# Evaluation of Multiple Biomarkers of Cardiovascular Stress for Risk Prediction and Guiding Medical Therapy in Patients With Stable Coronary Disease

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***Background***—Circulating biomarkers can offer insight into subclinical cardiovascular stress and thus have the potential to aid in risk stratification and tailoring of therapy.

***Methods and Results***—We measured plasma levels of 4 cardiovascular biomarkers, midregional pro-atrial natriuretic peptide (MR-proANP), midregional pro-adrenomedullin (MR-proADM), C-terminal pro-endothelin-1 (CT-proET-1), and copeptin, in 3717 patients with stable coronary artery disease and preserved left ventricular ejection fraction who were randomized to trandolapril or placebo as part of the Prevention of Events With Angiotensin Converting Enzyme (PEACE) trial. After adjustment for clinical cardiovascular risk predictors and left ventricular ejection fraction, elevated levels of MR-proANP, MR-proADM, and CT-proET-1 were independently associated with the risk of cardiovascular death or heart failure (hazard ratios per 1-SD increase in log-transformed biomarker levels of 1.97, 1.48, and 1.47, respectively; $P \leq 0.002$ for each biomarker). These 3 biomarkers also significantly improved metrics of discrimination when added to a clinical model. Trandolapril significantly reduced the risk of cardiovascular death or heart failure in patients who had elevated levels of $\geq 2$ biomarkers (hazard ratio, 0.53; 95% confidence interval, 0.36–0.80), whereas there was no benefit in patients with elevated levels of 0 or 1 biomarker (hazard ratio, 1.09; 95% confidence interval, 0.74–1.59; $P_{interaction} = 0.012$).

***Conclusions***—In patients with stable coronary artery disease and preserved left ventricular ejection fraction, our results suggest that elevated levels of novel biomarkers of cardiovascular stress may help identify patients who are at higher risk of cardiovascular death and heart failure and may be useful to select patients who derive significant benefit from angiotensin-converting enzyme inhibitor therapy. **(*Circulation*. 2012;125:233-240.)**

**Key Words:** angiotensin-converting enzyme inhibitors ■ biomarkers ■ coronary disease

Elevated levels of circulating biomarkers related to cardiac volume or pressure overload offer insight into subclinical cardiac stress and thus have the potential to aid in risk stratification.[1] Specifically, elevated levels of B-type natriuretic peptide (BNP; either the hormone or the amino-terminal fragment of the prohormone [NT-proBNP]) have been shown to be predictive of mortality and/or heart failure events across a broad range of individuals, ranging from the general population to patients with overt heart failure.[1–7]

**Clinical Perspective on p 240**

Development of newer assays that target more stable epitopes of hormones or prohormones that are released in relation to cardiomyocyte and/or vascular stress offers the potential for more refined risk assessment. Specifically, atrial natriuretic peptide (ANP) is a vasodilator and natriuretic that is synthesized in the myocardium in response to increased wall tension.[8] Adrenomedullin (ADM) is a potent vasodilator synthesized in the adrenal medulla, vascular endothelial cells, heart, and elsewhere in response to physical stretch and specific cytokines, with levels in the heart elevated in the setting of pressure and volume overload.[9,10] Endothelin-1 (ET-1) is a potent vasoconstrictor and profibrotic hormone that is secreted by vascular endothelial cells, with levels correlating with shear stress and pulmonary artery pressure.[11] Copeptin is a stable peptide derived from the precursor to

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arginine vasopressin, a vasoconstrictor that is secreted from the posterior pituitary in response not only to osmotic stimuli but also to hemodynamic changes detected by cardiac and vascular baroreceptors.[12] Higher levels of these biomarkers have been associated with an increased risk of death and/or heart failure events in patients with established heart failure.[13–16] The availability of an assay panel for these 4 biomarkers of cardiovascular stress that have shown promise in patients with established heart failure created the opportunity to investigate their utility in a broader population.

Angiotensin-converting enzyme (ACE) inhibitors substantially reduce the risk of death and heart failure events in patients with heart failure, with the greatest benefit in those patients with the most clinically severe heart failure.[17] Among patients with acute myocardial infarction (MI), the benefit of ACE inhibitors is greatest in those with high-risk clinical features such as anterior MI or depressed left ventricular systolic function.[18] In contrast, the role of ACE inhibitors in lower-risk patients with stable coronary artery disease (CAD) without heart failure is less clear.[19–21] We explored the hypotheses that in such patients, elevated levels of midregional (MR) pro-ANP, MR-proADM, C-terminal proET-1 (CT-proET-1), and copeptin would offer prognostic value for cardiovascular death and heart failure independently of clinical risk factors and would identify patients who derive greater clinical benefit from the use of an ACE inhibitor. We tested these hypotheses by measuring plasma levels of these novel biomarkers of cardiovascular stress in 3717 patients with stable CAD and preserved left ventricular ejection fraction (LVEF) who were randomized to trandolapril or placebo as part of the Prevention of Events With Angiotensin Converting Enzyme (PEACE) trial.

