Extracellular Cardiac Matrix Biomarkers in Patients With Acute Myocardial Infarction Complicated by Left Ventricular Dysfunction and Heart Failure Insights From the Eplerenone Post–Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) Study

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Background—Aldosterone stimulates cardiac collagen synthesis. Circulating biomarkers of collagen turnover provide a useful tool for the assessment of cardiac remodeling in patients with congestive heart failure and left ventricular systolic dysfunction after acute myocardial infarction.

Methods and Results—In a substudy of the Eplerenone Post–Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS), which evaluated the effects of the selective aldosterone receptor antagonist eplerenone versus placebo, serum levels of collagen biomarkers were measured in 476 patients with congestive heart failure after acute myocardial infarction complicated with left ventricular systolic dysfunction. The combination of the type I collagen telopeptide and brain natriuretic peptide levels above median at baseline was associated with all-cause mortality and the composite end point of cardiovascular death or heart failure hospitalization, with hazard ratios of 2.49 ($P=0.039$) and 3.03 ($P=0.002$), respectively. During follow-up, levels of aminoterminal propeptide of type I and type III procollagen were found to be consistently lower in the eplerenone group and significantly lower beginning at 6 months.

Conclusions—Changes in biomarkers of collagen synthesis and degradation suggest that extracellular matrix remodeling is an active process in patients with congestive heart failure and left ventricular systolic dysfunction after acute myocardial infarction. High type I collagen telopeptide and high brain natriuretic peptide serum levels are associated with the highest event rate. Eplerenone suppresses post–acute myocardial infarction collagen turnover changes. (Circulation. 2009;119:2471-2479.)

Key Words: biological markers ■ collagen ■ extracellular matrix ■ heart failure ■ myocardial infarction
remodeling is a consequence of changes in tissue structure (ie, cardiac extracellular collagen matrix [ECCM] synthesis or degradation) cannot be assessed by classic cardiac imaging. ECCM turnover is a major determinant of cardiac remodeling in various conditions such as HF, obesity, and AMI.2–4 Circulating biomarkers of collagen turnover may provide a simple tool to reliably assess this ECCM turnover.

In patients with CHF caused by systolic dysfunction after AMI, the kinetics of those biomarkers may be influenced by neurohormones involved in the pathophysiology of remodeling. In a substudy of the Randomized Aldactone Evaluation Study (RALES), biomarkers of matrix synthesis were found to be predictive of adverse outcome. These biomarkers were reduced by spironolactone, and patients with baseline concentrations above the median experienced a greater benefit.2

This process, however, has not been evaluated in detail in patients with CHF after AMI complicated by LVSD.5 In a substudy of EPHESUS, we examined the kinetics and predictive significance of markers of matrix synthesis and degradation and the effects of eplerenone on these parameters.

In this substudy, we examined the plasma time course profile of selected biomarkers over a period of 9 months and the effect of eplerenone on both the levels and time course of these biomarkers. In addition, we evaluated the relationship between baseline levels of some neurohormones and the biomarkers of interest and trial outcomes.

**Methods**

**Study Design and Patient Population**

The design and main results of EPHESUS have been reported.1,6 This substudy was conducted in 476 patients participating in EPHESUS enrolled in 65 centers that volunteered to participate in 17 countries. EPHESUS enrolled patients with CHF after AMI complicated by LVSD (ejection fraction ≤40%). HF had to be documented by at least one of the following: presence of pulmonary rales, chest radiography showing pulmonary venous congestion, or the presence of a third heart sound. Patients were entered in the study at any point from 3 to 14 days after infarction. Patients with diabetes mellitus were not required to have evidence of CHF. All patients were randomly assigned to treatment with eplerenone 25 mg/d or placebo for the first month uptitrated to 50 mg/d or placebo, depending on serum potassium levels. Treatment with eplerenone was in addition to standard medical therapy, which could include angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, β-blockers, diuretics, aspirin, statins, and coronary reperfusion therapy. EPHESUS was an event-driven study with a mean follow-up of 16 months.

Blood samples for all the biomarkers of collagen turnover were analyzed in 1 laboratory. Plasma BNP and other neurohormones were analyzed in a different laboratory. Correlation analysis of biomarkers and their predictability of outcomes took into account only the group treated with placebo.

