Episodes of Acute Heart Failure Syndrome Are Associated With Increased Levels of Troponin and Extracellular Matrix Markers

Andreia Biolo, MD; Mark Fisch, MD; Joshua Balog, MD; Tania Chao, MD; P. Christian Schulze, MD; Henry Ooi, MD; Deborah Siwik, PhD; Wilson S. Colucci, MD

Background—Increased myocyte loss and extracellular matrix (ECM) turnover are central mechanisms that contribute to pathological myocardial remodeling in chronic heart failure (HF). We tested the hypothesis that episodes of acute HF syndrome (AHFS) are associated with transient increases in markers of myocyte injury and ECM turnover beyond those observed in chronic stable HF.

Methods and Results—Markers of myocyte injury and ECM turnover were assessed in 80 patients prospectively divided into 3 groups: AHFS (n=39); chronic stable systolic HF (n=21); and control subjects without HF (n=20). Myocyte injury was assessed by measuring plasma troponin I. ECM turnover was assessed by measuring plasma matrix metalloproteinases, tissue inhibitors of matrix metalloproteinases, and procollagen N-terminal type I and procollagen type III N-terminal peptides. In the AHFS group, biomarkers were obtained (1) at the time of hospital admission for an episode of HF decompensation, (2) at the time of hospital discharge, and (3) several weeks after discharge in patients who had returned to a chronic stable compensated state. In patients with stable HF (versus non-HF controls), there was a small increase in troponin I and little or no difference in any marker of ECM turnover. In patients with AHFS, troponin I and 3 markers of ECM turnover (matrix metalloproteinases-2, tissue inhibitors of matrix metalloproteinases-1, and procollagen type III N-terminal peptides) were elevated (versus chronic stable HF), and all fell toward chronic HF levels in patients who returned to a compensated state.

Conclusion—Episodes of AHFS are associated with transient increases in markers of myocyte injury and ECM turnover that may reflect an acceleration of pathological myocardial remodeling during AHFS. (Circ Heart Fail. 2010;3:44-50.)

Key Words: troponin ■ matrix metalloproteinase ■ tissue inhibitor of metalloproteinase ■ heart failure
Methods

Subjects
Three groups of subjects were prospectively recruited into this study: (1) patients admitted to the hospital for treatment of AHFS, (2) patients with chronic stable HF, and (3) control subjects without HF. Patients with AHFS were identified from patients admitted to Boston University Medical Center for an episode of systolic HF complicated by volume overload. Systolic HF was defined as a previous diagnosis of HF and echocardiographic demonstration of systolic left ventricular (LV) dysfunction with an ejection fraction <45%. The diagnosis of AHFS with volume overload was defined clinically by the presence of worsening symptoms of dyspnea, paroxysmal nocturnal dyspnea, and/or orthopnea in conjunction with clinical signs of circulatory congestion (elevated jugular venous pressure, hepatoglujugular reflux, hepatomegaly, and/or peripheral edema). Patients with concomitant acute coronary syndromes within the previous 3 months, primary infectious or inflammatory processes, or severe hemodynamic instability requiring intravenous vasoactive drugs were excluded. Also excluded were patients with aortic stenosis, malignancy, significant renal (creatinine >3 mg/dL) or hepatic (cirrhosis or active hepatitis) dysfunction, and rheumatologic diseases.

Patients with chronic stable HF were recruited from the Cardiomyopathy Clinic at Boston University Medical Center. They had chronic stable symptoms, had not been hospitalized during the previous 2 months, were not volume overloaded by clinical examination, and did not require a change in diuretic therapy on that visit. Non-HF control subjects were recruited from the ambulatory clinic or inpatient services at Boston University Medical Center. They had no history, symptoms, or findings of HF. The research protocol was approved by the Institutional Review Board at the Boston University Medical Center. Written informed consent was obtained from all participants.

