Relations of Biomarkers of Extracellular Matrix Remodeling to Incident Cardiovascular Events and Mortality


Objective—To evaluate if biomarkers reflecting left ventricular/vascular extracellular matrix remodeling are associated with cardiovascular disease (CVD) and death in the community.

Methods and Results—In 922 Framingham Study participants (mean age, 58 years; 56% women), we related circulating concentrations of matrix metalloproteinase-9 (binary variable: detectable versus undetectable), log of tissue inhibitor of matrix metalloproteinase-1, and log of procollagen type III aminoterminal peptide (PIIINP) to incident CVD and death. On follow-up (mean, 9.9 years), 51 deaths and 81 CVD events occurred. Each SD increment of log of tissue inhibitor of matrix metalloproteinase-1 and log-PIIINP was associated with multivariable-adjusted hazards ratios of 1.72 (95% CI, 1.30 to 2.27) and 1.47 (95% CI, 1.11 to 1.96), respectively, for mortality risk. Log-PIIINP concentrations were also associated with CVD risk (hazard ratio [95% CI] per SD, 1.35 [1.05 to 1.74]). Death and CVD incidence rates were 2-fold higher in participants with both biomarkers higher than the median (corresponding hazard ratio [95% CI], 2.78 [1.43 to 5.40] and 1.77 [1.04 to 3.03], respectively) compared with those with either or both less than the median. The inclusion of both biomarkers improved the C-statistic (for predicting mortality) from 0.78 to 0.82 (P=0.03). Matrix metalloproteinase-9 was unrelated to either outcome.

Conclusion—Higher circulating tissue inhibitor of matrix metalloproteinase-1 and PIIINP concentrations are associated with mortality, and higher PIIINP is associated with incident CVD, in the community. (Arterioscler Thromb Vasc Biol. 2010;30:2283-2288.)

Key Words: extracellular matrix • remodeling • left ventricle • cardiovascular disease • matrix metalloproteinase-9 • tissue inhibitor of matrix metalloproteinase-1 • procollagen type III amino-terminal peptide

Left ventricular1 (LV) and systemic vascular2 remodeling precede and predict incident cardiovascular disease (CVD). Circulating biomarkers of ventricular and vascular remodeling may, therefore, aid in the prediction and stratification of CVD risk. Extracellular matrix (ECM) turnover is an integral component of cardiovascular remodeling. Consequently, 3 classes of proteins reflecting ECM synthesis and degradation (ie, the matrix metalloproteinases [MMPs], their tissue inhibitors [TIMPs], and the byproducts of collagen turnover [procollagen terminal peptides]) have received considerable attention in recent years.3 Several previous investigations reported associations of markers of ECM remodeling with cardiovascular risk factors,4–6 progression to hypertension,7 atherosclerotic coronary artery3 and cerebrovascular disease,8 and risk of death in patients with known CVD.9–11 However, it is unclear whether these biomarkers are associated with incident CVD events and all-cause mortality in people free of overt CVD in the community.

Accordingly, we evaluated the relations of circulating concentrations of MMP-9, TIMP-1, and procollagen type III aminoterminal peptide (PIIINP) to the incidence of CVD and all-cause mortality in a community-based sample. Of several possible biomarkers of ECM turnover, we chose these 3 because of previously demonstrated associations with ventricular and vascular remodeling.12–14 We hypothesized that elevated circulating levels of the previously mentioned biomarkers indicate active remodeling/turnover and will be associated with increased incidence of CVD and death. We further posited that these markers will improve risk prediction when added to statistical models with conventional cardiovascular risk factors.

Methods

Study Sample and Design
The details regarding the selection and sampling of the Framingham Heart Study offspring cohort have been previously published.15

Received on: April 25, 2010; final version accepted on: August 9, 2010. From the Framingham Heart Study (R.S.V., P.G., M.G.L., E.J.B., and R.S.V.), Framingham, Mass; the Department of Mathematics and Statistics (P.G. and M.G.L.), Boston University, Boston, Mass; the Department of Medical Sciences and Uppsala Clinical Research Center (J.S.), Uppsala University, Uppsala, Sweden; the Myocardial Biology Unit (D.S.), Boston University School of Medicine, Boston, Mass; the Epidemiology Department (W.S.C., E.J.B., and R.S.V.), School of Public Health, Boston University, Boston, Mass; the Cardiology Section (W.S.C., E.J.B., and R.S.V.), School of Medicine, Boston University, Boston, Mass; and the Preventive Medicine Section (E.J.B. and R.S.V.), School of Medicine, Boston University, Boston, Mass. Correspondence to Ramachandran S. Vasan, MD, Framingham Heart Study, 73 Mount Wayte Ave, Framingham, MA 01702–58. E-mail vasan@bu.edu © 2010 American Heart Association, Inc.