**Blood Sampling**

Blood samples were drawn at baseline, 4 weeks, and 3, 6, and 9 months or at the time of permanent discontinuation of the drug. All samples were centrifuged immediately at 3000 rpm for 10 minutes and stored at −80°C until assay. All samples were transported to the 2 respective central laboratories and assayed in 1 batch in each laboratory. A minimum of 2 samples were available per patient: 1 at baseline and 1 at follow-up.

**Table 1. Patient Outcomes**

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=236), n (%)</th>
<th>Eplerenone (n=240), n (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Died</td>
<td>22 (9.3)</td>
<td>27 (11.3)</td>
<td>0.28</td>
</tr>
<tr>
<td>Alive</td>
<td>214 (90.7)</td>
<td>213 (88.8)</td>
<td></td>
</tr>
<tr>
<td>Discontinued study medication</td>
<td>22 (9.3)</td>
<td>20 (8.3)</td>
<td>0.87</td>
</tr>
<tr>
<td>Protocol nonadherence</td>
<td>3 (1.3)</td>
<td>3 (1.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>Treated with spironolactone</td>
<td>4 (1.7)</td>
<td>0 (0.0)</td>
<td>0.42</td>
</tr>
<tr>
<td>Adverse sign or symptom</td>
<td>5 (2.1)</td>
<td>7 (2.9)</td>
<td>0.50</td>
</tr>
<tr>
<td>Adverse event &gt;7 d after last dose</td>
<td>0 (0.0)</td>
<td>1 (0.4)</td>
<td>0.72</td>
</tr>
<tr>
<td>Patient request</td>
<td>10 (4.2)</td>
<td>9 (3.8)</td>
<td>0.92</td>
</tr>
</tbody>
</table>

**Laboratory Analysis**

The following collagen biomarkers were measured: aminoterminal propeptide of type I procollagen (PINP) and aminoterminal propeptide of type III procollagen (PIIINP), which are markers of type I and III collagen synthesis, respectively; tissue inhibitor of matrix metalloproteinase 1 (TIMP-1), which inhibits matrix metalloproteinases (MMPs) involved in collagen degradation; and type I collagen telopeptide (ICTP), which is a marker of type I collagen degradation. All assays were performed by technicians blinded to clinical data and subject randomization.

Commercial radioimmunoassays (Orion Diagnostica, Espoo, Finland) were used to measure PIIINP, PINP, and ICTP. Determination of TIMP-1 was assessed with ELISA kits (Amersham Biosciences, Orsay, France).

The sensitivity (lowest concentration different from zero) was 0.3 μg/L for PIIINP, 2.0 μg/L for PINP, 0.4 μg/L for ICTP, and ≤3 ng/mL for TIMP-1.

Normal serum ranges for PIIINP, PINP, and ICTP were provided by the assay manufacturer and were based on a Finnish population. These ranges were 2.3 to 6.4 ng/mL for PIIINP; 22 to 87 and 19 to 83 ng/mL for PINP in men and women, respectively; and 3.2 and 3.5 ng/mL for ICTP in men and women, respectively. Normal serum ranges for TIMP-1 are 0 to 519 ng/mL.

Inter assay variations for PIIINP, PINP, and ICTP were <9.8% and 15% for TIMP-1. Their intra-assay variations were <10.2%.

**Statistical Analysis**

Analyzes were performed with SAS version 9.1.3 software (SAS Institute, Inc, Cary, NC). The 2-tailed significance level was set at 0.05. Categorical variables are given as percentages; continuous variables are given as mean±SD.

**Correlation Analyses**

To control for deviations from the normality assumption, correlation analyses were carried out with the nonparametric Spearman test.

**Biomarkers Kinetics**

Biomarker changes from baseline at months 1, 3, 6, and 12 were analyzed with a mixed-effect model, with change from baseline as the dependent variable and treatment (fixed effect) and patient (random effect) as covariables. Age and gender were tested in each model and included in the model when needed (The only significant association was found between PINP and gender). Treatment differences at each visit after baseline were assessed at the 1.25% significance level to preserve the 5% overall error rate (Bonferroni adjustment). Comparisons between baseline and month 1 were carried out with the paired Wilcoxon test.
Thus, a binary variable of BNP median versus median and ICTP patients with baseline values of BNP and ICTP above the median. The small relationship of BNP and ICTP values with risk levels, but further BNP alone in univariate analyses (data not shown). The small ICTP levels, and HF hospitalization was found to be associated with BNP and of cardiovascular death and the composite end point of cardiovascular death and HF hospitalization were found to be associated with BNP and composite death for the global population, we did not analyze the interaction biomarkers substudy and greater use of diuretics. Randomization was different patients in the global population had a patients in the biomarkers substudy were on average younger patients with biomarkers versus others.