## Methods

### Patient Population

This study involved 3717 patients with documented stable CAD who had been enrolled in the PEACE trial (www.ClinicalTrials.gov; unique identifier, NCT00000558) and provided a sample of blood at the time of enrollment. The design and main outcomes of the PEACE trial have been published previously,[21] and salient features are detailed in the Methods section and Table I in the online-only Data Supplement. In brief, subjects were free of heart failure at baseline, and none had been hospitalized with an acute coronary syndrome or had undergone coronary revascularization within the 3 months preceding trial entry. Both the parent clinical trial and this substudy were approved by the relevant institutional review boards, and informed consent was obtained from all patients.

### Biomarker Analyses

Baseline plasma levels of MR-proANP,[22] MR-proADM,[23] CT-proET-1,[24] and copeptin[25] (assays from B.R.A.H.M.S. GmbH, Henningsdorf, Germany) were determined in the Thrombolysis in Myocardial Infarction (TIMI) Clinical Trials Laboratory (Boston, MA) as detailed in the Methods section and Table II in the online-only Data Supplement. Baseline levels of NT-proBNP and cardiac troponin T (cTnT) measured with a highly sensitive assay had been determined in this population, as previously published and summarized in the Methods section in the online-only Data Supplement.[6,26] All testing was performed by personnel blinded to clinical outcomes and treatment allocation.

### Outcomes

On the basis of prior data regarding the predictive ability of biomarkers of cardiac stress,[6] the primary outcome in this analysis was the composite of cardiovascular death or hospitalization for heart failure. Additionally, we explored other major adverse cardiovascular events that had been recorded in patients in the trial, including all-cause death, acute MI, acute stroke, and coronary revascularization (percutaneous or surgical). Event adjudication is detailed in the Methods section in the online-only Data Supplement. All clinical events were classified before biomarkers were measured.

### Statistical Analyses

Baseline characteristics are reported as mean±SD for normally distributed continuous variables and as counts and percentages for categorical variables. Wilcoxon rank-sum and $\chi^2$ tests for trend were used to test for differences in continuous and categorical baseline characteristics between quartiles of biomarkers. The Spearman correlation was used to calculate the association between different biomarkers and categorized based on standard cut points.[27] The cumulative incidences of clinical outcomes across quartiles of each biomarker were compared by use of a log-rank test. Cox proportional-hazards models were used to examine the association between biomarker levels and outcome data. In these models, biomarker levels were examined both as a continuous variable (after natural logarithmic transformation) and as a categorical variable by quartiles. Associations were adjusted for age, sex, weight, history of hypertension, history of diabetes mellitus, current tobacco use, prior MI, prior percutaneous coronary intervention or coronary artery bypass grafting, systolic blood pressure, estimated glomerular filtration rate, ratio of apolipoprotein B to apolipoprotein A, LVEF, aspirin use, $\beta$-blocker use, and lipid-lowering medication use. Starting with a model containing the aforementioned clinical covariates, a forward selection algorithm ($P<0.05$ to enter the model) was used to select among the 4 novel biomarkers, as well as NT-proBNP and cTnT. The incremental performance of the biomarkers in addition to clinical predictors was further evaluated by calculating changes in the C statistic, integrated discrimination improvement, and category-free net reclassification improvement metrics (see the Methods section in the online-only Data Supplement for further details).[28–30]

To examine for heterogeneity in the effect of trandolapril on the risk of cardiovascular death or heart failure, hazard ratios (HRs) were calculated in patients who were and were not in the highest-risk category as defined by being in the top quartile of a biomarker level. To test for statistically significant effect modification, a Cox proportional hazards model was created that included a term for trandolapril, a term for biomarker risk category, and an interaction term.

A value of $P<0.05$ was considered to indicate statistical significance, and all tests were 2 sided. No adjustment for multiple comparisons was performed. Although based on previous work with these biomarkers in other populations, all of the analyses we have performed in this biomarker substudy are inherently exploratory. Analyses were performed with STATA/IC (version 10.1, STATA Corp, College Station, TX) and R (version 2.12.1).

## Results

### Baseline Characteristics of the Patients and Biomarker Levels

Baseline measurements of the 4 novel biomarkers were available for 3717 patients from the PEACE trial. The clinical characteristics of the patients are given in Table 1. By design, all patients had stable CAD, and LVEF was preserved at a mean±SD value of 58.7±9.6%. Median levels of MR-proANP, MR-proADM, CT-proET-1, and copeptin at baseline in patients in the PEACE trial were 90.45 pmol/L (25th–75th percentile, 63.68–128.3 pmol/L), 0.53 nmol/L (25th–75th percentile, 0.45–0.64 nmol/L), 47.82 pmol/L (25th–75th percentile, 39.04–57.02 pmol/L), and 6.47 pmol/L (25th–75th percentile, 0–10.67 pmol/L), respectively. The levels tended to be higher than those seen in healthy populations, but with the exception of MR-proADM, the majority of values were lower than the 97.5th percentile reported in healthy populations and lower than the values in patients with

