Serial Biomarker Measurements in Ambulatory Patients With Chronic Heart Failure
The Importance of Change Over Time
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Background—Cardiac troponin T (cTnT) and B-type natriuretic peptide (BNP) have been used to estimate prognosis in heart failure; however, most studies have evaluated decompensated patients with single measurements. To determine if there are advantages to serial measurements, we evaluated stable chronic heart failure patients every 3 months for 2 years.

Methods and Results—A cohort of 190 New York Heart Association class III–IV heart failure patients was prospectively enrolled from June 2001 to January 2004. Primary end points were death, cardiac transplantation, or hospitalization. At study enrollment cTnT was <0.01 ng/mL in 87 (45.8%) patients, 0.01 to 0.03 ng/mL in 50 (26.3%) patients, and >0.03 ng/mL in 53 (27.9%) patients. An increase in cTnT above normal (<0.01 ng/mL) carried a 3.4-fold increased risk (P=0.019). Further increases (>20%) from an elevated level worsened the overall risk (hazard ratio, 5.09; P<0.001). BNP was elevated (>95th percentile for age and gender normal population) in 122 (64.2%) patients. An elevation of BNP from normal at any time during the study was associated with a poor outcome, but, once elevated, further changes in BNP (increases or decreases) remained associated with the same risk (hazard ratio, 5.09; P<0.001). Combined elevations of cTnT (>0.03 ng/mL) and BNP defined the highest risk group (hazard ratio, 8.58; P<0.001).

Conclusions—Elevations of cTnT or BNP from normal detected at any time during clinical follow-up in ambulatory patients with chronic heart failure are highly associated with an increased risk of events. Further increases in cTnT contribute to additional risk. Combined elevations of cTnT and BNP contribute the highest risk. The ability to monitor changes by serial measurements adds substantially to the assessment of risk in this patient population. (Circulation. 2007;116:249-257.)

Key Words: heart failure ■ natriuretic peptides ■ prognosis ■ troponin

Despite advances in medical and device therapies, patients with chronic heart failure (HF) have an adverse prognosis over time.1–2 Progression often occurs even in the absence of overt clinical events.3,4 Because HF evokes the activation of multiple neurohormones such as the renin-angiotensin-aldosterone, endothelin, sympathetic, and natriuretic peptide systems,3 there has been enthusiasm for the use of related biomarkers to define prognosis. B-type natriuretic peptides (BNP and N-terminal proBNP [NT-proBNP]) and to a lesser extent cardiac troponin T (cTnT) have been used for this purpose, and elevations of these biomarkers identify patients at risk.6–11 Most studies to date, however, have examined acutely decompensated hospitalized patients rather than stable ambulatory HF patients and have evaluated only “single point in time” measurements. Only a few studies8,12–14 have evaluated changes over time, and most of these have monitored patients only for short periods (typically 6 months). Thus, how much additional information would be available to potentially guide management if values were monitored more frequently and for a longer period of time has not been well defined. In addition, the applicability of this approach to more stable patients is unclear. Accordingly, we evaluated serial measurements (every 3 months) of cardiac cTnT and BNP over a 2-year period in clinically stable, out-of-hospital patients with New York Heart Association (NYHA) class III–IV HF to determine the optimal way to use these biomarkers to follow ambulatory patients. Our hypothesis was that serial measurements of these biomarkers would provide added information to predict events such as death, cardiac transplantation, or hospitalization.

Methods

Patients and Study Design
A cohort of 200 NYHA class III and IV HF patients was prospectively enrolled during the period of June 2001 to January 2004.
These patients were recruited from the outpatient Mayo Heart Failure Clinic after initial evaluation and establishment of routine follow-up. Some patients were initially identified while in hospital but were not enrolled until they were stable outpatients. Informed consent was obtained from each patient after a primary medical evaluation had determined the clinical status of each patient. Patients were followed in our HF clinic with visits scheduled at 3-month intervals (±3 weeks) for a total of 24 months of follow-up. Patients were excluded if cardiac revascularization was anticipated within 6 months, if patients were awaiting cardiac transplantation, or if patients had experienced an episode of acute decompensation of HF <30 days prior to enrollment. All patients with chronic atrial fibrillation were treated with routine Coumadin therapy with International Normalized Ratio monitoring. The study was approved by the Mayo Foundation Institutional Review Board and included only those patients who provided written informed consent for clinical research analysis as required by Minnesota Statute 144.335/ CFR 21 (Part 50).