Results

Baseline Characteristics

Of the 476 patients included in the study, 236 were randomized to the placebo arm, and 240 received eplerenone. The disposition of patients during the course of the study is summarized in Table 1. In total, 49 patients died, and in terms of mortality and hospitalization for HF did not differ significantly between the placebo and eplerenone groups in this biomarkers substudy cohort.

There were no statistically significant differences between the eplerenone and placebo groups with respect to baseline demographic and clinical characteristics (Table 2).

Differences between the patient population in this study and the overall EPHESUS population are summarized in Table 3. Compared with the overall EPHESUS population, patients in the biomarkers substudy were on average younger and had higher diastolic blood pressure, LV ejection fraction, and potassium levels. Patients in the global population had a higher number of previous AMI and HF than patients in the biomarkers substudy and greater use of diuretics. Randomization occurred on average >1 day later in the biomarkers substudy. Because baseline characteristics were different from the global population, we did not analyze the interaction between the effects of eplerenone and study end points.

Table 2. Baseline Characteristics in Patients With Available Biomarkers

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Placebo Arm</th>
<th>Eplerenone Arm</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n</td>
<td>236</td>
<td>240</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>62±11</td>
<td>62±11</td>
<td>NS</td>
</tr>
<tr>
<td>&gt;65 y, n (%)</td>
<td>93/236 (39.4)</td>
<td>98/240 (40.8)</td>
<td>NS</td>
</tr>
<tr>
<td>&gt;75 y, n (%)</td>
<td>27/236 (11.4)</td>
<td>27/240 (11.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Male, %</td>
<td>75</td>
<td>73</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>121±16</td>
<td>118±16</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>75±10</td>
<td>73±10</td>
<td>NS</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>34±5</td>
<td>34±5</td>
<td>NS</td>
</tr>
<tr>
<td>Time from AMI to randomization, d</td>
<td>8.5±2.7</td>
<td>8.6±2.9</td>
<td>NS</td>
</tr>
<tr>
<td>Prior HF hospitalization, %</td>
<td>6</td>
<td>3</td>
<td>NS</td>
</tr>
<tr>
<td>Reperfusion/ revascularization, %</td>
<td>39</td>
<td>38</td>
<td>NS</td>
</tr>
<tr>
<td>HF symptoms, %</td>
<td>89</td>
<td>90</td>
<td>NS</td>
</tr>
<tr>
<td>Kaliemia, mmol/L</td>
<td>4.4±0.5</td>
<td>4.4±0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Creatininemia, µmol/L</td>
<td>101±32</td>
<td>99±28</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine clearance,*</td>
<td>81±36</td>
<td>80±29</td>
<td>NS</td>
</tr>
<tr>
<td>Medical history, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prior AMI</td>
<td>21</td>
<td>23</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes</td>
<td>29</td>
<td>30</td>
<td>NS</td>
</tr>
<tr>
<td>Prior HF episodes</td>
<td>11</td>
<td>9</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension</td>
<td>66</td>
<td>59</td>
<td>NS</td>
</tr>
<tr>
<td>Medications, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACEIs/ARB</td>
<td>89</td>
<td>83</td>
<td>0.043</td>
</tr>
<tr>
<td>β-blockers</td>
<td>78</td>
<td>77</td>
<td>NS</td>
</tr>
<tr>
<td>Diuretics</td>
<td>51</td>
<td>52</td>
<td>NS</td>
</tr>
<tr>
<td>Aspirin</td>
<td>85</td>
<td>91</td>
<td>0.029</td>
</tr>
<tr>
<td>Statins</td>
<td>50</td>
<td>46</td>
<td>NS</td>
</tr>
<tr>
<td>Biomarkers (n patients)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIIINP, ng/mL</td>
<td>35±17 (229)</td>
<td>33±14 (232)</td>
<td>NS</td>
</tr>
<tr>
<td>PiIIINP, ng/mL</td>
<td>4.2±1.5 (230)</td>
<td>4.2±1.7 (232)</td>
<td>NS</td>
</tr>
<tr>
<td>ICTP, ng/mL</td>
<td>6.7±4.0 (230)</td>
<td>6.4±3.4 (232)</td>
<td>NS</td>
</tr>
<tr>
<td>TIMP-1, ng/mL</td>
<td>1228±401 (230)</td>
<td>1164±317 (232)</td>
<td>NS</td>
</tr>
<tr>
<td>BNP, pg/mL</td>
<td>206±244 (226)</td>
<td>186±111 (232)</td>
<td>NS</td>
</tr>
</tbody>
</table>

