individuals died of vascular causes.

The "Inflammatory and Infectious Markers in HOPE" study protocols were approved by the Research Ethics Board at McMaster University and Hamilton Health Sciences, and written informed consent was obtained from patients.

Laboratory Analysis

Serum CRP was measured with a high-sensitivity, automated-rate nephelometric immunoassay (Dade Behring high-sensitivity CRP, BNII Nephelometer System, Marburg, Germany). Plasma fibrinogen was determined by the Clauss method on the Sigma Amax (Sigma, St. Louis, Mo) with reagent from Diagnostica Stago (Asnières-sur-Seine, France). Plasma sICAM-1, IL-6, sTNF-R-1 and -2 (R&D Systems, Minneapolis, Minn, and R&D Systems, Weisbaden, Germany), sIL-1Ra, sVCAM-1 (Biosource, Nivelles, Belgium), and IL-18 (MBL Co Ltd, Watertown, Mass) were determined by use of commercially available immunoassays. The intra-assay and interassay coefficients of variations for all immunoassays mentioned above ranged between 1.8% and 16.4%. Plasma Nt-proBNP was determined with an electrochemiluminescence sandwich immunoassay (ECLIA, Roche Diagnostics, Mannheim, Germany) on an Elecsys System 2010. Intra-assay and interassay precision for the assay was 0.8% to 3.0% and 2.2% to 5.8%, respectively. The linear range of detection of this assay was 5 to 35 000 pg/mL; cross-reactivity with BNP or atrial natriuretic peptide was <0.001%. The conversion factor into pmol/L is 0.118.

Fasting baseline glucose and creatinine was determined by use of routine methods of the respective study centers. Additionally, fasting serum lipids (total cholesterol, HDL cholesterol, and triglycerides) were measured centrally in Hamilton. LDL cholesterol was calculated according to the Friedewald formula. Urinary albumin was measured as described previously.15 All biochemical analyses were performed blinded to patient status.

Statistical Analysis

Inflammatory markers and lipid variables were log-transformed to improve normality of distribution, and geometric means are presented. Pearson correlations are presented on the log-transformed values. Student t tests and χ2 tests were used to compare baseline characteristics between those with and without events. The Kaplan-Meier curves for cumulative cardiovascular events were plotted by thirds of Nt-proBNP and compared with the log-rank test.

The association of different biomarkers with events was evaluated by Cox regression analysis and stratified by ramipril and vitamin E therapy. The Wald χ2 test was used to indicate differences across thirds. Age- and sex-adjusted and fully adjusted hazard ratios were estimated. In the adjusted model, those clinical and metabolic variables have been considered as covariates, which were significantly related to the primary outcome at univariate analyses: age, sex, ratio of LDL to HDL cholesterol, diabetes mellitus, smoking status, systolic blood pressure, waist-hip ratio, triglycerides, glucose, creatinine, microalbuminuria, lipid-lowering drugs, ramipril allocation, and peripheral vascular disease. We defined the model including these risk factors as the "baseline traditional risk factor model." Furthermore, backward regression models that initially included all biomarkers and confounders mentioned above were applied. To estimate the discriminative value of predictive models, we calculated the area under the receiver operating characteristic curve (AUC) for the predicted survival from the Cox regression analysis additionally including the respective biomarkers in risk factor–adjusted models. We present the AUC for single inflammatory variables and multiple biomarkers in addition to models that already included traditional risk factors. To compare AUCs, we used a nonparametric approach to the analysis of areas under correlated receiver operating characteristic curves.18 P<0.05 was considered significant. SAS 8.0 (SAS Institute, Cary, NC) and STATA 8.0 (Stata Corp, College Station, Tex) were used for data analysis.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.
Results

Baseline Characteristics and Traditional Risk Factors
The baseline characteristics of the 3199 individuals in whom biomarkers were determined were similar to those of the overall HOPE sample. As expected, in age- and sex-adjusted models, the strongest predictors of the combined primary outcome were traditional risk factors such as current smoking (hazard ratio [HR] 1.79; 95% CI 1.35 to 2.37; \(P<0.0001\)), diabetes mellitus (HR 1.48; 95% CI 1.23 to 1.77; \(P<0.0001\)), LDL:HDL cholesterol ratio (HR per 1-SD increment 1.24; 95% CI 1.13 to 1.36; \(P<0.0001\)), and glucose concentration (HR per 1-SD increment 1.22; 95% CI 1.12 to 1.33; \(P<0.0001\)). In individuals without diabetes, the association between glucose concentration and primary outcome was stronger (HR per 1-SD increment 1.33; 95% CI 1.07 to 1.66) than in those with diabetes (HR per 1-SD increment 1.10; 95% CI 0.96 to 1.26).