Data and Sample Collection
For control subjects and patients with stable HF, clinical data and blood samples were collected at a single time point. Patients with AHFS had clinical data and blood samples obtained at least twice: (1) during the first 24 hours of admission and (2) again just before hospital discharge. In a subset of the patients with AHFS, blood samples were obtained a third time, late after discharge, if they met the criteria for chronic stable compensation, which were (1) no evidence of volume overload by clinical examination, (2) no current need for diuretic adjustment, and (3) no hospital admission for AHFS within the previous 2 months. A subjective dyspnea score was used to access symptom severity. Using an analog visual scale (0, the most severe dyspnea ever experienced; and 100, no dyspnea), the patients were asked to score their symptoms at the time of admission and again at the time of discharge.

Biomarkers
Blood samples were centrifuged, and the plasma and serum were frozen at −70°C until the assays were performed. N-terminal pro-B-type natriuretic peptide (NT-pro-BNP) was analyzed in plasma using a commercially available ELISA kit (AlpcO Diagnostics, Salem, NH). Troponin I was measured in serum using a high-sensitivity commercial chemiluminescent assay (ADVIA Centaur; Siemens Medical Solutions Diagnostics). The detection threshold for this assay is 0.006 mg/mL, and abnormal levels are defined as values exceeding the 99th percentile of a reference control population (≥0.05 ng/mL). Procollagen type I N-terminal peptide (PINP) and procollagen type III N-terminal peptide (PIIINP) levels were assessed in serum samples using a radioimmunoassay (Orion Diagnostica, Finland). Gelatinases (matrix metalloproteinases [MMP]-2 and -9) and tissue inhibitors of MMPs (TIMP-1, -2, and -4) were measured with commercially available ELISA kits (Amersham Pharmacia Biotech, Buckinghamshire, UK). All specimens were processed in duplicate, and the mean intra-assay coefficient of variation was <7% for all assays.

Table 1. Demographics and Clinical Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=20)</th>
<th>Stable HF (n=21)</th>
<th>AHFS (n=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>54±10</td>
<td>61±14</td>
<td>61±13</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>12/8</td>
<td>19/3</td>
<td>30/9</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>30.7±5.5</td>
<td>29.7±7.6</td>
<td>30.8±6.7</td>
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<td>Creatinine, mg/dL</td>
<td>0.8±0.2</td>
<td>1.2±0.4</td>
<td>1.4±0.6†</td>
</tr>
<tr>
<td>Alkaline phosphate, U/L</td>
<td>75±20</td>
<td>82±21</td>
<td>113±69</td>
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<tr>
<td>NT-proBNP, fmol/mL</td>
<td>318±116</td>
<td>664±448</td>
<td>1869±1345†</td>
</tr>
<tr>
<td>Diabetes</td>
<td>35</td>
<td>48</td>
<td>38</td>
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<tr>
<td>Hypertension</td>
<td>60</td>
<td>71</td>
<td>77</td>
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<tr>
<td>Ischemic heart disease</td>
<td>25</td>
<td>48</td>
<td>46</td>
</tr>
<tr>
<td>ACEI/ARB</td>
<td>35</td>
<td>90*</td>
<td>72*</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>50</td>
<td>95*</td>
<td>77*</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>0</td>
<td>19</td>
<td>8</td>
</tr>
</tbody>
</table>

Values are mean±SD. No. patients or percentages. ACEI indicates angiotensin converting enzyme inhibitors; ARB, angiotensin II receptor blockers; NYHA, New York Heart Association. *P<0.05 versus stable HF. †P<0.05 versus controls.