BP indicates blood pressure; LVEF, LV ejection fraction; ACEI, angiotensin-converting enzyme inhibitor; and ARB, angiotensin receptor blocker. Values are mean±SD when appropriate.

*Cockcroft-Gault.

Time-to-Event Analyses

Analysis of the association between baseline biomarkers and outcomes was performed with the Cox proportional-hazards model. All-cause death and the composite end point of cardiovascular death or HF hospitalization were found to be associated with BNP and ICTP levels, and HF hospitalization was found to be associated with BNP alone in univariate analyses (data not shown). The small number of events prevented any reliable assessment of the linear relationship of BNP and ICTP values with risk levels, but further explorations showed that ~50% of outcomes were observed in patients with baseline values of BNP and ICTP above the median. Thus, a binary variable of BNP>median and ICTP>median versus BNP<median and/or ICTP<median was constructed and used as a
In the main study population and the present substudy population, although patients with diabetes could be enrolled only with the LVSD criteria and were not required to have evidence of CHF, only a small minority of these patients did not have CHF signs and symptoms in the placebo and eplerenone arms (15 of 236 [6.4%] and 16 of 240 [6.7%), respectively).

Baseline Levels and Evolution of Biomarkers in the Placebo Group
Baseline levels of the matrix biomarkers assessed in this study are summarized in Table 3. Baseline levels of all biomarkers were comparable between the placebo and eplerenone groups. Markers of collagen synthesis, including PINP and PIIINP, were all within reference limits at baseline, whereas TIMP-1 and ICTP were significantly elevated. Details of the plasma collagen biomarker patterns are shown in Figure 1. For all the analyzed biomarkers, important changes occurred early after AMI (baseline to the 1-month follow-up).

Markers of Matrix Synthesis
With respect to markers of collagen synthesis, PINP levels increased significantly from baseline to month 1 ($P<0.0001$) and declined modestly thereafter. The overall profile was similar for PIIINP but remained above baseline through month 9.

Markers of Matrix Degradation
With regard to collagen degradation, levels of TIMP-1 were above reference values throughout the course of the study. They decreased significantly between baseline and month 1 ($P<0.0001$) and month 3 and did not change significantly thereafter.

At baseline, ICTP levels were significantly higher than reference levels. There was a sustained and statistically significant fall in ICTP levels from baseline to month 1 ($P<0.0001$) and subsequent stabilization at levels slightly lower (5.3±2.8 ng/mL) than the upper reference value (5.6 ng/mL) through month 9.

Relation of Biomarkers to Other Biomarkers of Increased Mortality Risk at Baseline
Baseline collagen biomarkers were correlated with one another and with BNP (Table 4). Nevertheless, PINP was associated only with PIIINP ($r=0.32$, $P<0.0001$), and TIMP-1 was not correlated with PINP. ICTP was positively and significantly associated with PIIINP ($r=0.40$, $P<0.0001$) and BNP ($r=0.32$, $P<0.0001$). All collagen biomarkers and
BNP were correlated positively with high-sensitivity C-reactive protein levels at baseline.

LV ejection fraction was associated with levels of all biomarkers but PINP at baseline, especially with BNP ($r=0.24$, $P<0.0001$).

Prognostic Significance of Baseline Biomarker Levels

Fifty percent of outcomes were observed in patients with baseline values of BNP and ICTP above the median. The predictive value of baseline BNP and ICTP levels for the risks of all-cause death and composite end point cardiovascular death or HF hospitalization in the placebo group was examined with the Cox proportional-hazards model with medians as cutoffs. Results are presented in Figures 2 and 3.