The risk factors that were significantly associated with stroke were microalbuminuria (HR 1.67; 95% CI 1.06 to 2.63; \(P=0.03\)), glucose concentration (HR per 1-SD increment 2.27; 95% CI 1.07 to 4.85; \(P<0.0001\)), PVD (HR 1.84, 95% CI 1.23 to 2.74; \(P=0.003\)), and age (HR 1.08, 95% CI 1.05 to 1.1; \(P<0.0001\)). The baseline characteristics according to cardiovascular outcome are outlined in Table 1.

Correlation Coefficients
Of all biomarkers evaluated, Nt-proBNP presented the strongest correlation with age (\(r=0.33\)). The strongest association between the anthropometric variable of body mass index and inflammatory variables was observed for CRP and sIL-1Ra antagonist (\(r=0.30\) each). Between the biomarkers, correlations \(r>0.4\) were observed within the cluster of the 3 acute-phase reactants: CRP, fibrinogen, and IL-6. In addition, strong interdependence was observed between sTNF-R-1 and -2. Correlation coefficients are presented in detail in Figure 1. No correlation \(r>0.3\) has been observed between any biomarker and lipid levels, glucose, or blood pressure.

Primary Outcome
Baseline levels of inflammatory variables and Nt-proBNP were significantly higher among individuals who subsequently developed cardiovascular outcomes than among those who did not (Table 1). To assess the independent association between each biomarker and primary outcome, a series of Cox regression models was performed (online-only Data Supplement). In models that accounted for all traditional risk factors (PVD, ramipril allocation, lipid-lowering drugs, glucose, microalbuminuria, and creatinine), the following variables were independently associated with the primary cardiovascular outcome when the upper versus the lower third were compared: Nt-proBNP (HR 2.25; 95% CI 1.74 to 2.89), sICAM-1 (HR 1.32; 95% CI 1.04 to 1.66), IL-1Ra (HR 1.38; 95% CI 1.11 to 1.73), and IL-18 (HR 1.29; 95% CI 1.03 to 1.62). If the variables are considered per increment SD, results are virtually identical. In particular, Nt-proBNP was associated with a 1.52-fold increase in risk (95% CI 1.37 to 1.67; \(P<0.0001\)) for developing the primary cardiovascular outcome in a fully adjusted model. The Kaplan-Meier rate of the primary outcome according to thirds of Nt-proBNP is displayed in Figure 2. The optimal cutpoints from the receiver operating characteristic curve for the clinically relevant biomarkers CRP and Nt-proBNP were 6 mg/L and 18.4 pmol/L, respectively. In the fully adjusted Cox predictive model, individuals with CRP >6 mg/L had a 1.26-fold (95% CI 1.01 to 1.56; \(P=0.04\)) increase in risk, and individuals with Nt-proBNP >18.4 pmol/L had a 1.83-fold (95% CI 1.50 to 2.23; \(P<0.0001\)) increase in risk.

Myocardial Infarction
When an end point of fatal and nonfatal myocardial infarction (coronary events) was used rather than the composite primary outcome, the relationship between biomarker concentrations and coronary end point was of similar magnitude to that obtained by evaluating the primary outcome. Specifically, in fully adjusted models, the following variables were independently associated with the coronary end point if the upper versus the lower third were compared: Nt-proBNP (HR 2.56, 95% CI 1.91 to 3.44), sVCAM-1 (HR 1.39, 95% CI 1.07 to 1.81), IL-1Ra (HR 1.49, 95% CI 1.15 to 1.93), and IL-18 (HR 1.36; 95% CI 1.05 to 1.77).

Stroke
Age- and sex-adjusted and fully adjusted HRs for future strokes are outlined in the Data Supplement. In particular, only sICAM-1 (HR 1.91, 95% CI 1.12 to 3.25) and CRP (HR 2.16, 95% CI 1.18 to 4.00) were related to future stroke. Age-and sex-adjusted and fully adjusted rates of the primary outcome, coronary events, and stroke by increment of 1 SD and thirds of each respective biomarker are displayed in the Data Supplement.