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Briefly, 5124 participants who are the children of the Framingham original cohort, and the spouses of these children, were enrolled in 1971 and have been evaluated approximately every 4 years. Of these participants, 3532 who attended examination cycle 6 (1995–1998) were eligible for the present investigation.

Given the relative novelty of the ECM biomarkers under consideration, we measured them only in a subsample of examination cycle 6 attendees to conserve nonrenewable precious serological resources and maximize statistical efficiency. The description of, and rationale for, our sampling strategy have been previously published and were determined by our primary objective of relating these ECM biomarkers to echocardiographic indexes of LV remodeling. Briefly, we identified candidates for ECM biomarker measurement by examining the distributions of echocardiographically measured LV wall thickness (LVWT) and LV end-diastolic dimension (LVEDD) and sampled participants with both LVWT and LVEDD less than the 50th percentile and with either higher than the 90th percentile of the distributions. Thus, plasma MMP-9, TIMP-1, and PIIINP were measured in 700, 1032, and 944 participants, respectively. Of these participants, we excluded those with prevalent CVD (definition given later). Relations of MMP-9, TIMP-1, and PIIINP to incident CVD and death were evaluated in the remaining 607, 922, and 840 participants, respectively. All participants provided written informed consent, and the study protocol was approved by the institutional review board of Boston Medical Center, Boston, Mass.

**Measurement of Biomarkers**

Plasma samples for biomarker measurement were drawn after an overnight fast, typically between 8 and 9 AM, and stored at 80°C without any freeze-thaw cycles. We measured the biomarkers in duplicate, using 2-site sandwich ELISA assays (Amersham Pharmacia Biotech, Piscataway, NJ) for MMP-9 and TIMP-1 and a radioimmunoassay (Orion Diagnostica, Espoo, Finland) for PIIINP. The plasma total MMP-9 assay measured MMP-9, ProMMP-9, and the ProMMP-9/TIMP-1 complex. Plasma total TIMP-1 assays measured free TIMP-1 and complexes of TIMP-1 with various MMPs. The intra-assay coefficients of variation were as follows: less than 18% for MMP-9, less than 5% for TIMP-1, and 6% for PIIINP.

**Echocardiographic Methods**

We measured LVEDD and the end-diastolic thicknesses of the interventricular septum (IVST) and posterior wall (PWT) using an American Society of Echocardiography–recommended leading-edge technique and calculated LV mass (LVM) and LVWT as follows:

\[
LVM \text{ (in grams)} = 0.814[(\text{LVEDD} + \text{IVST} + \text{PWT})^3 - (\text{LVEDD})^3] + 0.6
\]

\[
\text{LVWT (in centimeters)} = \text{IVST} + \text{PWT}
\]

Participants with both LVEDD and LVWT less than the 50th percentile of distribution were classified as the “referent” group, and those with either of these measures higher than the 90th percentile were classified as the “remodeled” group (we termed these groups “LVW and PWT” or “LV sampling groups”).

**Assessment of Outcomes**

A panel of 3 Heart Study investigators reviewed all cardiovascular events and validated them according to prespecified criteria. A neurologist examined participants with suspected stroke, and a separate panel that included a neurologist validated all cerebrovascular events. The 2 end points for this investigation are as follows: (1) all-cause mortality and (2) all fatal or nonfatal CVD events. CVD included recognized and unrecognized myocardial infarction, coronary insufficiency (unstable angina), angina pectoris, nonhemorrhagic stroke, transient ischemic attack, intermittent claudication, and heart failure. The Framingham Heart Study criteria for validating these events have been previously published.

**Statistical Analysis**

Because of the low rates of MMP-9 detection, we modeled this biomarker as a dichotomous variable (detectable versus undetectable) only. We natural-logarithmically transformed (to account for skewed distributions) TIMP-1 and PIIINP. We evaluated correlations between the latter 2 biomarkers by estimating age- and sex-adjusted Pearson correlation coefficients.