Patients with both BNP and ICTP levels above the median displayed a significant increase in all-cause mortality with a hazard ratio (HR) of 2.49 ($P=0.039$) and in the composite end point of cardiovascular death or HF hospitalization with an HR of 3.03 ($P=0.002$).

Of note, PINP and PIIINP levels were not associated with any of the end points.

Effects of Eplerenone

Details of the collagen biomarker patterns are shown in Figure 1.

The mean changes in PINP and PIIINP levels throughout the study period were significantly different between the placebo and eplerenone groups (overall $P=0.001$ and 0.003 for PINP and PIIINP, respectively). PINP levels increased significantly in the eplerenone group from baseline to month 1 ($P<0.0001$) and declined modestly thereafter. Levels of PINP and PIIINP were consistently lower in the eplerenone group, significantly so beginning at month 6 ($P=0.007$ at month 6 for both PINP and PIIINP; $P<0.0004$ and 0.008 at month 9 for PINP and PIIINP, respectively).

With regard to collagen degradation, levels of TIMP-1 were above reference values in the eplerenone group throughout the course of the study. They tended to decrease subsequently, but the decrease was not statistically significant. Levels at all time points did not differ statistically from levels in the placebo group.

Similar to levels in the placebo group, ICTP baseline levels in the eplerenone group were significantly higher than reference levels and declined subsequently. Levels at all time points did not differ between the 2 treatment groups.

\begin{table}
\centering
\caption{ECCM Biomarkers and Risk Factor Correlations at Baseline for All Patients With Biomarkers} 
\begin{tabular}{|l|l|l|l|l|l|l|l|}
\hline
 & PINP & PIIINP & ICTP & TIMP-1 & BNP & hs-CRP & LVEF \\
\hline
\textbf{PINP} \\
$r$ & 1.00 & 0.32 & 0.09 & $-0.01$ & $-0.08$ & $-0.21$ & 0.06 \\
$P$ & $<0.0001$ & 0.056 & 0.76 & 0.084 & $<0.0001$ & 0.24 & 0.24 \\
n & 461 & 461 & 461 & 461 & 456 & 414 & 461 \\
\hline
\textbf{PIIINP} \\
r & 0.32 & 1.00 & 0.40 & 0.29 & 0.31 & 0.26 & $-0.12$ \\
$P$ & $<0.0001$ & $<0.0001$ & $<0.0001$ & $<0.0001$ & $<0.0001$ & 0.011 & 0.011 \\
n & 461 & 462 & 462 & 462 & 457 & 414 & 462 \\
\hline
\textbf{ICTP} \\
r & 0.09 & 0.40 & 1.00 & 0.25 & 0.32 & 0.21 & $-0.15$ \\
$P$ & 0.056 & $<0.0001$ & $<0.0001$ & $<0.0001$ & $<0.0001$ & 0.002 & 0.002 \\
n & 461 & 462 & 462 & 462 & 457 & 414 & 462 \\
\hline
\textbf{TIMP-1} \\
r & $-0.01$ & 0.29 & 0.25 & 1.00 & 0.37 & 0.37 & $-0.16$ \\
$P$ & 0.76 & $<0.0001$ & $<0.0001$ & $<0.0001$ & $<0.0001$ & 0.0008 & 0.0008 \\
n & 481 & 462 & 462 & 462 & 457 & 414 & 462 \\
\hline
\textbf{BNP} \\
r & $-0.08$ & 0.31 & 0.32 & 0.37 & 1.00 & 0.33 & $-0.24$ \\
$P$ & 0.084 & $<0.0001$ & $<0.0001$ & $<0.0001$ & $<0.0001$ & 0.001 & 0.001 \\
n & 456 & 457 & 457 & 457 & 458 & 414 & 458 \\
\hline
\textbf{PIIINP} \\
r & $-0.21$ & 0.26 & 0.21 & 0.37 & 0.33 & 0.33 & $-0.10$ \\
$P$ & $<0.0001$ & $<0.0001$ & $<0.0001$ & $<0.0001$ & $<0.0001$ & 0.037 & 0.037 \\
n & 414 & 414 & 414 & 414 & 414 & 415 & 415 \\
\hline
\textbf{ICTP} \\
r & 0.06 & $-0.12$ & $-0.15$ & $-0.16$ & $-0.24$ & $-0.10$ & 1.00 \\
$P$ & 0.24 & 0.01 & 0.002 & 0.0008 & $<0.0001$ & 0.037 & 0.037 \\
n & 461 & 462 & 462 & 462 & 458 & 415 & 476 \\
\hline
\end{tabular}
\ \footnotesize{hs-CRP indicates high-sensitivity C-reactive protein; LVEF, LV ejection fraction.}
\end{table}
Discussion