Study Protocol

Blood samples for BNP and cardiac cTnT were drawn at study entry (baseline) and every 3 months thereafter for 2 years. Blood samples for BNP were collected in EDTA and immediately stored on ice, processed, and subsequently stored at −70°C until batch analysis (every 3 to 4 weeks) was performed during the course of the study. Troponin samples were assayed when samples were obtained at each patient visit. Clinicians and investigators were blinded to biomarker results. Blood samples were also obtained for plasma electrolytes, serum creatinine, and hemoglobin as clinically indicated. Left ventricular ejection fraction was derived from 2-dimensional echocardiography performed within 3 months of study enrollment and subsequently during routine clinical follow-up as determined by the patient’s HF clinic provider. Patients were seen in the Mayo Heart Failure Clinic for routine clinical follow-up as well as for study visits, and every effort was made to coordinate these activities. In addition to blood samples obtained for the measurement of biomarkers, an updated patient history, interim clinical status (such as visit to the Failure Clinic for routine clinical follow-up as well as for study enrollment) data that were obtained at the time of study enrollment for each of the end points. Results from all Cox models are presented as hazard ratios (HR) with corresponding 95% CIs and probability values. Proportional hazard assumptions were tested with the scaled Schoenfeld residuals. Forward stepwise methods were used to construct multivariable models to adjust for characteristics at study enrollment. As a result of repeated data collection for cTnT and BNP and variety of enrollment times, time-dependent analysis methods were also used. The survival curves presented represent the serial measurements of cTnT and BNP and account for changes a patient demonstrated during the course of the study. Likewise, in the time-dependent Cox multivariable models with cTnT and BNP, patients are included in the proper risk set and can alternate between risk sets. All other variables in these models are based on the value at the time of the study enrollment. Time-dependent Cox models were also used for models that evaluated the importance of changes in the biomarkers between 3-month time periods. To be included in this analysis an individual would have to have been followed through at least 1 visit after the baseline (study enrollment) visit.

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

Statistical Analysis

Analyses were performed with SAS software (version 9; SAS Institute Inc, Cary, NC) or with S-plus Version 7 (Insightful Corporation, Seattle, Wash). Data for continuous variables are reported as mean±SD and median with 25% and 75% percentiles. Categorical variables are reported as a percent of the total values. Associations between each biomarker at baseline (study enrollment) and over the 2-year time interval of follow-up and the other parameters such as GFR, body mass index, and vital signs (systolic/diastolic blood pressure and heart rate) were assessed by Spearman rank correlation. The primary study end point was the time until death or cardiac transplantation. A second end point was time until first hospitalization for decompensated HF, death, or cardiac transplantation. The goal was to compare the relationship over time between these end points and cTnT and BNP levels. The data were collected at 3-month intervals throughout the study period with 2 years of follow-up for all surviving patients. To facilitate the use of time-dependent modeling and because there were large numbers of observations that were recorded as zero or nondetectable for cTnT, we categorized the data into 3 intervals (<0.01, 0.01 to 0.03, and >0.03 ng/mL). BNP levels were dependent on age and gender, and for comparative purposes we categorized BNP levels as either elevated or not elevated. We defined an elevated BNP as any value greater than the 95th percentile of normal adjusted for age and gender referenced for the Shionogi assay (Shionogi Pharmaceuticals, Osaka, Japan). Patients were considered at risk for cardiac events from the time of enrollment in the study through the last follow-up visit. Univariate Cox proportional hazard models were run with study enrollment (baseline) data that were obtained at the time of study enrollment for each of the end points. Results from all Cox models are presented as hazard ratios (HR) with corresponding 95% CIs and probability values. Proportional hazard assumptions were tested with the scaled Schoenfeld residuals. Forward stepwise methods were used to construct multivariable models to adjust for characteristics at study enrollment. As a result of repeated data collection for cTnT and BNP and variety of enrollment times, time-dependent analysis methods were also used. The survival curves presented represent the serial measurements of cTnT and BNP and account for changes a patient demonstrated during the course of the study. Likewise, in the time-dependent Cox multivariable models with cTnT and BNP, patients are included in the proper risk set and can alternate between risk sets. All other variables in these models are based on the value at the time of the study enrollment. Time-dependent Cox models were also used for models that evaluated the importance of changes in the biomarkers between 3-month time periods. To be included in this analysis an individual would have to have been followed through at least 1 visit after the baseline (study enrollment) visit.