To examine the associations of biomarkers with the end points of interest, we estimated Cox proportional hazards regression models (after confirming the assumption of proportionality of hazards) and related MMP-9, log-TIMP-1, and log-PIIINP individually to all-cause mortality and incident CVD (separate models for each outcome) in age- and sex-adjusted models and in multivariable models. In addition, we adjusted for body mass index, systolic blood pressure, hypertension treatment, diabetes mellitus, the total cholesterol/high-density lipoprotein cholesterol ratio, current smoking, LVM, and LV sampling group. We also evaluated the relations of the biomarkers to both end points, separately in the referent and remodeled LV sampling groups.

We evaluated the relative contributions of TIMP-1 and PIIINP to mortality and CVD risk by including both biomarkers together in multivariable models and relating them to each end point separately. To determine the incremental value of ECM markers over clinical factors in predicting death and incident CVD, we calculated the C-statistics for models with and without biomarkers and tested if the inclusion of biomarkers significantly improved the C-statistic. We also evaluated net reclassification improvement and integrated discrimination improvement using the methods described by Pencina et al.

In additional analyses, we grouped participants into those with biomarker levels at or less than versus higher than the median value and constructed cumulative mortality incidence curves for each group. Also, we evaluated the hazards for mortality and CVD in those with both biomarkers higher than the median, compared with participants with either biomarker at or less than the median, and evaluated if hazards for increment in the risk of death or CVD (when both biomarkers are elevated) are additive or synergistic by including the product of median TIMP-1 and median PIIINP as an interaction term in the model. In addition, we evaluated whether biomarker-outcome relations varied according to LV sampling group by including first-order interaction terms (biomarker × LV sampling group) in the multivariable models relating each biomarker separately to 2 end points (death and CVD). Finally, because B-type natriuretic peptide (BNP) and C-reactive protein (CRP) have been consistently reported to predict death and CVD, we added them to the multivariable models to evaluate if ECM markers predicted either outcome independent of these established biomarkers. All analyses were performed using computer software (SAS, version 8.2) and a 2-sided probability value (<0.05) denoted statistical significance.

**Results**

The baseline clinical, echocardiographic, and ECM biomarker characteristics of the participants are presented in Table 1. The overall sample of 922 individuals included 572 in the reference group and 350 in the remodeled group. During a follow-up of up to 12.7 years (mean, 9.9 years), 51 deaths and 81 first CVD events occurred. The age- and sex-adjusted Pearson correlation coefficient between log-TIMP-1 and log-PIIINP was 0.18 (P<0.001).

**Relations of Biomarkers to Mortality Risk**

In age- and sex-adjusted models, we observed that each SD increment of log-TIMP-1 and log-PIIINP levels was associated with a 97% and 48% increased risk of death, respectively (Table 2). Adjustment for other covariates only slightly attenuated the risk associated with log-TIMP-1 but did not alter the estimates for log-PIIINP (Table 2); further adjustment for BNP and CRP did not substantially alter these results (data not shown). MMP-9 was not significantly associated with mortality risk.
Table 1. Baseline Characteristics of Study Participants*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Whole Sample (n=922)</th>
<th>Reference Group (n=572)</th>
<th>Remodeled Group (n=350)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>58 (10)</td>
<td>55 (9)</td>
<td>59 (10)</td>
</tr>
<tr>
<td>Women, %</td>
<td>58</td>
<td>57</td>
<td>61</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.1 (5.0)</td>
<td>25.2 (3.7)</td>
<td>29.6 (5.5)</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>126 (19)</td>
<td>121 (17)</td>
<td>134 (21)</td>
</tr>
<tr>
<td>Hypertension treatment, %</td>
<td>ACE inhibitors</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>β-blockers</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Calcium channel blockers</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Diuretics</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Lipid-modifying treatment, %</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Diabetes mellitus, %</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Total/HDL cholesterol ratio, %</td>
<td>4.3 (1.4)</td>
<td>4.2 (1.5)</td>
</tr>
<tr>
<td></td>
<td>Current smoking, %</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Estimated glomerular filtration rate, mL/min/1.73 m²</td>
<td>92 (50)</td>
<td>93 (58)</td>
</tr>
<tr>
<td></td>
<td>LV mass, g</td>
<td>159 (55)</td>
<td>125 (26)</td>
</tr>
<tr>
<td></td>
<td>LV wall thickness, cm</td>
<td>1.89 (0.31)</td>
<td>1.72 (0.14)</td>
</tr>
<tr>
<td></td>
<td>LV end-diastolic dimension, cm</td>
<td>4.74 (0.61)</td>
<td>4.46 (0.35)</td>
</tr>
<tr>
<td>Biomarker factors</td>
<td>MMP-9, % detectable</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>TIMP-1, ng/mL</td>
<td>20.0 (4.0)</td>
<td>19.2 (3.2)</td>
</tr>
<tr>
<td></td>
<td>PIIINP, ng/mL</td>
<td>4.0 (3.8)</td>
<td>4.0 (4.1)</td>
</tr>
</tbody>
</table>