Risk Prediction Model
To determine the most predictive variables, we performed backward stepwise regression analyses that included the predictive risk factors similar to those in the fully adjusted model of the Data Supplement and determined biomarkers in the initial model. Biomarkers have been introduced in categorized variables that compared the highest versus the lowest two thirds. Age, male sex, LDL:HDL cholesterol ratio, glucose, current smoking, PVD, and microalbuminuria, as well as fibrinogen, sIL-1RA, sICAM-1, and Nt-proBNP, remained significantly related to primary cardiovascular outcome (Table 2). The selected biomarkers did not show a significant pairwise correlation, which suggests that they were indicative of different biological processes.

Multiple Biomarkers and Cardiovascular Outcome
As outlined in Table 3, we performed analyses to evaluate the composite measure of the aggregate number of biomarkers in the highest third with respect to cardiovascular outcome. We considered the biomarkers selected from the backward logistic regression analyses, such as Nt-proBNP, fibrinogen, sICAM-1, and sIL-1RA. The HR increased with rising numbers of “positive” biomarkers, and individuals with all 4 markers in their highest third had a 2.50-fold (95% CI 1.52 to 4.12) increase in risk.
Incremental Effects of Biomarkers in Addition to Traditional Risk Factors

To explore whether any of these biomarkers added to the predictive value of traditional risk factor screening, we computed the AUC associated with risk prediction models based on a model that included all covariates outlined in the fully adjusted model (see Data Supplement or Table 3). We compared this basic model with models that additionally