Biomarker Measurements

BNP (32 amino acid residues; half-life, 20 minutes) was measured by the Shionoria assay method (intra- and interassay variability of 7.2±1.7 pg/mL and 8.0±1.4 pg/mL, respectively) with normal range in plasma of 12±4 pg/mL in the laboratory of Dr. John C. Burnett, Jr., of the Mayo Clinic. Cardiac cTnT (limit of detection <0.01 ng/mL with coefficient of variability 10% at a value of 0.035 ng/mL) was measured on the Roche Elecsys third generation troponin T immunoassay (Roche Diagnostics, Indianapolis, Ind).

An elevated cTnT was defined as a value ≥99th percentile of the normal reference population (≥0.01 ng/mL). Analyses were performed with this recommended European Society of Cardiology/ American College Of Cardiology Committee cutoff guideline and the conventional clinical practice cutoff value of >0.03 ng/mL (10% coefficient of variability value) used at the Mayo Clinic. Elevated BNP levels were defined as those values above the 95% percentile of normal on the basis of age and gender as previously reported with this BNP assay. Renal function was determined at study enrollment (baseline) and at subsequent visits every 3 months by calculation of estimated glomerular filtration rate (GFR, mL/min per 1.73 m²) by use of the Modification of Diet in Renal Disease equation.

Results

Table 1 shows the clinical and demographic characteristics of the patients enrolled in the present study. Of the 200 patients enrolled, 10 patients had insufficient data to be included in the study (no study enrollment biomarker data); therefore, results are reported on 190 participants. The majority of patients were male (76%), were categorized as NYHA class III (92%), and had an ischemic etiology of HF (55%). Most patients were mildly anemic and with at least moderate renal insufficiency (estimated GFR, 46±15 mL/min per 1.73 m²). Fifteen percent of patients were diabetic and more than half were hypertensive and hyperlipidemic. Average duration of HF at study enrollment was 41.9±44.2 months with a median of 31 months.