Statistical Analysis
Continuous variables are expressed as mean±SD or median and interquartile (IQ) range, and categorical variables are expressed as the number of patients or percentage. Comparisons among all groups for clinical variables were performed using ANOVA or χ² tests as appropriate. Comparisons among groups for all biomarkers were performed using ANOVA and Tukey multiple-comparison post hoc tests, or Kruskal-Wallis test for nonnormally distributed variables. Admission values were used as the reference for AHFS group for all between-group comparisons. Troponin I values were also categorized according to the detectable and abnormal thresholds of the assay and analyzed using χ² statistics. Within AHFS group, admission values were compared with values at discharge and with stable compensation by using a paired t test or a Wilcoxon rank test. We further compared both compensated groups (chronic compensation values for AHFS and stable group) by using a Student t test. The effects of medications, renal function, and hepatic function on biomarkers were assessed by univariate, followed by multivariate, regression analysis. Medications and markers of renal or hepatic function with significant relationships were added to models with the respective biomarker as the dependent variable. A value of P<0.05 was considered significant.

Results

Patient Demographics and Clinical Characteristics
A total of 80 subjects were included in this study as follows: AHFS=39, chronic stable HF=21, and non-HF control=20 (Table 1). The groups were not different with regard to age or gender distribution and had similar frequencies of diabetes, hypertension, and ischemic heart disease. The use of HF medications was lower in non-HF controls, as expected, but similar in the AHFS and stable HF groups. Patients in the chronic stable HF group were predominantly in New York Heart Association class I and II, whereas patients with AHFS were all in New York Heart Association class III or IV. NT-pro-BNP levels in patients with AHFS were markedly elevated and higher than in chronic patients with stable HF (Figure 1A).
Hemodynamic and Echocardiographic Characteristics

LV size and function, although markedly abnormal, were similar in the chronic HF and AHFS groups (Table 2). In both HF groups, the mean LV end-diastolic dimension was 59 to 60 mm, and the ejection fraction was 24%.

Troponin I

The mean troponin I level was elevated in both HF groups (versus non-HF controls) and was highest in the AHFS group (Figure 1B). Compared with non-HF controls, a higher proportion of patients in both HF groups had detectable troponin levels (>0.006 ng/mL): 91% of stable HF and 98% of AHFS group (versus 53% of non-HF controls; P<0.001 for both). Similarly, troponin I levels ≥0.05 ng/mL, the institutional criteria for an abnormal elevation, were present in 27% of patients with stable HF and 49% of patients with AHFS but in none of the non-HF controls (P<0.001 versus controls for both).

ECM Markers

PINP and PIIINP reflect collagen synthesis. PIIINP was markedly increased in patients with AHFS compared with both patients with stable HF and non-HF controls (Figure 2A), whereas PINP levels were similar in the 3 groups (Figure 3A). Neither PIIINP nor PINP was elevated in patients with stable HF compared with control subjects without HF.

ECM degradation is regulated by MMPs and TIMPs. None of the MMPs or TIMPs was elevated in patients with stable HF compared with non-HF controls. In patients with AHFS, MMP-2 and TIMP-1 levels were increased compared with patients with stable HF or non-HF controls (Figure 2B and 2C). In contrast, MMP-9 and TIMP-2 were not different in patients with AHFS versus stable HF (Figure 3B and 3C). TIMP-4 levels were increased in patients with AHFS compared with non-HF controls but were not different from patients with stable HF (Figure 3D).

HF Etiology

We evaluated whether the observed differences in biomarkers were related to the etiology of HF. Patients with ischemic HF represented 37% of patients with HF (45% of stable HF and 34% of AHFS, P=0.34). Troponin I levels tended (P=0.19) to be higher in the patients with nonischemic HF (0.046 ng/mL; IQ range, 0.025 to 0.087 ng/mL) compared with patients with an ischemic etiology (0.028 ng/mL; IQ range, 0.022 to 0.068 ng/mL). Similarly, all ECM markers were similar (P>0.4 for all) in patients with ischemic versus nonischemic HF. Among patients with AHFS, the presence or absence of coronary artery disease (CAD) was not associated with troponin levels (patients with CAD=0.063 ng/mL; IQ range, 0.028 to 0.10 ng/mL; patients without CAD=0.044