<table>
<thead>
<tr>
<th>TABLE 1. Baseline Characteristics of the Study Participants</th>
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<tbody>
<tr>
<td>Characteristics*</td>
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<tr>
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<tr>
<td>Without Primary Outcome (n=2698)</td>
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<tr>
<td>With Primary Outcome (n=501)</td>
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<tr>
<td>P</td>
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<tr>
<td>Age, mean±SD, y</td>
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<tr>
<td>65.1±6.4</td>
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<tr>
<td>67.0±7.1</td>
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<tr>
<td>&lt;0.0001</td>
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<td>Male sex, n (%)</td>
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<tr>
<td>2072 (76.8)</td>
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<td>385 (83.2)</td>
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<tr>
<td>0.002</td>
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<td>Body mass index, mean±SD, kg/m²</td>
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<tr>
<td>27.8±4.3</td>
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<tr>
<td>27.8±4.3</td>
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<tr>
<td>0.88</td>
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<tr>
<td>Waist-hip ratio, mean±SD</td>
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<tr>
<td>0.93±0.08</td>
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<tr>
<td>0.94±0.07</td>
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<tr>
<td>0.001</td>
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<td>Systolic blood pressure, mean±SD, mm Hg</td>
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<tr>
<td>134.8±17.8</td>
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<tr>
<td>135.5±18.8</td>
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<tr>
<td>0.06</td>
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<tr>
<td>Diastolic blood pressure, mean±SD, mm Hg</td>
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<tr>
<td>77.5±9.5</td>
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<tr>
<td>77.8±9.5</td>
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<tr>
<td>0.5</td>
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<tr>
<td>History of hypertension, n (%)</td>
</tr>
<tr>
<td>1093 (40.5)</td>
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<tr>
<td>218 (43.7)</td>
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<tr>
<td>0.19</td>
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<tr>
<td>History of diabetes mellitus, n (%)</td>
</tr>
<tr>
<td>887 (32.9)</td>
</tr>
<tr>
<td>201 (40.3)</td>
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<tr>
<td>0.001</td>
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<tr>
<td>Smoking status, n (%)</td>
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<tr>
<td>631 (23.4)</td>
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<tr>
<td>110 (22.0)</td>
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<tr>
<td>&lt;0.0001</td>
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<tr>
<td>Never</td>
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<tr>
<td>1721 (63.8)</td>
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<tr>
<td>294 (58.7)</td>
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<tr>
<td>Prevalence of</td>
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<tr>
<td>CAD, n (%)</td>
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<tr>
<td>2307 (85.5)</td>
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<tr>
<td>436 (87.0)</td>
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<tr>
<td>0.37</td>
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<tr>
<td>PVD, n (%)</td>
</tr>
<tr>
<td>1039 (38.5)</td>
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<tr>
<td>259 (51.7)</td>
</tr>
<tr>
<td>&lt;0.0001</td>
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<tr>
<td>CVD, n (%)</td>
</tr>
<tr>
<td>2522 (93.5)</td>
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<tr>
<td>481 (96.0)</td>
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<tr>
<td>0.03</td>
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<tr>
<td>Medical treatment</td>
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<tr>
<td>Lipid-lowering agent, n (%)</td>
</tr>
<tr>
<td>890 (33.0)</td>
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<tr>
<td>127 (25.3)</td>
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<tr>
<td>0.008</td>
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<tr>
<td>Never</td>
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<tr>
<td>1276 (47.3)</td>
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<tr>
<td>207 (41.3)</td>
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<tr>
<td>0.01</td>
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<tr>
<td>Never</td>
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<tr>
<td>Total cholesterol, median (IQR), mmol/L*</td>
</tr>
<tr>
<td>5.37 (5.34–5.41)</td>
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<tr>
<td>5.44 (5.35–5.52)</td>
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<tr>
<td>0.19</td>
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<tr>
<td>LDL cholesterol, median (IQR), mmol/L</td>
</tr>
<tr>
<td>3.91 (3.35–4.48)</td>
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<tr>
<td>4.01 (3.46–4.57)</td>
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<tr>
<td>0.02</td>
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<tr>
<td>HDL cholesterol, median (IQR), mmol/L*</td>
</tr>
<tr>
<td>1.02 (1.01–1.03)</td>
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<tr>
<td>0.97 (0.95–0.99)</td>
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<tr>
<td>0.001</td>
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<tr>
<td>Ratio of LDL to HDL cholesterol (IQR) *</td>
</tr>
<tr>
<td>3.84 (3.01–4.79)</td>
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<tr>
<td>4.16 (3.34–5.06)</td>
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<tr>
<td>&lt;0.0001</td>
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<tr>
<td>Triglycerides, median (IQR), mmol/L*</td>
</tr>
<tr>
<td>1.94 (1.91–1.98)</td>
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<tr>
<td>2.03 (1.94–2.12)</td>
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<tr>
<td>0.07</td>
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<tr>
<td>Glucose, median (IQR), mmol/L*</td>
</tr>
<tr>
<td>6.41 (6.33–6.48)</td>
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<tr>
<td>6.81 (6.61–7.02)</td>
</tr>
<tr>
<td>0.002</td>
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<tr>
<td>Microalbuminuria, n (%)</td>
</tr>
<tr>
<td>367 (13.6)</td>
</tr>
<tr>
<td>123 (24.6)</td>
</tr>
<tr>
<td>&lt;0.0001</td>
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<tr>
<td>Creatinine, mean±SD, mg/dL</td>
</tr>
<tr>
<td>1.11±0.23</td>
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<tr>
<td>1.16±0.26</td>
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<tr>
<td>0.0006</td>
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<tr>
<td>Inflammatory cytokines</td>
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<tr>
<td>IL-1 receptor antagonist, median (IQR), pg/mL</td>
</tr>
<tr>
<td>192.1 (187.3–197.1)</td>
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<tr>
<td>207.7 (196.2–219.9)</td>
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<tr>
<td>0.02</td>
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<tr>
<td>sTNF-R1, median (IQR), pg/mL</td>
</tr>
<tr>
<td>1258 (1237–1278)</td>
</tr>
<tr>
<td>1366 (1312–1422)</td>
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<tr>
<td>&lt;0.0001</td>
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<tr>
<td>sTNF-R2, median (IQR), pg/mL</td>
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<tr>
<td>3156 (3119–3193)</td>
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<tr>
<td>3416 (3325–3510)</td>
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<td>&lt;0.0001</td>
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<tr>
<td>IL-18, median (IQR), pg/mL</td>
</tr>
<tr>
<td>370.2 (362.3–378.3)</td>
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<tr>
<td>396.7 (378.7–415.5)</td>
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<tr>
<td>0.008</td>
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<tr>
<td>Acute-phase reactants</td>
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<tr>
<td>Fibrinogen, median (IQR), g/L</td>
</tr>
<tr>
<td>3.51 (3.48–3.71)</td>
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<tr>
<td>3.64 (3.57–3.71)</td>
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<tr>
<td>&lt;0.0001</td>
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<tr>
<td>IL-6, median (IQR), pg/mL</td>
</tr>
<tr>
<td>3.23 (3.15–3.31)</td>
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<tr>
<td>3.47 (3.29–3.67)</td>
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<tr>
<td>0.02</td>
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<tr>
<td>CRP, median (IQR), pg/L</td>
</tr>
<tr>
<td>2.59 (2.49–2.69)</td>
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<tr>
<td>2.98 (2.71–3.28)</td>
</tr>
<tr>
<td>0.007</td>
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<tr>
<td>Soluble adhesion molecules</td>
</tr>
<tr>
<td>sICAM-1, median (IQR), ng/mL</td>
</tr>
<tr>
<td>326.0 (321.4–330.7)</td>
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<tr>
<td>369.7 (354.1–386.1)</td>
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<tr>
<td>&lt;0.0001</td>
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<tr>
<td>sVCAM-1, median (IQR), ng/mL</td>
</tr>
<tr>
<td>1171 (1146–1196)</td>
</tr>
<tr>
<td>1264 (1201–1330)</td>
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<tr>
<td>0.007</td>
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<tr>
<td>NT-proBNP, median (IQR), pmol/L</td>
</tr>
<tr>
<td>17.1 (16.4–17.8)</td>
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<tr>
<td>28.4 (25.8–31.2)</td>
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<tr>
<td>&lt;0.0001</td>
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</tbody>
</table>