In the present study, we describe serial short-term and long-term changes in collagen biomarkers in patients after AMI complicated by HF and LVSD. We also show that at baseline, all collagen biomarkers correlated significantly with one another, BNP, and high-sensitivity C-reactive protein. Combination of both ICTP and BNP levels above median at baseline was a significant predictor of all-cause mortality and cardiovascular death or HF hospitalization in multivariable analyses.

During follow-up, eplerenone significantly reduced PINP and PIINP levels, with a significant reduction from month 6 to 9. ICTP and TIMP-1 biomarkers were not affected by eplerenone throughout the study period.

Baseline Characteristics and Kinetics of Collagen Biomarkers

Previous studies have shown that plasma levels of procollagen peptides and metalloproteinases can be used for monitoring cardiac collagen turnover.7 Because PINP and PIINP are released during collagen biosynthesis, it is possible to use them as biomarkers of collagen synthesis.8,9 Baseline values of those biomarkers of collagen synthesis among these patients (2.3 to 6.4 ng/mL for PIINP; 22 to 87 ng/mL for PINP in men and 19 to 83 ng/mL in women) were consistent with values of PINP and PIINP reported in a number of small AMI observational studies.10–16 In studies with repeated assessments, synthesis biomarkers rose as early as day 1 after MI12 and peaked between day 4 to 14.10–16 They remained high for periods of up to 1 year.14 Our results are supported by experimental work in which increases in types I and III procollagen mRNA in both infarcted and noninfarcted myocardium, followed by an increased collagen deposition, were reported as early as day 2 and peaked at day 14.17,18

Of note, our study enrolled only patients after AMI with LVSD and predominantly with HF. In addition, baseline values were not assessed in the very acute (first day) phase of AMI. Indeed, samples were taken at the randomization visit at an average of 8.5 days after AMI. Therefore, comparison of our results with those already reported should be made with caution. Interestingly, in chronic cardiac failure with established reductions in ejection fraction and LV chamber dilation, a substudy of the RALES trial,2 PIINP values were...
comparable to those reported in the present EPHESUS substudy population during long-term follow-up.

TIMP-1 levels were elevated above reference values in both the placebo and eplerenone groups throughout the course of the study. In the present study, because of limitations of blood sample volume, we did not assess the MMPs, compounding the interpretation of the TIMP-1 levels. MMP expression is known to increase in HF, and this increase usually exceeds the modest changes in levels of TIMP-1. The findings in this trial are consistent with an increase in matrix synthesis but suggest that eplerenone does not act via suppression of the TIMP pathway and is therefore unlikely to adversely affect the quality of collagen during the process of remodeling. Future studies should directly assess MMP activity, especially MMP-2 and MMP-9, simultaneously with TIMP in post-AMI HF.

High baseline levels of ICTP of 6.9±3.6 ng/mL in post-AMI patients were previously associated with LV remodeling. So far, no study has assessed the long-term kinetics of ICTP. In our study, ICTP levels were elevated at baseline and decreased significantly during the first month after AMI. However, ICTP levels remained higher than reference values during the 9-month follow-up period. High serum concentrations during the first month suggest increased degradation of the ECCM in the early phase after AMI. In HF resulting from dilated cardiomyopathy, high levels of ICTP at baseline have been reported, with levels up to a mean of 7.6±3.6 ng/mL. Spinale suggests that increased activity of MMP after infarction contributes to the remodeling process, which is consistent with a rise in the end product of PICP matrix degradation. The results of our study suggest that ICTP may be a marker of early post-AMI remodeling leading to LV dilation.