ACE indicates angiotensin converting enzyme; HDL, high-density lipoprotein.
*Data are given as mean (SD) unless otherwise indicated.
†The total sample for this factor was 840.

Relations of Biomarkers to Incidence of CVD

We observed that higher PIIINP levels were associated with a 31% increased risk of CVD per SD increment log-marker in age- and sex-adjusted models (Table 2). Multivariable adjustment did not attenuate these findings, but relations of PIIINP to CVD were no longer statistically significant after additional adjustment for BNP and CRP. Relations of log-TIMP-1 to CVD were no longer statistically significant after additional adjustment for BNP and CRP. Relations of log-TIMP-1 and log-PIIINP were associated with a 4% and 35% increase in hazards, respectively (Table 3). In multivariable analyses relating both biomarkers together to CVD risk, log-TIMP-1 and log-PIIINP were associated with a 4% and 35% increase in hazards, respectively, but the relations of log-TIMP-1 were no longer statistically significant after adjustment for BNP and CRP. Relations of log-TIMP-1 and log-PIIINP together were significantly related to mortality; each SD increase of log-TIMP-1 and log-PIIINP was associated with a 4% and 35% increase in risk of mortality, respectively (Table 3). In multivariable analyses relating both biomarkers together to CVD risk, log-TIMP-1 and log-PIIINP were associated with a 4% and 35% increase in hazards, respectively, but the relations of log-TIMP-1 were not statistically significant (P=0.77; Table 3).

The inclusion of biomarkers did not improve the model C-statistic. The inclusion of TIMP-1 and PIIINP to the model with clinical covariates did not improve net reclassification improvement statistic that was of borderline significance for predicting death (integrated discrimination improvement statistic, 0.18; P=0.07) and statistically significant for predicting CVD (integrated discrimination improvement statistic, 0.009; P=0.03).

Additional Analyses

The cumulative incidence of death by median PIINP and median TIMP-1 is presented in Figure 1A and B, respectively. Participants with both biomarkers higher than the median experienced mortality and CVD at rates that were twice as high compared with those with either biomarker less than the median (Figure 2 and Table 4). However, the

Table 2. Relations of ECM Biomarkers to CVD and Mortality

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adjusted HR (95% CI)*</th>
<th>P Value</th>
<th>Adjusted HR (95% CI)*</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death (n=808, 44 events)</td>
<td>Log-TIMP-1</td>
<td>1.60 (1.19–2.15)</td>
<td>0.002</td>
<td>Log-PPIINP</td>
</tr>
<tr>
<td>CVD (n=808, 64 events)</td>
<td>Log-TIMP-1</td>
<td>1.04 (0.79–1.37)</td>
<td>0.77</td>
<td>Log-PPIINP</td>
</tr>
</tbody>
</table>

HR indicates hazards ratio.
*HR per SD change in biomarker levels, adjusted for age, sex, body mass index, systolic blood pressure, hypertension treatment, diabetes mellitus, total/HDL cholesterol ratio, current smoking, LV mass, and LV sampling group.

Table 3. Conjoint Relations of TIMP-1 and PIIINP to the Risk of Death and CVD

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adjusted HR (95% CI)*</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death (n=808, 44 events)</td>
<td>Log-TIMP-1</td>
<td>1.60 (1.19–2.15)</td>
</tr>
<tr>
<td>CVD (n=808, 64 events)</td>
<td>Log-TIMP-1</td>
<td>1.04 (0.79–1.37)</td>
</tr>
</tbody>
</table>

HR indicates hazards ratio.
*HR per SD change in biomarker levels, adjusted for age, sex, body mass index, systolic blood pressure, hypertension treatment, diabetes mellitus, total/HDL cholesterol ratio, current smoking, LV mass, and LV sampling group.
interaction term for the product of median TIMP-1 and median PIIINP was not significant, suggesting that the hazards for death and CVD portended by elevation of both biomarkers are additive, not synergistic.