Table 2. Hemodynamic and Echocardiographic Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=20)</th>
<th>Stable HF (n=21)</th>
<th>AHFS (n=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>128±14</td>
<td>119±26</td>
<td>123±20</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>74±8</td>
<td>71±9</td>
<td>75±13</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>69±15</td>
<td>71±13</td>
<td>77±17</td>
</tr>
<tr>
<td>LV end-diastolic diameter, mm</td>
<td>47±5</td>
<td>59±7*</td>
<td>60±10*</td>
</tr>
<tr>
<td>LV end-systolic diameter, mm</td>
<td>31±3</td>
<td>48±9*</td>
<td>50±13*</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>63±3</td>
<td>24±10*</td>
<td>24±10*</td>
</tr>
</tbody>
</table>

Values are mean±SD. No. patients or percentages. NYHA indicates New York Heart Association.

*P<0.05 versus control group.
ng/mL; IQ range, 0.027 to 0.07 ng/mL; \( P = 0.49 \) or any of the ECM markers \( ( P > 0.3 \) for all markers).

**Medications, Renal and Liver Function**

When patients were grouped by medications, there were no significant differences for troponin or any of the ECM markers with regard to use of angiotensin-converting enzyme inhibitor/angiotensin receptor blockers, \( \beta \)-blockers, or spironolactone. Similarly, controlling for angiotensin-converting enzyme inhibitor/angiotensin receptor blockers or \( \beta \)-blocker use had no effect on the intergroup differences observed for troponin and ECM markers (data not shown).

Serum creatinine concentration was similar in the chronic stable HF group and the AHFS group at the time of admission (Table 1), and within the AHFS group, creatinine was unchanged on recompensation (1.32 ± 0.5 mg/dL; \( P = 0.93 \) versus admission). Within the AHFS group, creatinine correlated with TIMP-1 \( ( r = 0.36; \ P = 0.04 \) but not with troponin \( ( r = -0.041; \ P = 0.812 \) or any other ECM marker \( ( P > 0.50 \) for all). When the analysis was performed across all patients with HF, there was no correlation with troponin \( ( r = 0.044; \ P = 0.748 \) or any ECM marker \( ( P > 0.50 \) for all). Controlling for creatinine in the TIMP-1 regression model had no effect on the observed intergroup differences in TIMP-1. Alkaline phosphatase was similar in all 3 groups (Table 1) and was unchanged on recompensation (117 ± 55; \( P = 0.47 \) versus admission).

**Effect of Acute Therapy**

For the patients with AHFS, the duration of hospital admission averaged 5 ± 6 days. The predominant treatment during the admission was the use of intravenous diuretics leading to an average weight loss of 3.6 ± 0.7 kg. This diuresis was associated with a marked improvement in HF symptoms as reflected by an increase in the dyspnea subjective score from 33 to 85 \( ( P < 0.0001 \)).

Troponin I values were unchanged at the time of discharge (0.055 ng/mL; IQ range, 0.027 to 0.1 ng/mL) compared admission (0.046 ng/mL; IQ range, 0.028 to 0.08 ng/mL, \( P = 0.77 \); Figure 1D). Among the ECM markers, MMP-2 was decreased at discharge, whereas PIIINP and TIMP-1 remained elevated (Figure 2D through 2F).

**Chronic Compensation**

In a subgroup of 16 patients with AHFS who subsequently met the criteria for chronic stable HF (see Methods), biomarkers were measured again an average of 8 ± 3 months after discharge. NT-pro-BNP levels returned to values similar to the stable HF group \( ( P = 0.43 \); Figure 1C). Compared with levels observed during decompensation, both troponin I (Figure 1D) and all 3 of the ECM markers that were elevated with AHFS (PIIINP, MMP-2, and TIMP-1) were decreased (the \( P \) values for admission versus chronic compensation were 0.04, 0.04, 0.005, and 0.008 for troponin I, PIIINP, MMP-2, and TIMP-1, respectively; Figure 2D through 2F). In contrast, the markers that were not increased with AHFS (PINP, MMP-9, and TIMP-2) remained unchanged from admission values (the \( P \) values for admission versus chronic
compensation were 0.95, 0.40, and 0.34 for PINP, MMP-9, and TIMP-2, respectively; Figure 3E through 3H). Creatinine levels did not change at the follow-up visit (admission = 1.36 ± 0.6 mg/dL; discharge = 1.31 ± 0.5 mg/dL; late = 1.32 ± 0.5 mg/dL; P = 0.93). Similarly, hepatic enzymes did not change at the follow-up visit (data not shown).