At study enrollment (Table 1), cTnT level was <0.01 ng/mL in 87 (45.8%) patients, 0.01 to 0.03 ng/mL in 50
<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Median, Q1 to Q3 (25% to 75%)</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, y</strong></td>
<td>71 ± 10</td>
<td>73 (64 to 79)</td>
<td>190</td>
</tr>
<tr>
<td><strong>Gender, male</strong></td>
<td>...</td>
<td>...</td>
<td>145 (76.3%)</td>
</tr>
<tr>
<td><strong>Race, white/Native American</strong></td>
<td>...</td>
<td>...</td>
<td>188/2</td>
</tr>
<tr>
<td><strong>NYHA class III</strong></td>
<td>...</td>
<td>...</td>
<td>175 (92.1%)</td>
</tr>
<tr>
<td><strong>NYHA class IV</strong></td>
<td>...</td>
<td>...</td>
<td>15 (7.9%)</td>
</tr>
<tr>
<td><strong>Duration of heart failure, mo</strong></td>
<td>42.5±44.3</td>
<td>32 (7 to 66)</td>
<td>190</td>
</tr>
<tr>
<td><strong>LVEF, %</strong></td>
<td>27.3±12.2</td>
<td>24 (18 to 33)</td>
<td>190</td>
</tr>
<tr>
<td><strong>Weight, kg</strong></td>
<td>87±23</td>
<td>62 (73 to 97)</td>
<td>190</td>
</tr>
<tr>
<td><strong>Height, cm</strong></td>
<td>172±9</td>
<td>174 (166 to 178)</td>
<td>190</td>
</tr>
<tr>
<td><strong>Body mass index, kg/m²</strong></td>
<td>29.2±6.7</td>
<td>28 (25 to 32.5)</td>
<td>190</td>
</tr>
<tr>
<td><strong>Heart rate, bpm</strong></td>
<td>71±13</td>
<td>71 (62 to 80)</td>
<td>190</td>
</tr>
<tr>
<td><strong>SBP, mm Hg</strong></td>
<td>113±21</td>
<td>110 (98 to 124)</td>
<td>190</td>
</tr>
<tr>
<td><strong>DBP, mm Hg</strong></td>
<td>63±11</td>
<td>60 (58 to 70)</td>
<td>190</td>
</tr>
<tr>
<td><strong>Hemoglobin, g/dL</strong></td>
<td>12.5±1.7</td>
<td>12.4 (11.1 to 13.7)</td>
<td>99</td>
</tr>
<tr>
<td><strong>Serum creatinine, mg/dL</strong></td>
<td>1.6±0.6</td>
<td>1.5 (1.3 to 1.9)</td>
<td>188</td>
</tr>
<tr>
<td><strong>GFR, mL/min per 1.73 m²</strong></td>
<td>46±15</td>
<td>45 (35 to 57)</td>
<td>188</td>
</tr>
<tr>
<td><strong>Potassium, mEq/L</strong></td>
<td>4.4±0.9</td>
<td>4.4 (4.1 to 4.7)</td>
<td>187</td>
</tr>
<tr>
<td><strong>Sodium, mEq/L</strong></td>
<td>137±16</td>
<td>140 (137 to 142)</td>
<td>184</td>
</tr>
<tr>
<td><strong>BNP, pg/mL</strong></td>
<td>408±423</td>
<td>305 (118 to 521)</td>
<td>190</td>
</tr>
<tr>
<td><strong>cTnT, ng/mL</strong></td>
<td>0.074±0.491</td>
<td>0.013 (0.005 to 0.035)</td>
<td>190</td>
</tr>
</tbody>
</table>

**Etiology of HF**

- **Ischemic**... 104 (54.7%)
- **IDCM**... 56 (29.5%)
- **Hypertension**... 5 (2.6%)
- **Valvular**... 7 (3.7%)
- **Other**... 18 (9.5%)
- **Diabetes**... 28 (15%)
- **Hypertension**... 120 (63%)
- **Hyperlipidemia**... 122 (64%)
- **COPD**... 49 (26%)
- **S/P CABG**... 82 (43%)
- **BIV PPM**... 27 (14%)
- **AICD**... 50 (26%)
- **Hx MI**... 91 (48%)
- **Hx CVA**... 12 (6%)
- **Never smoker**... 68 (36%)
- **Atrial fibrillation**... 87 (46%)
- **Aortic stenosis**... 20 (11%)
- **Aortic regurgitation**... 46 (24%)
- **Mitra regurgitation**... 142 (75%)
- **Tricuspid regurgitation**... 130 (68%)
- **S/P valve replacement surgery**... 17 (9%)

**Medications**

- **ACEI**... 140 (74%)
- **ARB**... 34 (18%)
- **β-Blocker**... 147 (77%)
- **Aldosterone blocker**... 50 (26%)
- **Digoxin**... 113 (59%)
- **Diuretic**... 178 (94%)
- **Aspirin**... 114 (60%)
- **Nitrates**... 55 (29%)
- **Antidyssrhythmics**... 40 (21%)