**Table 1. Baseline Characteristics of Patients**

| Baseline Characteristic | All | Placebo | Trandolapril |
|---|---|---|---|
| Patients, n | 3717 | 1868 | 1849 |
| Age, y | 64.1±8.2 | 64.1±8.2 | 64.2±8.1 |
| Female sex, n (%) | 701 (18.9) | 334 (17.9) | 367 (19.9) |
| Weight, kg | 83.9±15.7 | 83.7±15.7 | 84.2±15.6 |
| Hypertension, n (%) | 1658 (44.6) | 835 (44.7) | 823 (44.5) |
| Diabetes mellitus, n (%) | 602 (16.2) | 294 (15.7) | 308 (16.7) |
| Current smoker, n (%) | 564 (15.2) | 290 (15.5) | 274 (14.8) |
| Prior MI, n (%) | 2087 (56.2) | 1076 (57.6) | 1011 (54.7) |
| Prior PCI or CABG, n (%) | 2697 (72.6) | 1367 (73.2) | 1330 (72.0) |
| Aspirin use, n (%) | 3389 (91.2) | 1721 (92.2) | 1668 (90.3) |
| $\beta$-blocker use, n (%) | 2303 (62.0) | 1156 (61.9) | 1147 (62.1) |
| Lipid-lowering therapy use, n (%) | 2667 (71.8) | 1334 (71.5) | 1333 (72.2) |
| SBP, mm Hg | 133.4±16.8 | 133.4±16.8 | 133.3±16.8 |
| DBP, mm Hg | 78.1±10.0 | 78.2±10.2 | 78.0±9.8 |
| GFR, mL · min$^{-1}$ · 1.73 m$^{-2}$ | 77.9±19.4 | 78.3±19.4 | 77.6±19.3 |
| ApoB, mg/dL | 107.2±23.1 | 107.6±22.9 | 106.8±23.2 |
| ApoA, mg/dL | 138.2±24.6 | 138.6±24.5 | 137.8±24.7 |
| LVEF, % | 58.7±9.6 | 58.7±9.6 | 58.8±9.7 |

MI indicates myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; SBP, systolic blood pressure; DBP, diastolic blood pressure; GFR, glomerular filtration rate; ApoB, apolipoprotein B; ApoA, apolipoprotein A; and LVEF, left ventricular ejection fraction. Data are presented as mean±SD for normally distributed continuous variables and n (%) for dichotomous variables.

overt heart failure (Table II in the online-only Data Supplement). Characteristics of patients according to quartiles of biomarker levels are shown in Tables III through VI in the online-only Data Supplement. In general, higher levels of biomarkers of cardiovascular stress were positively associated with greater age and prevalence of hypertension and lower estimated glomerular filtration rate. LVEF was inversely associated with MR-proANP and copeptin levels but differed by only 2.0 and 1.0 absolute percentage points between the top and bottom quartiles for the 2 biomarkers, respectively. Among the novel biomarkers, the only moderately strong correlation was between MR-proADM and CT-proET-1 ($r$=0.63); the others were moderate to low ($r\leq$0.44; Table VII in the online-only Data Supplement). As expected, there was a strong positive correlation between levels of MR-proANP and NT-proBNP ($r$=0.76), but correlations of NT-proBNP and cTnT with other markers were weak ($r\leq$0.38; Table VII in the online-only Data Supplement).

## Clinical Outcomes

Among patients allocated to the placebo arm of the PEACE trial, higher baseline levels of each of the 4 novel biomarker of cardiovascular stress were strongly associated with the subsequent risk of cardiovascular death or heart failure (the composite of which occurred in 114 patients), with up to approximately a doubling of the risk per each 1-SD increase in log-transformed biomarker levels ($P\leq$0.002 for each biomarker; Table 2). Risk increased across quartiles, especially the fourth quartile (Figure 1). Similar associations were seen between biomarker levels and the risk of cardiovascular death (which occurred in 67 patients) and of heart failure individually (which occurred in 56 patients; Table VIII in the online-only Data Supplement).

After adjustment for traditional clinical risk predictors, estimated glomerular filtration rate, and LVEF (see Methods for a detailed list of covariates), elevated levels of MR-proANP, MR-proADM, and CT-proET-1 remained significantly associated with an increased risk of cardiovascular death or heart failure, ranging from 47% higher risk to a near doubling of the risk per each 1-SD increase in log-transformed biomarker levels ($P\leq$0.002 for each biomarker); in terms of quartile analysis, the risk was most pronounced for those patients in the top quartile, who had almost 3 times to >5 times the risk seen for patients in the lowest quartile. In contrast, after multivariable adjustment, the association with copeptin was no longer significant (Table 3). As was the case for the unadjusted analyses, similar associations were seen between biomarker levels and the risk of cardiovascular death and of heart failure individually (Table VIII in the online-only Data Supplement). Compared with cardiovascular death, the associations with the less cardiovascular-specific end point of all-cause death were significant but weaker (Table IX in the online-only Data Supplement). As expected on the basis of prior work,[6,26] there were nonsignificant adjusted associations between levels of novel biomarkers of cardiovascular stress and the risk of acute MI, stroke, or coronary revascularization, with the exception of MR-proANP and stroke ($P$=0.043; Table IX in the online-only Data Supplement).