IQR indicates interquartile range; CAD, coronary artery disease. CVD included CAD, PVD, or stroke. Data presented are percentages for categorical variables, mean±SD for continuous variables, and *geometric means (95% CI) for log-transformed variables. The t test was used for continuous variables and Mantel-Haenszel χ² test for discrete variables.
included the respective individual or multiple biomarkers simultaneously. Figure 3 displays HRs for biomarkers per increment in SD, adjusted for the basic model risk factors outlined above. Most inflammatory markers, including CRP, provided little or no additional predictive information over that obtained from assessment of traditional risk factors. By contrast, inclusion of Nt-proBNP improved the model accuracy such that the AUC increased significantly from 0.65 (95% CI 0.63 to 0.69) to 0.69 (95% CI 0.67 to 0.72; \( P < 0.001 \)), whereas inclusion of the biomarker score that additionally included sICAM-1, sIL-1Ra, and fibrinogen did not further improve the model accuracy.

To determine the most predictive and parsimonious model and to assess whether determination of multiple biomarkers adds any information for cardiovascular risk prediction, we plotted receiver operating characteristic curves for the primary cardiovascular outcome. Compared with the basic model that included the easily assessable risk factors (Figure 4, model A), simultaneous inclusion of the 3 inflammatory variables selected from the backward stepwise regression analyses (fibrinogen, sICAM-1, and sIL-1Ra; Figure 4, model B) did not improve the model accuracy. By contrast, additional inclusion of Nt-proBNP (Figure 4, model C) significantly increased the AUC (0.71 versus 0.66; \( P < 0.0001 \)).

**Discussion**

Our data indicate first that among a panel of 10 biomarkers, plasma Nt-proBNP level predicts future fatal and nonfatal cardiac events and adds significantly to the information obtained from determination of traditional risk factors in individuals with existing CVD. Second, although various inflammatory biomarkers and microalbuminuria were individually significantly related to future cardiovascular risk, they added very little additional prognostic information to the traditional markers. Third, the present data illustrate that models that include simple and readily available risk factors do not gain accuracy by the inclusion of any inflammatory biomarker for prediction of cardiovascular events in this setting of preexisting disease.