Q1 indicates quartile 1; Q3, quartile 3; LVEF, left ventricular ejection fraction; IDCM, idiopathic dilated cardiomyopathy; COPD, chronic obstructive pulmonary disease; S/P CABG, status post–coronary artery bypass graft surgery; BIV PPM, biventricular permanent pacemaker; AICD, automatic implantable cardioverter defibrillators; Hx MI, history of myocardial infarction; Hx CVA, history of cerebral vascular accident; ACEI, angiotensin-converting enzyme inhibitor; and ARB, angiotensin receptor blocker.
TABLE 2. Univariate Models: Risk of Death/Cardiac Transplantation Based on Elevated Baseline Values of Troponin T and BNP

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Troponin T</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥0.01 ng/mL</td>
<td>2.69</td>
<td>1.54 to 4.72</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt;0.03 ng/mL</td>
<td>2.48</td>
<td>1.50 to 4.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BNP, pg/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&gt;95th percentile for normal age, gender-based population)</td>
<td>2.54</td>
<td>1.35 to 4.78</td>
<td>0.0038</td>
</tr>
</tbody>
</table>

HRs were calculated with Cox proportional hazards models and are relative to measurements below detectable limits (<0.01 ng/mL) for cTnT and to nonelevated baseline BNP values.

(26.3%) patients, and elevated above the 10% coefficient of variability cutoff value of 0.03 ng/mL in 53 (27.9%) patients. On the basis of age and gender, BNP was elevated (>95th percentile for normal population for age and gender) in 122 (64.2%) patients at study enrollment.

Study enrollment (baseline) cTnT showed a modest inverse correlation with GFR such that the lower the estimated GFR, the higher the observed cTnT ($r = -0.32; P < 0.001$). The baseline BNP also demonstrated a modest inverse relationship to GFR ($r = -0.20; P = 0.006$). cTnT value at enrollment was not correlated with body mass index, heart rate, or systolic blood pressure. There was a statistically significant but modest correlation with diastolic blood pressure ($r = -0.16; P = 0.02$). Enrollment BNP was not correlated with heart rate or systolic and diastolic blood pressures. There was a modest inverse correlation between enrollment BNP and body mass index ($r = -0.13; P = 0.10$).

Primary End Point (Death or Cardiac Transplantation)

Eight patients died after enrollment and before the initial 3-month follow-up visit and provided, therefore, only study enrollment (baseline) data. Six (3%) patients went on to cardiac transplantation, and 55 (29%) patients died during the course of study follow-up.

The univariate HRs for the risk of death and transplantation that were based on the enrollment biomarker levels are shown in Table 2. In an interaction model (Figure 1) that incorporated only study enrollment cTnT and BNP with cutoff cTnT values of ≥0.01 ng/mL and elevated BNP level of >95th percentile for age and gender of normal population, the highest risk of death or cardiac transplantation was associated with the elevation of both troponin T ≥0.01 ng/mL and BNP (HR, 6.37).

A total of 103 (54%) patients had a cTnT elevation ≥0.01 ng/mL at study enrollment, and an additional 31 patients developed a new elevation in cTnT ≥0.01 ng/mL during the subsequent course of the study. Overall, 134 (103 + 31 patients; 70.5%) patients had a cTnT elevation at enrollment or during the course of the study. A total of 122 (64%) patients had an elevated BNP level at enrollment, and another 31 patients developed a new elevation of BNP over the course of the study; overall, 153 (122 + 31 patients; 80.5%) patients had an elevation in BNP at enrollment or during the study period.

Primary End Point (Death or Cardiac Transplantation)

Survival for the patients grouped by the cTnT level obtained at the last follow-up visit that preceded an event is shown in Figure 2A. Survival was markedly worse in patients with a cTnT value ≥0.03 ng/mL measured at any point in time during the study (P < 0.001). Figure 2B illustrates that survival is also worse in patients who have an elevated BNP obtained at the last follow-up visit that preceded an event (P < 0.001).