In some recent studies, ICTP levels were reported to increase in the 10 days after AMI, but profiles beyond this timeframe were not reported. In our study, ICTP levels were elevated at baseline but declined in parallel after 30 days concomitantly with decreases in the levels of PINP and PIIINP, suggesting a relationship between these biomarkers. Interestingly, in studies of patients with chronic cardiac failure with established LVSD and chronic ischemia, collagen synthesis was the main issue because a rise in PIIINP and its association with outcome were the main findings, compared with patients with AMI and LVSD such as in the present study, in which geometric remodeling of the LV was in evolution and collagen degradation seems to play a larger role.

Correlations Between Baseline Characteristics of ECCM Biomarkers
PINP was correlated only with PIIINP at baseline. This positive correlation between PINP and PIIINP levels implies a parallel or interrelated process for the synthesis and degradation of these biomarkers. Nevertheless, other associations between biomarkers according to our results underline the fact that the remodeling process consists of a continuous balance between various activators of ECCM collagen turnover.

Prognostic Significance in the Placebo Group
In our study, basal values of ICTP and BNP above median were associated with increased risks of all-cause death and cardiovascular death or HF hospitalization. High levels of ICTP reflect an intense breakdown of cicatricial zonal collagen after AMI, resulting in an increased release of ICTP. With a loss of matricial support in this zone, the patient would be more exposed to cardiac risk of dilation or even to LV rupture. Furthermore, in the present study, BNP was correlated with all ECCM biomarkers (except PINP), including ICTP. This is consistent with the findings of previous studies. Correlation between BNP and ICTP may suggest that this natriuretic peptide modulates collagen scar formation after AMI. Alternatively, the association between raised ICTP combined with raised BNP and adverse outcome may be explained by both biomarkers being covariates associated with a larger infarct extension, involving greater degradation of ECCM (hence high ICTP levels) and increased LV wall tension (thus an elevated BNP). Interestingly, the presence of high ICTP levels combined with low BNP levels at baseline (and reciprocally) was not associated with adverse outcomes, suggesting that a combination of increased ECCM degradation and myocyte stretch is critical to cardiovascular outcome and may be a potential differential therapeutic target.

The present study did not highlight any correlation between PIIINP and events. High basal values of PIIINP were correlated with cardiovascular mortality in CHF patients in the RALES study. This may imply that PIIINP is a more accurate biomarker of long-term cardiovascular events in chronic conditions such as HF rather than early-phase events such as AMI.

Effects of Eplerenone
Biomarkers of matrix synthesis were lower in the eplerenone than in the placebo group. PINP and PIIINP levels were consistently lower in the eplerenone group, significantly lower beginning at 6 months. Aldosterone has been shown to stimulate cardiac collagen synthesis and fibroblast proliferation via activation of local mineralocorticoid receptors. Aldosterone has been shown to promote cardiac fibrosis in experimental models and in humans. Eplerenone selectively blocks the mineralocorticoid receptor, thereby reversing these deleterious effects of aldosterone. In the RALES study of chronic cardiac failure with LVSD, high baseline serum levels of markers of cardiac fibrosis synthesis were significantly associated with poor outcome and decreased during spironolactone therapy. The benefit from spironolactone was associated with higher levels of collagen synthesis markers. Tsutamoto et al reported that 4 months of treatment with spironolactone improved LV volume and mass and decreased the plasma levels of BNP and PIIINP.

These results suggest that endogenous aldosterone has an important role in the process of LV remodeling in patients with CHF and that limitation of the excessive extracellular matrix turnover may be one of the various mechanisms contributing to the beneficial effect of spironolactone.

In patients with AMI, Hayashi et al demonstrated that aldosterone was extracted through the infarct heart and that extracting aldosterone stimulated postinfarct LV remodeling.
They randomized 134 patients with first anterior AMI to receive potassium canrenone (the active metabolite of spironolactone) in addition to an angiotensin-converting enzyme inhibitor or control treatment. LV ejection fraction was significantly improved and LV end-diastolic volume dilatation was significantly suppressed in the aldosterone antagonist group compared with the control group. Transcardiac extraction of aldosterone through the heart was significantly suppressed and the plasma PIIINP level was significantly lower in the aldosterone antagonist group. The authors suggest that aldosterone antagonist therapy, combined with angiotensin-converting enzyme inhibitor, can prevent postinfarct LV remodeling better than angiotensin-converting enzyme inhibitor alone, along with the suppression of a marker of collagen synthesis.29

Therefore, the results of our study with eplerenone are consistent with and extend the results of previous experimental and clinical observations with aldosterone antagonists in HF and after AMI and suggest that the effect of aldosterone receptor blockade on ECCM remodeling may contribute to the clinical benefits of this therapy.