**Table 2. Association of Biomarker Levels and Clinical Outcomes in the Placebo Arm**

| | Risk for CV Death or Heart Failure | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | HR (95% CI) per 1-SD Increase in Log-Transformed | | HR (95% CI) Across Quartiles | | | | *P* | |
| Biomarker | Biomarker Values | *P* | 1 | 2 | 3 | 4 | Multiple Partial | Trend |
| MR-proANP | 2.25 (1.89–2.42) | <0.001 | Referent | 1.92 (0.85–4.30) | 3.10 (1.46–6.59) | 7.30 (3.62–14.70) | <0.0001 | <0.0001 |
| MR-proADM | 1.69 (1.52–1.88) | <0.001 | Referent | 2.15 (0.88–5.28) | 3.65 (1.58–8.45) | 10.25 (4.71–22.33) | <0.0001 | <0.0001 |
| CT-proET-1 | 1.96 (1.57–2.44) | <0.001 | Referent | 2.25 (1.14–4.45) | 1.42 (0.68–2.98) | 5.07 (2.72–9.44) | <0.0001 | <0.0001 |
| Copeptin | 1.30 (1.10–1.55) | 0.002 | Referent | 0.88 (0.43–1.79) | 1.23 (0.74–2.05) | 2.09 (1.32–3.28) | 0.0072 | 0.0013 |

CV indicates cardiovascular; HR, hazard ratio; CI, confidence interval; MR-proANP, midregional pro-atrial natriuretic peptide; MR-proADM, midregional pro-adrenomedullin; and CT-proET-1, C-terminal pro-endothelin-1. A total of 114 of the 1868 patients allocated to placebo experienced CV death or heart failure. Each biomarker was analyzed separately. In quartile analyses, "multiple partial" refers to a 3-*df* test for the addition of all quartiles and "trend" refers to a 1-*df* test for linear trend across quartiles.

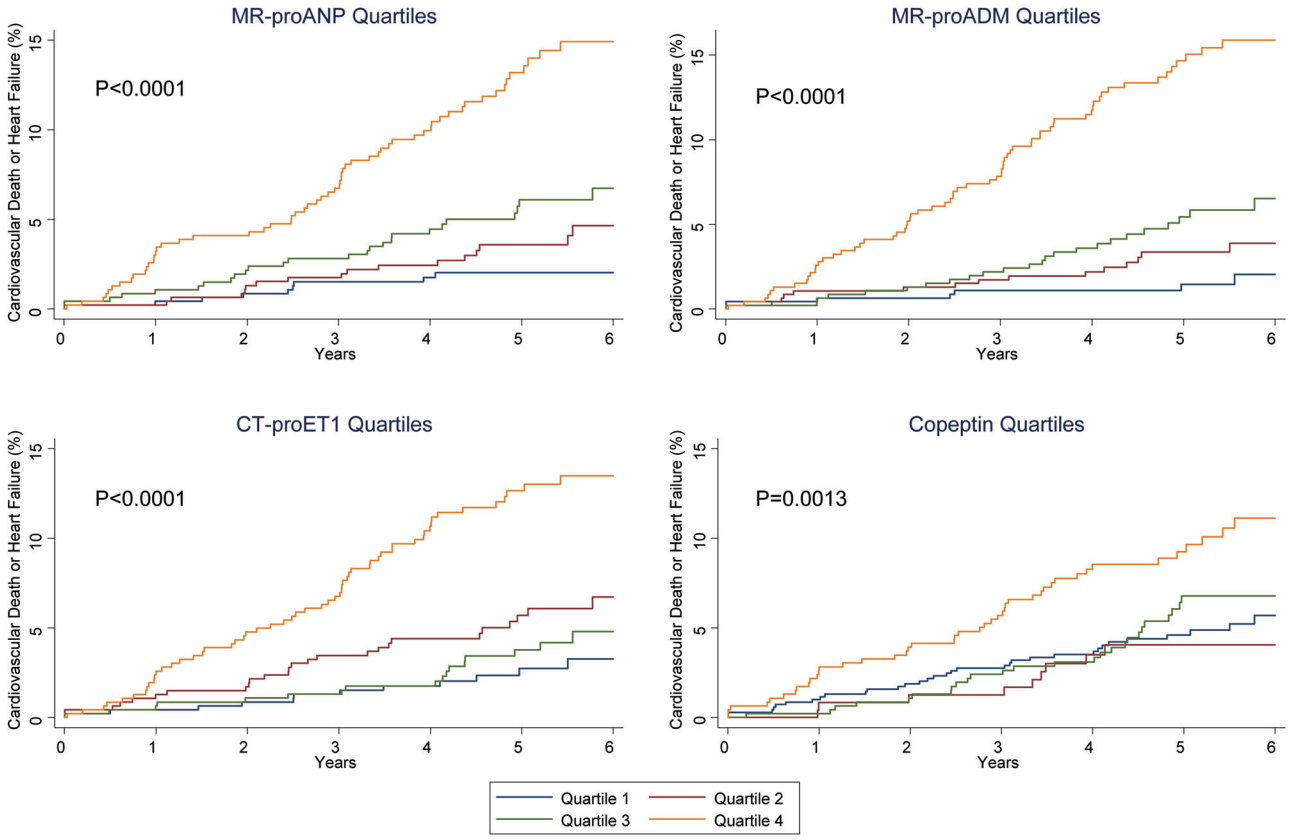


**Figure 1.** Cumulative incidence curves for the composite of cardiovascular death or heart failure among patients in the placebo arm of the Prevention of Events With Angiotensin Converting Enzyme (PEACE) trial (n=1868) categorized by quartiles of midregional pro-atrial natriuretic peptide (MR-proANP), midregional pro-adrenomedullin (MR-proADM), C-terminal pro-endothelin-1 (CT-proET-1), or copeptin. *P* values are for log-rank test for trend across quartiles.