An increasing number of biomarkers have been proposed to identify patients at high risk for future cardiovascular events in the setting of primary\(^3\)–\(^6\),\(^19\) and secondary\(^7\)–\(^9\),\(^20\) prevention, as well as acute coronary syndrome.\(^10\),\(^21\)–\(^25\) From a clinical perspective, the utility of CRP has been proposed for risk stratification in healthy individuals.\(^26\) However, only limited data are available to support the predictive value of CRP beyond that of traditional risk factors in individuals with established CVD or at high risk for CVD.\(^27\) In addition, recent data challenged the value of CRP as an important predictor of future cardiovascular risk.\(^28\)–\(^30\) Whereas Danesh et al\(^28\) report data from individuals without previous CVD, the present study primarily included patients with prevalent CVD. In both study populations, the relative risks and the increase of c-statistics attributed to elevated CRP were modest and nearly identical. In addition, data from the Framingham Heart Study suggest that an elevated CRP level does not provide further prognostic information beyond traditional risk factor assess-

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**Table 2. HR for the Association of Various Risk Markers With Primary Outcome in the Multivariable Model Derived From Cox Proportional-Hazard Regression Model**

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR</th>
<th>95% CI</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.03</td>
<td>1.01–1.05</td>
<td>0.0005</td>
</tr>
<tr>
<td>Male</td>
<td>2.05</td>
<td>1.54–2.73</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ratio LDL-C:HDL-C</td>
<td>1.38</td>
<td>1.02–1.87</td>
<td>0.04</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.63</td>
<td>1.20–2.22</td>
<td>0.002</td>
</tr>
<tr>
<td>Current smoking</td>
<td>1.65</td>
<td>1.27–2.14</td>
<td>0.0002</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>1.55</td>
<td>1.22–1.98</td>
<td>0.0004</td>
</tr>
<tr>
<td>PVD</td>
<td>1.31</td>
<td>1.06–1.61</td>
<td>0.01</td>
</tr>
<tr>
<td>sIL-1RA</td>
<td>1.30</td>
<td>1.05–1.61</td>
<td>0.02</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>1.31</td>
<td>1.05–1.62</td>
<td>0.02</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>1.46</td>
<td>1.19–1.80</td>
<td>0.0003</td>
</tr>
<tr>
<td>Nt-proBNP</td>
<td>1.72</td>
<td>1.39–2.12</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

LDL-C:HDL-C indicates LDL cholesterol:HDL cholesterol.

*The model initially included age, sex, ramipril, waist-hip ratio, diabetes, systolic blood pressure, ratio of fasting LDL-C:HDL-C, smoking status, lipid-lowering drugs, fasting glucose, microalbuminuria, triglycerides, creatinine, and PVD and the biomarkers sTNFR-1 and -2, IL-18, IL-1Ra, IL-6, CRP, fibrinogen, sICAM-1, sVCAM-1, and Nt-proBNP, which have entered the model as categorized variables (upper vs lower two thirds). Glucose, triglycerides, and LDL-C:HDL-C ratio have been standardized by log transformation. \( P \) values were obtained by Wald tests from Cox regression. Retention criterion was \( P < 0.05 \). For tertile values see the online-only Data Supplement.
ment to predict major CVD and coronary heart disease, indicating similar risk estimates compared with the HOPE study.30 Thus, these studies, together with the recently published results of the Third National Health and Nutrition Examination Survey,31 question the value of CRP as a useful tool for clinical risk prediction over and above simple risk predictors.

In addition to fibrinogen and CRP, several inflammatory cytokines and soluble adhesion molecules have been assessed in individuals with established CVD. The present data are largely in accordance with those obtained from other studies that included individuals with existing atherosclerotic disease.7,9,10,32,33 In particular, sIL-1RA, sVCAM-1, and IL-18 remained significantly associated with future fatal and non-fatal myocardial infarction. However, the present study indicates that neither (pro)inflammatory cytokines nor the soluble adhesion molecules contribute to cardiovascular risk stratification beyond traditional risk factors. The stronger association of IL-6,32 sVCAM-1,7 IL-18,9 or IL-1RA10 with outcome than in the HOPE study individuals might be explained by the inclusion of acute coronary syndrome patients in the respective studies.