The results of analyses relating TIMP-1 and PIIINP to all-cause mortality separately in the referent and remodeled groups were consistent with those from the overall sample (supplemental Table I and supplemental Table II; available online at http://atvb.ahajournals.org). For incident CVD, results for both markers in the remodeled group paralleled those from the combined sample (supplemental Table II). In the referent group, TIMP-1 and PIIINP were not associated with CVD (supplemental Table I). In addition, none of the interaction terms in the models evaluating if relations of biomarkers to outcomes varied by LV sampling group were statistically significant ($P \geq 0.05$ for all interactions).

**Discussion**

**Principal Findings**

In a community-based sample free of baseline CVD, we observed that higher circulating levels of TIMP-1 and PIIINP were associated with increased risk of death and that higher PIIINP concentrations were associated with incident CVD events. Although ECM biomarkers have been reported to be associated with LV and vascular remodeling,6,16 we noted that the associations of these markers with death and CVD were maintained even after adjustment for LVM and LV remodeling and that their relations to death endured after additional adjustment for BNP and CRP. Not surprisingly, PIIINP (a direct measure of tissue turnover) had the strongest and most consistent associations with death and CVD in all models. The results of analyses stratified by LV sampling group were not significantly different from our main results (for the total sample), and none of the interaction terms were statistically significant; however, given the modest event density in our sample, we may be unable to completely rule out heterogeneity in the association of matrix markers with outcomes according to LV group. To our knowledge, this is one of the first investigations that demonstrated the association of these biomarkers with incident CVD and all-cause mortality in a community-based sample.

**Relations of ECM Remodeling Markers to CVD: Insights From Previous Reports**

As previously noted, several previous investigations4–7 reported the associations of ECM remodeling markers with CVD risk factors. Prior reports also described the associations of ECM biomarkers with LV structural change,20 LV remodeling after MI,21,22 measures of carotid atherosclerosis,23 hypertension-related systolic and diastolic heart failure,24 functional status measures in those with overt heart failure,25

<table>
<thead>
<tr>
<th>Variable</th>
<th>Level at or Less than the Median</th>
<th>Both Levels Higher than the Median</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Event proportions* 17/585 (2.9) 27/223 (12.1)</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Event rates† 7.4 (0.0–14.2) 14.1 (5.7–21.4)</td>
<td>Referent 2.78 (1.43–5.40) 0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (95% CI)‡</td>
<td>Referent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CVD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Event proportions* 29/585 (5.0) 35/223 (15.7)</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Event rates† 7.8 (3.3–11.8) 13.6 (7.3–19.2)</td>
<td>Referent 1.77 (1.04–3.03) 0.04</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HR indicates hazards ratio; NA, not applicable.

*Data are given as number of events/total number at risk (percentage).
†Age- and sex-adjusted event rates per 100 person-years of follow-up.
‡Data are for participants with both biomarker levels higher than the median ($n=223$), compared with those with either at or less than (referent; $n=585$), adjusted for age, sex, body mass index, systolic blood pressure, hypertension treatment, diabetes mellitus, total/HD cholesterol ratio, current smoking, LV mass, and LV sampling group.

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Figure 1. A, Cumulative incidence of all-cause mortality in participants with a PIIINP higher than and at or less than the median. B, Cumulative incidence of all-cause mortality in participants with a TIMP-1 higher than and at or less than the median.

Figure 2. Cumulative incidence of all-cause mortality in participants with both TIMP-1 and PIIINP higher than the median and either or both biomarkers at or less than the median.
and progression of heart failure because of volume overload.26 Other investigators27 have explored the quantitative differences in circulating ECM marker levels between healthy individuals, those with risk factor substrate for heart failure, and those with clinical heart failure. One report28 identified the utility of these markers in the diagnosis of diastolic dysfunction and heart failure with preserved ejection. However, previous investigations did not evaluate the association of ECM biomarkers with CVD risk in those free of prevalent disease.

Our investigation is relatively novel in 3 respects. First, we demonstrate the independent relations of ECM biomarkers to death and incident CVD in a population free of preexisting CVD. Second, we report the incremental value of these biomarkers over conventional cardiovascular risk factors in predicting risk of death, as evidenced by a significant improvement in model C-statistic. Third, we observe that these markers are related to death and CVD even after adjusting for echocardiographic indexes of LV remodeling, suggesting that the adverse prognosis related to matrix remodeling may not be fully captured by imaging measures.