We have previously measured NT-proBNP and cTnT in this population, and the association of those biomarkers with cardiovascular death or heart failure in a model adjusted for the aforementioned clinical covariates is shown in Table X in the online-only Data Supplement. Ranking each biomarker individually on the basis of the magnitude of risk (HR) per 1 SD gives the following order: MR-proANP (1.97), NT-proBNP (1.73), MR-proADM (1.48), CT-proET-1 (1.47), and cTnT (1.37). Given the correlation between the biomarkers and that none is established for routine use in this population, we used an unbiased forward selection algorithm to create a multimarker model. The only 2 biomarkers to enter and remain in a model already containing clinical covariates were MR-proANP (adjusted HR, 1.79; 95% confidence interval [CI], 1.41–2.26; $P<0.001$) and MR-proADM (adjusted HR, 1.27; 95% CI, 1.07–1.51; $P=0.007$).

The addition of MR-proANP, MR-proADM, and CT-proET-1 individually to the clinical model significantly improved metrics of discrimination (Table 4). In contrast, the addition of copeptin did not improve these metrics. The addition

**Table 3. Multivariable-Adjusted Association of Biomarker Levels and Clinical Outcomes in the Placebo Arm Adjusted for Clinical Factors**

| | Adjusted Risk for CV Death or Heart Failure | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | HR (95% CI) per 1-SD Increase in Log-Transformed | | HR (95% CI) Across Quartiles | | | | *P* | |
| Biomarker | Biomarker Values | *P* | 1 | 2 | 3 | 4 | Multiple Partial | Trend |
| MR-proANP | 1.97 (1.58–2.46) | <0.001 | Referent | 1.60 (0.70–3.66) | 2.72 (1.24–5.96) | 4.35 (1.96–9.62) | <0.0001 | <0.0001 |
| MR-proADM | 1.48 (1.27–1.73) | <0.001 | Referent | 1.90 (0.77–4.69) | 2.45 (1.03–5.82) | 5.51 (2.38–12.75) | <0.0001 | <0.0001 |
| CT-proET-1 | 1.47 (1.15–1.88) | 0.002 | Referent | 2.03 (1.03–4.04) | 0.99 (0.46–2.11) | 2.73 (1.41–5.27) | <0.001 | 0.01 |
| Copeptin | 1.10 (0.91–1.33) | 0.32 | Referent | 0.77 (0.37–1.57) | 1.11 (0.66–1.86) | 1.41 (0.87–2.28) | 0.30 | 0.11 |

CV indicates cardiovascular; HR, hazard ratio; CI, confidence interval; MR-proANP, midregional pro-atrial natriuretic peptide; MR-proADM, midregional pro-adrenomedullin; and CT-proET-1, C-terminal pro-endothelin-1. Covariates in the model include standard clinical factors: age, sex, weight, history of hypertension, history of diabetes mellitus, current tobacco use, prior myocardial infarction, prior percutaneous coronary intervention or coronary artery bypass graft surgery, systolic blood pressure, estimated glomerular filtration rate, ratio of apolipoprotein B to A, left ventricular ejection fraction, aspirin use, $\beta$-blocker use, and lipid-lowering medication use. Each biomarker was analyzed separately in the placebo arm. In quartile analyses, "multiple partial" refers to a 3-*df* test for the addition of all quartiles and "trend" refers to a 1-*df* test for linear trend across quartiles.

**Table 4. Impact of Biomarker Levels and Metrics of Discrimination and Reclassification in the Placebo Arm**

| | C Statistic | | Integrated Discrimination Index | | Net Reclassification Improvement | |
|---|---|---|---|---|---|---|
| Model | Value | *P* | Value, % | *P* | Value | *P* |
| Clinical model alone | 0.768 | N/A | N/A | N/A | N/A | N/A |
| Clinical model+ MR-proANP | 0.804 | 0.0018 | 3.8 | <0.0001 | 0.412 | <0.0001 |
| Clinical model+ MR-proADM | 0.788 | 0.0064 | 1.9 | 0.0027 | 0.362 | 0.0003 |
| Clinical model+ CT-proET-1 | 0.779 | 0.23 | 1.2 | 0.047 | 0.205 | 0.039 |
| Clinical model+ copeptin | 0.769 | 0.85 | 0.2 | 0.14 | 0.061 | 0.54 |