The results of univariate analyses for predictors of death or cardiac transplantation were significant for NYHA class (HR, 3.46; 95% CI, 1.75 to 6.83; P < 0.001), biventricular pace-
TABLE 3. Time-Dependent Cox Model Multivariable Analysis: Death/Cardiac Transplantation and Baseline Risk Factors

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated BNP</td>
<td>1.53</td>
<td>0.86 to 2.72</td>
<td>0.146</td>
</tr>
<tr>
<td>Troponin T&gt;0.03 ng/mL</td>
<td>4.37</td>
<td>2.55 to 7.49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NYHA class (risk of class IV vs class III)</td>
<td>4.18</td>
<td>2.03 to 8.62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of myocardial infarction</td>
<td>1.40</td>
<td>0.82 to 2.39</td>
<td>0.213</td>
</tr>
<tr>
<td>Biventricular pacing</td>
<td>3.42</td>
<td>1.52 to 7.70</td>
<td>0.003</td>
</tr>
</tbody>
</table>

maker (HR, 2.79; 95% CI, 1.27 to 6.14; P=0.011), idiopathic etiology of HF (HR, 0.52; 95% CI, 0.28 to 0.98; P=0.043), myocardial infarction history (HR, 1.90; 95% CI, 1.13 to 3.19; P=0.015), stroke history (HR, 2.29; 95% CI, 1.04 to 5.04; P=0.039), GFR (HR, 0.98; 95% CI, 0.96 to 0.99; P=0.005), elevated BNP (HR, 2.54; 95% CI, 1.35 to 4.78; P=0.004), and elevated cTnT (≥0.01 ng/mL) (HR, 2.69; 95% CI, 1.54 to 4.72; P=0.001). The multivariable model for covariate correction was developed prior to assessment of the effect of cTnT or BNP and included NYHA class, history of myocardial infarction, and biventricular pacing. When the indicator variable for cTnT >0.03 ng/mL was added to this model, the HR for cTnT was 4.8 (P<0.001). When an elevated BNP was added to the model (without cTnT), the HR was 2.4 (P=0.003). Table 3 contains the multivariable model with the time-dependent variables for elevated BNP and cTnT >0.03 ng/mL. cTnT demonstrates a highly significant increased risk of an event relative to patients with a cTnT >0.03 ng/mL (HR, 4.37). An elevated BNP demonstrated a 1.53-fold (HR) increased risk.

End Point (Death or Cardiac Transplantation) Analysis of Change in Serial Troponin T and BNP Levels Over Time

Table 4 shows the risk prediction of changes in cTnT from 1 follow-up visit to the next visit relative to a persisting normal level of cTnT (<0.01 ng/mL). Both an increase >0.01 ng/mL from normal or a persistently elevated cTnT level over time carried a 3.38-fold increase in risk for death or cardiac transplantation. Further increases from an already elevated level also increased the risk (<20% change: HR, 3.40; ≥20% increase: HR, 5.09). A reduction of cTnT contributed very little unless it was reduced to a normal level. If so, the risk diminished (HR, 2.02).

TABLE 4. Effect of Change in Serial Troponin T Values Over the Study Period on Outcome of Death/Cardiac Transplantation

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal to elevated troponin T (≥0.01 ng/mL)</td>
<td>3.38</td>
<td>1.23 to 9.34</td>
<td>0.019</td>
</tr>
<tr>
<td>Elevated troponin T to normal value</td>
<td>2.02</td>
<td>0.63 to 6.49</td>
<td>0.235</td>
</tr>
<tr>
<td>Elevated troponin T to &lt;20% change (increase or decrease but none back to normal value)</td>
<td>3.40</td>
<td>1.51 to 7.70</td>
<td>0.003</td>
</tr>
<tr>
<td>Elevated troponin T to ≥20% further increase in troponin T</td>
<td>5.09</td>
<td>2.27 to 11.40</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

All values are relative to normal values (<0.01 ng/mL) that remained normal.

The same analysis was carried out for BNP (Table 5). An elevated BNP level from normal at any time during follow-up predicted an increased risk (HR, 2.90) and subsequent changes in BNP, either increases or decreases, were not associated with any further changes in risk level. Even normalization of the BNP level from a previously elevated value was not associated with reduced risk (HR, 4.94).