**Study Limitations**

The principal limitation of this study is that the substudy population differed from the main EPHESUS study population with respect to baseline demographic and clinical characteristics, the timing of treatment initiation, and clinical outcomes. Therefore, extrapolating the results of this study to be reflective of EPHESUS should be done cautiously.

Decisions to reduce the variables of measured BNP and ICTP to binary form and to create a combination of these 2 variables were not made before data analysis and were ad hoc choices. Therefore, the HR and probability values from the corresponding Cox model leading to Kaplan-Meier curves may be biased by these choices.

Additionally, the power of the time to event analyses was limited by the small number of outcomes. With 226 patients, the power to detect an HR of 1.25 per 1 SD varied from 24% (HF hospitalization) to 35% (combined end point). Furthermore, background therapies of the patients involved in the present study also were shown to affect collagen turnover. Therefore, the kinetic changes in ECCM serological markers we report are to be interpreted within this context. However, background therapy was similar in both groups, and, if any, changes related to eplerenone were observed beyond the effects of background therapy. In addition, we were unable to investigate the interaction between the effects of eplerenone on collagen biomarkers and its clinical benefit. Most likely, the results of this study provide a representation of matrix dynamics after AMI and of the effects of eplerenone. Of note, the effects of eplerenone on biomarkers in this study were broadly consistent with those observed with other aldosterone antagonists in other experimental and clinical studies. For logistical and analytical reasons, we did not assess serum levels of MMPs, which could have yielded a better understanding of changes in the degradation of ECCM. In our experience and as reported previously, the results of assessment of some MMPs may be influenced significantly by the duration of storage.30

**Conclusions**

This is the first study describing long-term kinetics of ECCM collagen biomarkers in patients with AMI complicated by LV dysfunction and congestive HF. Changes in biomarkers of collagen synthesis and degradation suggest that ECCM remodeling is an active process in patients with CHF. Key biomarkers of matrix synthesis were lower in the eplerenone than the placebo group throughout the study, implying that treatment with eplerenone suppresses collagen turnover.

Furthermore, the associations of collagen biomarkers, BNP, and high-sensitivity C-reactive protein with each other and with determinants of disease severity indicate that AMI involves a complicated process of ECCM remodeling and myocyte stretch and inflammation. High serum levels of ICTP, a marker of cardiac collagen degradation, and high serum levels of BNP were associated with poor outcomes, supporting the concept that increased ECCM degradation and increased LV wall tension result in progressive remodeling and a worsening of HF.

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**Disclosures**

Drs Pitt and Zannad have received honoraria from and served on advisory boards for Pfizer Inc. Dr Vincent is an employee of Pfizer Inc. The other authors report no conflicts.

**References**


**CLINICAL PERSPECTIVE**

In the present substudy of the Eplerenone Post–Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS), we evaluated serum levels of selected biomarkers of extracellular cardiac matrix turnover in 476 patients. In the main EPHESUS, the aldosterone antagonist eplerenone plus standard of care was found to be superior to standard of care plus placebo in preventing cardiovascular outcomes in patients with left ventricular dysfunction and heart failure after an acute myocardial infarction. We describe the short- and long-term dynamic processes of collagen turnover from the changes reported in serological markers of type I and III collagen synthesis and degradation and their association with biomarkers of ventricular stress and inflammation (serum brain natriuretic peptide and high-sensitivity C-reactive protein). High serum levels of type I collagen telopeptide, a marker of cardiac collagen degradation, and high serum levels of brain natriuretic peptide were associated with poor outcomes. Eplerenone interfered mainly with serum levels of aminoterminal propeptide for type III collagen by blunting its 1-month rise after acute myocardial infarction. The findings of the present study may have several clinical implications, especially concerning stratification of risk in patients with post–myocardial infarction heart failure, and provide insights into the mechanisms of the beneficial effects of aldosterone antagonist therapy in such patients.
Extracellular Cardiac Matrix Biomarkers in Patients With Acute Myocardial Infarction Complicated by Left Ventricular Dysfunction and Heart Failure: Insights From the Eplerenone Post–Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) Study

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