MR-proANP indicates midregional pro-atrial natriuretic peptide; MR-proADM, midregional pro-adrenomedullin; and CT-proET-1, C-terminal pro-endothelin-1. Terms in the clinical model include age, sex, weight, history of hypertension, history of diabetes mellitus, current tobacco use, prior myocardial infarction, prior percutaneous coronary intervention or coronary artery bypass graft surgery, systolic blood pressure, estimated glomerular filtration rate, ratio of apolipoprotein B to A, left ventricular ejection fraction, aspirin use, β-blocker use, and lipid-lowering medication use. Each biomarker was analyzed separately in the placebo arm. *P* values are for comparison with clinical model alone.

of all 3 biomarkers to the clinical model improved the C statistic from 0.768 to 0.809 and yielded an integrated discrimination improvement of 4.6% and an net reclassification improvement of 0.435 (all $P \leq 0.0005$). Adding MR-proANP, MR-proADM, and CT-proET-1 uniformly and significantly improved the C statistic of multivariable models already containing clinical covariates, regardless of whether NT-proBNP, cTnT, or both were also in the model; conversely, adding NT-proBNP and cTnT to a model containing clinical covariates as well as MR-proANP, MR-proADM, and CT-proET-1 did not improve the C statistic (Table XI in the online-only Data Supplement).

## Interaction With Trandolapril Therapy

In the overall biomarker cohort, treatment with trandolapril resulted in an HR of 0.80 (95% CI, 0.61–1.05) for cardiovascular death or heart failure. Notably, however, among patients having an MR-proANP, MR-proADM, or CT-proET-1 level in the top quartile and thus at the highest risk of cardiovascular death or heart failure based on these biomarkers, trandolapril significantly reduced the risk of cardiovascular death or heart failure by 34% to 44%, whereas no benefit was observed among those with lower levels (Figure 2A). In contrast, there was no significant benefit from treatment with trandolapril among patients in the highest quartiles of either NT-proBNP or cTnT (Figure I in the online-only Data Supplement).

A gradient of benefit ($P_{interaction}=0.016$) with trandolapril therapy was observed in patients categorized according to whether they had elevated levels of 0 (n=2037), 1 (n=891), 2 (n=472), or all 3 (n=317) novel biomarkers that we found to be associated with cardiovascular death or heart failure in adjusted analyses (Figure 2B). When the results were dichotomized, among the 2928 patients (79% of the biomarker cohort) with ≤1 elevated biomarker, there was no benefit of trandolapril therapy on the risk of cardiovascular death or heart failure (HR, 1.09;