**Potential Mechanisms**

Cardiovascular remodeling is an active process consisting of adaptive and maladaptive changes in response to pressure or volume overload; it antedates the development of overt CVD.1,20 In addition to hemodynamic load, several biological processes (ie, inflammation, oxidative stress, a prothrombotic state, or activation of the renin-angiotensin-aldosterone and natriuretic peptide systems) are associated with both ventricular and vascular remodeling and the subsequent risk for CVD. Because injury from any or all cardiovascular risk factors leads to remodeling, ECM turnover can potentially be viewed as a final common process that identifies the cumulative effects of several risk factors and pathways.

Thus, there are several potential explanations for our results. First, elevated levels of ECM turnover markers in individuals without clinically apparent CVD could reflect subclinical disease and, thus, are identifying those with greater risk. Second, ECM markers are related to several conventional CVD risk factors; however, in our study, these markers were related to CVD risk above and beyond these factors. Third, ventricular remodeling (as measured by changes in LV structure via echocardiography) is independently related to risk of death and CVD10–32 and, therefore, the biomarkers (reflecting this process) are related to mortality and CVD risk. However, we observed that ECM markers are associated with mortality and CVD risk after adjusting for remodeling indexes. Fourth, apart from structural change, other processes (eg, inflammation, oxidative stress, and renin-angiotensin-aldosterone system activation) are associated with CVD risk; ECM markers are associated with markers of these pathways33 and, thereby, may be capturing the risk associated with them. Indeed, relations of PIIINP to CVD were no longer significant after adjustment for BNP and CRP. Last, conventional CVD risk factors do not completely explain CVD risk, and ECM markers may be identifying the residual risk.

Although the results of our investigation draw attention to several pathophysiological mechanisms involved in cardiovascular events, as previously noted, the low-risk clinical profile of our sample and the low event density suggest caution in generalizing these findings to clinical settings. However, our findings do imply that ECM biomarkers merit further investigation for their role in risk prediction in individuals with average or higher CVD risk.

**Limitations**

From a large family of markers of ECM turnover, we measured only 3 biomarkers; other markers may be equally or more informative. We did not measure the biomarkers in a sample that included the entire range of LVWT and LVEDD and, therefore, our results may not be generalized to individuals with LVEDD and LVWT in the intermediate range. We were hampered in the evaluation of the relations of MMP-9 to CVD and death because of low rates of detectability. Preanalytical factors related to degradation of MMP-9 in frozen specimens may have contributed to the low detectability.34 Because of the modest numbers of mortality and CVD events, we did not have adequate power to detect relations of biomarkers to individual CVD event types (eg, coronary heart disease and heart failure) and separately to risk of cardiovascular versus noncardiovascular death. Statistical modeling cannot completely account for the impact of comorbidities, and there may be residual confounding not accounted for by the covariates in our models. Last, our sample is composed of middle-aged white individuals of European descent and our results cannot be generalized to individuals of other age groups or ethnicities.

In conclusion, in a free-living cohort of middle-aged individuals, we demonstrate that circulating biomarkers of ECM turnover (specifically, TIMP-1 and PIIINP) predict incident CVD and death. Participants with both markers higher than the median are at markedly higher risk of death and CVD. Our findings confirm and extend previous research that demonstrated that ventricular and vascular remodeling antedates clinical CVD. Additional research is needed to delineate the utility of these measures for screening, to estimate the risk of individual CVD events, and to use the measures in clinical settings.

**Acknowledgments**

We had full access to the data and take responsibility for the integrity of the data. We also all have read and agree to the manuscript as written.

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**Disclosures**

None.