**Discussion**

The major finding of this study is that episodes of AHFS are associated with transient increases in the blood levels of troponin I, a marker for cardiac myocyte injury, and 3 markers for ECM turnover (MMP-2, TIMP-1, and PIIINP). Compared with patients with chronic stable HF, troponin I and these ECM markers were elevated in patients with AHFS. Of note, when patients with AHFS returned to chronic stable HF, all of the elevated markers returned to or toward the levels observed in the chronic stable HF group. In contrast, patients with stable HF had only a modest increase of troponin I levels above that in non-HF controls and little or no alteration in any of the ECM markers.

Troponin, a marker of myocyte injury, is known to be elevated in patients with HF in the absence of epicardial CAD.6,7 In patients with HF, elevated troponin levels are associated with a worse prognosis.7–11 Although troponin I is highly specific for cardiac myocytes, circulating levels may also be elevated due to renal insufficiency. However, this does not seem to underlie our observations, because serum creatinine levels were similar in the chronic stable HF and AHFS groups (Table 1). Furthermore, there was no relationship between troponin and creatinine levels within the AHFS group and across all patients with HF. Thus, we believe that the transient elevation in circulating troponin I in our patients with AHFS primarily reflects increased release from the myocardium, and thus, may indicate myocyte injury and/or death. A common cause of cardiac myocyte death is ischemia due to CAD.12 However, troponin levels in our study were...
similar in patients with nonischemic and ischemic HF, suggesting that other factors might be responsible. Of note, episodes of AHFS are associated with nonischemic processes that are known to cause myocyte death including mechanical strain, oxidative stress, and neurohormonal activation.13–16

Quantitative and qualitative alterations in the composition of the cardiac ECM are another important component of pathological myocardial remodeling.1 ECM composition is determined by the balance of degradative and synthetic processes, and accordingly, circulating levels of MMPs, TIMPs, and collagen fragments have proved useful in providing evidence of increased ECM turnover in patients with HF.17–24 In cross-sectional studies of patients with systolic HF, alterations in circulating MMP and TIMP levels are related to the extent of LV remodeling and predict clinical outcomes,19,25,26 thus supporting the clinical relevance of these biomarkers.

No previous study has assessed the relationship of ECM turnover to episodes of AHFS, nor it is known whether ECM turnover is increased during an episode of AHFS. In this regard, our study provides 2 new observations about ECM turnover in HF. First, markers of ECM turnover were not increased in our patients with chronic stable HF. Second, during episodes of AHFS there were marked increases in 3 markers of ECM turnover (MMP-2, TIMP-1, and PIIINP). The relationship of these biomarkers to AHFS was supported by the sequential demonstration in a subgroup of patients with AHFS that these markers returned to or toward the levels observed in compensated patients with chronic stable HF. The levels of matrix markers can be influenced by alterations in renal and/or hepatic clearance that may occur in AHFS. An effect on ECM levels related to renal function seems unlikely, because serum creatinine and hepatic enzymes were similar in the chronic stable and AHFS groups.