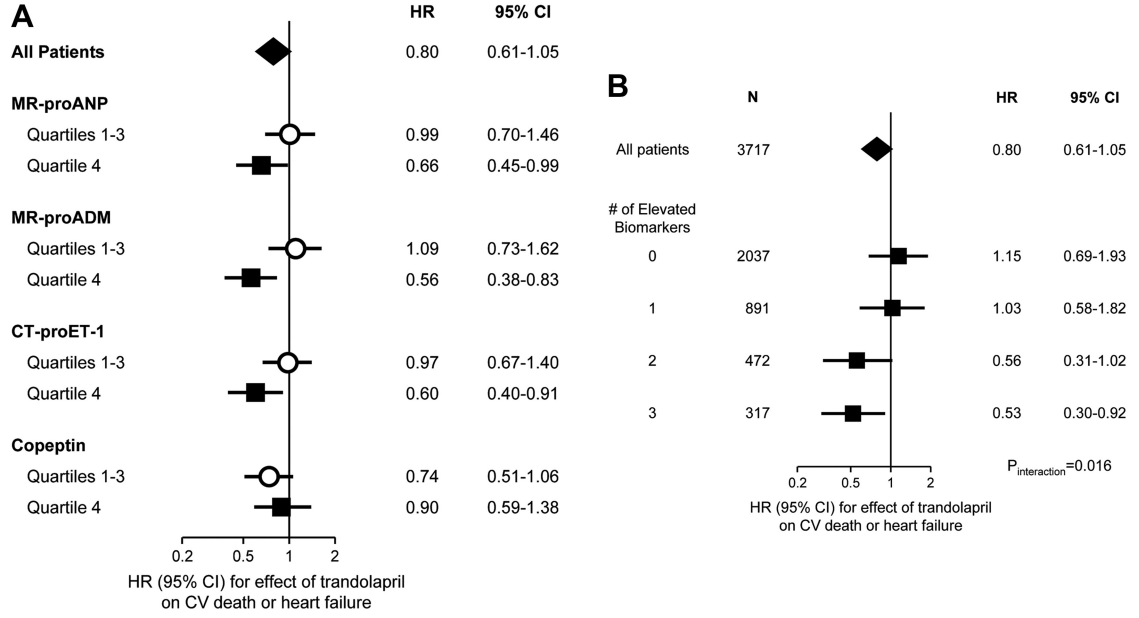


**Figure 2.** Benefit of trandolapril on the risk of the composite of cardiovascular (CV) death or heart failure in 3717 patients from the Prevention of Events With Angiotensin Converting Enzyme (PEACE) trial categorized according to their levels of biomarkers of cardiovascular stress. **A**, Patients are categorized according to whether their level of each biomarker of cardiovascular stress was in the top quartile (quartile 4) or not (quartiles 1–3). The *P* values for interaction were 0.16, 0.02, 0.09, and 0.72 for midregional pro-atrial natriuretic peptide (MR-proANP), midregional pro-adrenomedullin (MR-proADM), C-terminal pro-endothelin-1 (CT-proET-1), and copeptin, respectively. **B**, Patients are categorized by the number of biomarkers (MR-proANP, MR-proADM, and CT-proET-1) in the top quartile; the *P* value for interaction is 0.016. In **A** and **B**, the diamonds indicate the effect in the entire biomarker cohort, with the center indicating the point estimate and the left and right ends indicating the 95% confidence interval (CI). The squares and circles indicate the point estimate, and the horizontal lines indicate the 95% CIs for the effect in each subgroup. HR indicates hazard ratio.

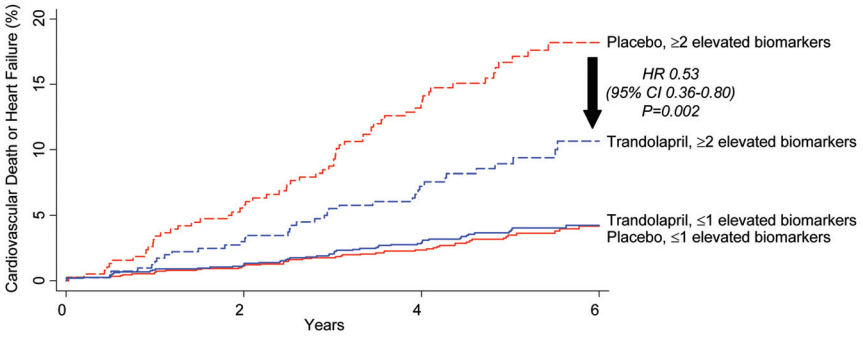


**Figure 3.** Cumulative incidence curves for the composite of cardiovascular death or heart failure in 3717 patients from the Prevention of Events with Angiotensin Converting Enzyme (PEACE) trial categorized by whether they had ≤1 elevated biomarkers (solid lines; red indicates 1487 patients treated with placebo; blue, 1441 patients treated with trandolapril) or ≥2 elevated biomarkers (dashed lines; red indicates 381 patients treated with placebo; blue, 408 patients treated with trandolapril). HR indicates hazard ratio; CI, confidence interval.

95% CI, 0.74–1.59), whereas among the 789 patients (21% of the biomarker cohort) with ≥2 elevated biomarkers, trandolapril significantly reduced the rate of cardiovascular death or heart failure (HR, 0.53; 95% CI, 0.36–0.80; $P$=0.002, $P_{interaction}$=0.012; Figure 3). The absolute risk reduction over 6 years in this latter group was 7.5%; thus, in this subset, 14 patients would need to be treated with trandolapril for 6 years to prevent a cardiovascular death or hospitalization for heart failure.

## Discussion

In an exploratory analysis among a large cohort of patients with stable CAD and preserved LVEF, we have demonstrated that elevated levels of 3 novel biomarkers of cardiovascular stress are independently associated with the subsequent risk of cardiovascular death and heart failure. Specifically, MR-proANP, MR-proADM, and CT-proET-1 were associated with cardiovascular death or heart failure independently of clinical factors, renal function, and LVEF, ranging from 47% higher risk to a near doubling of the risk per each 1-SD increase in log-transformed biomarker levels and almost 3 times to >5 times the risk for patients in the highest compared with the lowest quartile. In contrast, a fourth biomarker, copeptin, was not independently associated with the risk of cardiovascular events. Moreover, and in contrast to previous results with other biomarkers, including NT-proBNP and cTnT,[6,26,31] elevated levels of these 3 biomarkers identified patients in whom, despite appearing to be at low risk clinically, therapy with an ACE inhibitor resulted in a significant reduction in the risk of cardiovascular death or heart failure.

We used assays for the prohormones ANP, ADM, and ET-1 because the prohormones are released in an equimolar ratio to the vasoactive hormones but have a longer half-life. When possible, we also used assays for a midregional fragment because these fragments are more stable in vivo and ex vivo than the amino- or carboxy-terminal part of the prohormone, thereby minimizing the risk of underestimation of levels as a result of early degradation of crucial epitopes at the extreme ends of the molecule.[32] In studies of patients with established heart failure, elevated levels of MR-proANP, MR-proADM, and CT-proET-1 have been shown to be associated with mortality independently of clinical variables, and the biomarkers have displayed prognostic and discriminatory value that has compared favorably with BNP and/or NT-proBNP.[13–15]

Concordant with those observations, in our data set, we found that during the creation of a multimarker model adjusted for clinical factors, MR-proANP and MR-proADM proved to be the strongest 2 biomarkers, superior to NT-proBNP and cTnT measured with a highly sensitive assay. Because this was a clinical rather than a mechanistic study, we can only speculate as to the reasons for the superior performance, which could be related to subtle differences in the respective pathobiology underlying elevation of each of the biomarkers or could stem from more favorable analytic properties that translate into a better reflection of subclinical cardiovascular pathology. Regardless, our data are supported by and extend previous findings regarding these biomarkers and atherosclerosis reported by Schnabel and colleagues[7] in several ways, including studying patients who were free of heart failure at baseline and whose LVEF was known and incorporated into all multivariable models, using patients enrolled from a much broader number of clinical centers, and examining the specific clinical events that biomarkers of cardiac stress are best suited to predict, namely cardiovascular death and heart failure, rather than a composite of death or MI.