Only a few studies have simultaneously assessed the value of a number of additional inflammatory markers.29,34 Whereas single-marker determination only provides a borderline association with future cardiovascular risk, an aggregate number of inflammatory markers are independent predictors of cardiovascular events in older persons.34 Our hypothesis was that a multimarker strategy involving different pathophysiological aspects would be more informative; however, the present study did not support this. Although circulating inflammatory markers and the score were related to future cardiovascular events, they did not provide an incremental value over and above that of traditional risk factors without

![Figure 3. HRs for primary cardiovascular outcome according to inflammatory variables, microalbuminuria, and Nt-proBNP. Squares denotes HRs; horizontal lines represent 95% CI. HRs for biomarkers indicate the increase in risk per increment SD, adjusted for variables of the basic model, which included age, sex, ramipril, waist-hip ratio, diabetes, systolic blood pressure, ratio of fasting LDL to HDL cholesterol, smoking status, lipid-lowering drugs, fasting glucose, microalbuminuria, triglycerides, creatinine, and PVD. Glucose and creatinine entered the model as log-transformed continuous variables. AUCs are derived from logistic regression analyses that included the covariates as outlined above and the respective biomarker.](http://circ.ahajournals.org/)}
inclusion of Nt-proBNP. Determination of whether inflammation processes and their markers represent an independent causal pathway for atherogenesis and its clinical complications will require interventional trials with specific agents that primarily affect inflammation and have little impact on other known beneficial mechanisms such as lipids or blood pressure.

Determination of BNP provides information about cardiac function and supports diagnosis of heart failure. Data from the AtheroGene study extended the potential clinical application of Nt-proBNP to patients presenting with stable angina pectoris. Of particular importance, Nt-proBNP constitutes the only biomarker that adds significant information beyond models that include the contemporary and easily assessable risk factors. Irrespective of being considered as a continuous or categorical variable, the HR for Nt-proBNP is higher than those of diabetes, LDL-HDL ratio, or other classic risk factors. Whether its strong association with the primary outcome is due to the fact that elevated levels of BNP or Nt-proBNP simply reflect the presence of multiple risk factors or directly indicate left ventricular or early diastolic dysfunction as strong prognostic factors is still a matter of controversy. Emerging data from the Framingham Heart Study population further broaden its indication by demonstrating that a single BNP determination provides additional information beyond traditional risk factors and the new biomarkers.

Several limitations of the present study should be considered: First, we only performed baseline measurements and therefore cannot clarify the variability of the inflammatory markers during the course of the study; however, this limitation is likely to affect both traditional risk factors and the new biomarkers. Second, measurement of all inflammatory markers during the course of the study; however, this limitation is likely to affect both traditional risk factors and the new biomarkers. Therefore, simple, readily available, and conventional risk factors should be the mainstay of prediction in patients with known CVD.

In conclusion, the present study data indicate that the traditional risk factors provide the most powerful predictive information for CVD events, with significant incremental prediction obtained by the addition of Nt-proBNP. Therefore, simple, readily available, and conventional risk factors should be the mainstay of prediction in patients with known CVD.

Sources of Funding
This work was supported by the Heart and Stroke Foundation of Ontario (grant No. NA4192), by a grant of the Stiftung Rheinland-Pfalz für Innovation, Ministry for Science and Education (AZ 15202-386261/545), Mainz, Germany, and by the MAIFOR grant of the Johannes Gutenberg-University Mainz. Dr Blankenberg was supported by an NIH grant (R01 HL-076229). This work was supported by the Heart and Stroke Foundation of Ontario (grant No. NA4192), by a grant of the Stiftung Rheinland-Pfalz für Innovation, Ministry for Science and Education (AZ 15202-386261/545), Mainz, Germany, and by the MAIFOR grant of the Johannes Gutenberg-University Mainz. Dr Blankenberg was supported by a grant of the Institut National de la Santé et de la Recherche Médicale (INSERM), Paris, France. Dr Smieja is supported by a Career Scientist Award from the Father Sean O’Sullivan Research Centre, St. Joseph’s Healthcare, Hamilton, Canada. Dr Yusuf is a senior scientist of the Canadian Institute of Health Research and holds a Research Chair of the Heart and Stroke Foundation of Ontario.

Disclosures
Dr McQueen received an honorarium from Roche Diagnostics for presenting data of the predictive value of Nt-proBNP at the American Heart Association annual meeting in Orlando, Fla, in 2003. The remaining authors report no conflicts.

References

Individuals at highest risk for recurrent cardiovascular events beyond assessment of traditional cardiovascular risk factors.


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for the HOPE Study Investigators

_Circulation._ 2006;114:201-208; originally published online July 10, 2006;
doi: 10.1161/CIRCULATIONAHA.105.590927
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/114/3/201

Data Supplement (unedited) at:
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