We believe that our ability to identify differences in troponin and ECM biomarkers in the patients with AHFS compared with those with chronic stable HF reflects 2 relatively unique aspects of our study design. First, we prospectively grouped patients as compensated (ie, chronic stable) or decompensated (ie, AHFS requiring admission to the hospital). In this regard, it is noteworthy that, although the chronic stable and decompensated HF groups had identical degrees of LV dilation and systolic dysfunction, they differed markedly with regard to symptom severity, New York Heart Association functional class, and BNP levels. Previous studies have generally examined patients with heterogeneous or ill-defined levels of clinical stability and compensation. A second important feature of our design is the use of a non-HF control group that had a similar incidence of concomitant cardiovascular risk factors and conditions that are associated with ECM turnover, including hypertension,25 diabetes,26 and CAD.27

Several limitations of this study need to be appreciated. First, although troponin is highly specific for the myocardi-um, circulating matrix markers may reflect events in other organs. A second limitation is the relatively small number of patients, which may have decreased our ability to detect small changes in ECM markers in the stable HF group and to correctly completely for factors that affect these biomarkers. However, the number of patients was adequate to detect increased troponin levels in the stable group, and thus, it is unlikely that an important difference in ECM biomarkers was missed in that group. A third limitation is that we cannot completely exclude the possibility that small changes in renal or hepatic function contributed to altered levels by means of effects on clearance. However, in this regard, it is helpful to note that the observed changes in 3 ECM markers were not associated with changes in 4 other, structurally similar ECM markers (ie, PIII-NP, MMP-9, TIMP-2, and TIMP-4), which should have been subject to similar effects related to clearance pathways.

When the symptoms of AHFS are sufficient to preclude ambulatory management, patients are admitted to the hospital for intensive therapy that focuses on fluid removal, and in some cases, the bolstering of hemodynamic function with vasodilators and positive inotropic agents. The primary goal of therapy is to alleviate symptoms and to restore the compensated state. Ongoing cardiac myocyte injury and/or death and qualitative and quantitative changes in ECM composition are central mechanisms in myocardial remodeling. Accordingly, an important implication of our observations is that episodes of decompen-sation may be associated with an acceleration of pathological myocardial remodeling.

Gheorghiadie et al28 recently concluded that “should further research establish the presence and magnitude of myocardial injury in AHFS, preventing or limiting it with acute interventions may result in improvement in long-term outcome.” We believe that our prospective study provides some of the first direct support for this notion by demonstrating that episodes of AHFS are associated with transient increases in markers of both myocyte injury and ECM turnover. These observations should stimulate further studies of the pathobiology of AHFS and, ultimately, may have implications regarding the importance of preventing episodes of acute decompensated heart failure and the identification of therapeutic targets related to cell death and ECM turnover in this setting.

Acknowledgment
We thank Dr Flora Sam for providing blood samples that were used in the preliminary studies that led to this project.

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Disclosures
None.

References


CLINICAL PERSPECTIVE

Increased myocyte loss and extracellular matrix (ECM) turnover are central mechanisms that contribute to pathologic myocardial remodeling in chronic heart failure (HF). Patients with chronic HF frequently experience episodes of acute HF syndromes (AHFS), with interstitial fluid overload, elevated cardiac filling pressures, depressed cardiac output, and the attendant symptoms, with a negative prognostic impact in HF course. Despite the importance of AHFS, little is known about its pathobiology. The major finding of this study is that episodes of AHFS are associated with transient increases in the blood levels of troponin I, a marker for cardiac myocyte injury, and 3 markers for ECM turnover (MMP-2, TIMP-1, and PIINP). Compared with patients with chronic stable HF, troponin I and these ECM markers were elevated in patients with AHFS. Furthermore, when patients with AHFS returned to chronic stable HF, all of the elevated markers returned to or toward the levels observed in the chronic stable HF group. In contrast, patients with stable HF had only a modest increase of troponin I levels above that in non-HF controls and little or no alteration in any of the ECM markers. An important implication of our observations is that episodes of decompensation may be associated with an acceleration of pathological myocardial remodeling. These observations should stimulate further studies of the pathobiology of AHFS and, ultimately, may have implications regarding the importance of preventing episodes of acute decompensated heart failure and the identification of therapeutic targets related to cell death and ECM turnover in this setting.
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