Critically, whereas other biomarker analyses have been embedded in observational cohorts, we had the benefit of studying these biomarkers in a randomized clinical trial, allowing us to examine the interaction between baseline biomarker levels and the efficacy of the randomized therapy without concern for the inherent bias in examining nonrandomly allocated therapies. Using a panel of these novel biomarkers of cardiovascular stress, we were able to identify approximately one fifth of enrolled patients with stable CAD in whom ACE inhibitor therapy nearly halved the risk of cardiovascular death or heart failure. Our findings are conceptually analogous to the results of Richards and colleagues,[33,34] who showed that elevated levels of biomarkers of cardiovascular stress identified patients with ischemic left ventricular dysfunction who benefited from $\beta$-blockade.

Current practice guidelines for the management of patients with stable CAD recommend ACE inhibitor therapy in those patients with an LVEF <40%; in addition, in part on the basis of data from the Heart Outcomes Prevention Evaluation (HOPE) trial, ACE inhibitors are recommended for patients who are relatively high risk and/or have another compelling clinical indication (eg, hypertension, diabetes mellitus, or chronic kidney disease).[35] In contrast, for lower-risk patients like those in the PEACE trial, in which the event rate in the placebo arm was lower than the event rate in the ACE inhibitor arm from the HOPE trial, the guidelines note that it is reasonable but not recommended to use ACE inhibitors when cardiovascular risk factors are well controlled and revascularization has been performed. Our data now support the hypothesis that within this very large population of patients who appear to be of lower risk

clinically, biomarkers of cardiovascular stress levels may be useful to help guide such decision making. Although additional prospective analyses will need to be done if these biomarkers become available for routine clinical use in the United States, targeting long-term drug therapy based on a panel of biomarkers should be cost effective.

Several potential limitations of our study deserve consideration. The PEACE clinical trial population, which was predominantly a white, male population >50 years of age, is not representative of the general population. However, the clinical and laboratory characteristics of patients in this study are typical of patients with stable coronary disease, and a high proportion of patients were treated with β-blockers and lipid-lowering therapy. Blood samples were obtained from only a subgroup of the participants in the overall PEACE trial, but there were no clinically relevant differences between patients who did and did not participate in the biomarker substudy. Banked biosamples were used, but any sample degradation should be random with respect to cardiovascular outcomes, and thus any resultant misclassification should only bias toward the null hypothesis. The formation of the multimarker score for interaction with therapy should be considered exploratory, and the optimal combination of biomarkers and their cut points merits validation in additional populations. Heart failure events were not a component of the prespecified primary outcome in the original trial design but are a well-established outcome predicted by biomarkers of cardiac stress and prevented by ACE inhibitors in other populations.[6,19,20,26]

## Conclusion

In apparently low-risk patients with stable CAD and preserved LVEF, elevated levels of novel biomarkers reflecting cardiovascular stress may be useful both to identify patients who are at higher risk of cardiovascular death and heart failure and to select patients who derive a significant benefit from ACE inhibitor therapy.

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## Disclosures

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## CLINICAL PERSPECTIVE

The benefit of angiotensin-converting enzyme inhibitors in low-risk patients with stable coronary artery disease without heart failure remains controversial, and current practice guidelines note that it is reasonable but not recommended to use angiotensin-converting enzyme inhibitors when cardiovascular risk factors are well controlled and revascularization has been performed. We now demonstrate that elevated levels of 3 novel biomarkers of cardiovascular stress, midregional pro-atrial natriuretic peptide, midregional pro-adrenomedullin, and C-terminal pro-endothelin-1, are associated with the subsequent risk of cardiovascular death and heart failure independently of clinical factors (adjusted hazard ratios per 1-SD increase of 1.97, 1.48, and 1.47, respectively; $P \leq 0.002$ for each biomarker). Furthermore, elevated levels of these biomarkers identified patients in whom therapy with an angiotensin-converting enzyme inhibitor resulted in a significant reduction in the risk of cardiovascular death or heart failure. Specifically, trandolapril significantly reduced the risk of cardiovascular death or heart failure in patients who had elevated levels of $\geq 2$ biomarkers (hazard ratio, 0.53; 95% confidence interval, 0.36–0.80), whereas there was no benefit in patients with elevated levels of 0 or 1 biomarker (hazard ratio, 1.09; 95% confidence interval, 0.74–1.59; $P_{\text{interaction}} = 0.012$). Thus, in patients with stable coronary artery disease and preserved left ventricular ejection fraction, elevated levels of novel biomarkers of cardiovascular stress identify patients who are at higher risk of cardiovascular death and heart failure and may be useful to select patients who derive significant benefit from angiotensin-converting enzyme inhibitor therapy.