**References**

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Supplementary Information: Relations of Biomarkers of Extracellular Matrix Remodeling to Incident Cardiovascular Events and Mortality

I. Definitions of covariates

II. Supplementary Table I. Results in referent group

III. Supplementary Table II. Results in remodeled group
Definitions of Covariates

We defined all covariates at examination cycle 6, which served as the baseline for the present investigation. We calculated body mass index (BMI) as the weight in kilograms divided by the square of height in meters. A physician measured blood pressure twice during the Heart Study clinic visit on the left arm of seated participants using a mercury-column sphygmomanometer and a cuff of appropriate size; we used the average of these two readings as the examination blood pressure. We defined diabetes as fasting plasma glucose of 126 mg/dl or greater, or the use of insulin or other hypoglycemic therapy. We defined “current smoking” as smoking on average at least one cigarette per day in the year preceding the baseline examination.
Supplementary Table I. Relations of ECM biomarkers to CVD and mortality – Referent Group

<table>
<thead>
<tr>
<th></th>
<th>CVD</th>
<th></th>
<th>Death</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adjusted HR (CI)</td>
<td>P-value</td>
<td>Adjusted HR (CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>A. MMP-9 as binary covariate (n = 271; 12 CVD events and 9 deaths) *</td>
<td>Age- and sex-adjusted</td>
<td>3.52 (1.03 – 12.10)</td>
<td>0.045</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>Multivariable-adjusted</td>
<td>4.29 (1.02 – 18.00)</td>
<td>0.047</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>B. log-TIMP-1 (n = 572; 26 CVD events and 17 deaths) †</td>
<td>Age- and sex-adjusted</td>
<td>1.10 (0.72 – 1.69)</td>
<td>0.65</td>
<td>2.52 (1.49 – 4.27)</td>
</tr>
<tr>
<td></td>
<td>Multivariable-adjusted</td>
<td>1.09 (0.75 – 1.60)</td>
<td>0.64</td>
<td>2.33 (1.35 – 4.00)</td>
</tr>
<tr>
<td>C. log-PIIINP (n = 512; 24 CVD events and 15 deaths) †</td>
<td>Age- and sex-adjusted</td>
<td>1.09 (0.73 – 1.64)</td>
<td>0.67</td>
<td>1.54 (1.04 – 2.27)</td>
</tr>
<tr>
<td></td>
<td>Multivariable-adjusted</td>
<td>1.06 (0.68 – 1.66)</td>
<td>0.81</td>
<td>1.56 (1.02 – 2.37)</td>
</tr>
</tbody>
</table>

Multivariable model included age, sex, BMI, systolic blood pressure, hypertension treatment, diabetes, total cholesterol/high density lipoprotein cholesterol ratio, current smoking and LV mass (continuous variable).

* HR indicates hazards in those with detectable MMP-9 compared to those without.
† HR per standard deviation change in biomarker levels.
**Supplementary Table II. Relations of ECM biomarkers to CVD and mortality – Remodeled Group**

<table>
<thead>
<tr>
<th></th>
<th>CVD</th>
<th></th>
<th>Death</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adjusted HR (CI)</td>
<td>P-value</td>
<td>Adjusted HR (CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>A. MMP-9 as binary covariate (n = 335; 52 CVD events and 32 deaths) *</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age- and sex-adjusted</td>
<td>1.13 (0.61 – 2.09)</td>
<td>0.71</td>
<td>1.42 (0.67 – 2.98)</td>
<td>0.84</td>
</tr>
<tr>
<td>Multivariable-adjusted</td>
<td>0.95 (0.50 – 1.80)</td>
<td>0.87</td>
<td>1.10 (0.50 – 2.38)</td>
<td>0.82</td>
</tr>
<tr>
<td>B. log-TIMP-1 (n = 350; 55 CVD events and 34 deaths) †</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age- and sex-adjusted</td>
<td>1.38 (1.07 – 1.77)</td>
<td>0.01</td>
<td>1.73 (1.29 – 2.32)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Multivariable-adjusted</td>
<td>1.11 (0.84 – 1.47)</td>
<td>0.45</td>
<td>1.64 (1.16 – 2.30)</td>
<td>0.005</td>
</tr>
<tr>
<td>C. log-PIIINP (n = 323; 50 CVD events and 31 deaths) †</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age- and sex-adjusted</td>
<td>1.52 (1.16 – 1.99)</td>
<td>0.003</td>
<td>1.50 (1.01 – 2.23)</td>
<td>0.04</td>
</tr>
<tr>
<td>Multivariable-adjusted</td>
<td>1.50 (1.09 – 2.06)</td>
<td>0.01</td>
<td>1.80 (1.23 – 2.70)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Multivariable model included age, sex, BMI, systolic blood pressure, hypertension treatment, diabetes, total cholesterol/high density lipoprotein cholesterol ratio, current smoking and LV mass (continuous variable).

* HR indicates hazards in those with detectable MMP-9 compared to those without.
† HR per standard deviation change in biomarker levels.