End Point (Death or Cardiac Transplantation) Time-Dependent Analysis With the Combination of Troponin T and BNP Levels

Figure 3 shows the risk prediction capacity when the combined levels of cTnT and BNP are analyzed at any time point during follow-up. When cTnT alone is stratified at levels ≥0.01 ng/mL and >0.03 ng/mL, the risk increases markedly at values ≥0.03 ng/mL. BNP elevations alone predict a substantial increased risk of death or transplantation but were less powerful. The combined elevations of cTnT and BNP augment risk substantially when a cTnT cutoff level ≥0.01 ng/mL is used (HR, 5.01); however, when a cTnT cutoff of >0.03 ng/mL is used (HR, 8.26), the addition of an elevated BNP level to the analysis does not contribute significantly to risk prediction (HR, 8.58).

End Point (Hospitalization for HF Decompensation, Death, or Transplantation)

Hospitalization for decompensated HF occurred in 71 (37%) patients with a total of 150 HF-related hospitalizations during the course of the study.

Figure 4A contains the curves that depict survival free of hospitalization for HF decompensation, death, or transplantation for each of the cTnT groups predicated on elevations at any time during the follow-up period. Patients with any elevation of cTnT (≥0.01 ng/mL) during the study had an increased risk of an event compared with patients who did not have an elevation in cTnT (P<0.001). An elevated BNP was also associated with an increased risk of an event (Figure 4B; P=0.07).

In a univariate model, only NYHA class and an idiopathic etiology of HF were associated with events. Addition of cTnT ≥0.01 ng/mL to this model, however, produced a 2.9-fold increased risk (P<0.001). When an elevated BNP was added to the model with NYHA class and idiopathic etiology, the HR was 1.6 (P=0.02). When both cTnT ≥0.01 ng/mL and elevated BNP were included in the multivariable model, the HR for cTnT ≥0.01 ng/mL was 2.43 (P<0.001), and the HR for elevated BNP was 1.37 (P=0.153).

Effects of Medication Changes and Interventions on Outcomes

In patients who died (55 patients), we assessed whether changes in medications during follow-up may have been associated with their poor outcome. In 71% of patients, no changes in medication occurred within 6 months of death. In 15 patients, a change in medication (diuretic, β-blocker, converting enzyme inhibitor, or angiotensin-receptor blocker) occurred at or within 6 weeks to 6 months of death. We also assessed whether a temporal relationship existed between any procedure (coronary angiography, coronary intervention,
defibrillator, permanent pacemaker implantation, intraaortic balloon pump, or pulmonary artery catheterization) and date of death. No associations were demonstrated.

**Discussion**

The results of the present study add significantly to the available data regarding the use of biomarkers to evaluate and follow patients with chronic HF. The potent prognostic effects of single enrollment elevations in cTnT and BNP levels were observed as in previous studies. In our data set, 103 (54%) patients had enrollment (baseline) elevations of cTnT above 0.01 ng/mL. Among these, 53 (28%) patients demonstrated elevations >0.03 ng/mL, which is consistent with the frequency seen in other studies. However, elevations in cTnT (especially >0.03 ng/mL) and BNP (above the 95th percentile of the normal population for age and gender) that occurred at any time during clinical follow-up are independently associated with an increased short-term risk of death or exacerbation of HF that possibly required cardiac transplantation. Of potential importance was the fact that changes in cTnT that occurred during follow-up seemed to modulate risk. New elevations shifted patients to substantially higher risk. An additional 31 (16%) patients developed elevations over the subsequent course of the study. The risk, however, was also mitigated when the cTnT level returned to normal. These results, if confirmed, would suggest that not only are enrollment (baseline) values and follow-up values of cTnT useful, but that strategies to improve prognosis may need to be developed to preclude new elevations or to intervene when they occur.

Surprisingly, we did not see as much of an impact of changes in BNP over time. New increases in BNP above normal did move patients into a higher risk category, but once elevated at any time, subsequent changes in BNP, whether increases or decreases, did not modulate risk further. This suggests that chronic monitoring of BNP after an initial elevation may not be as efficacious as prior studies have suggested. We do not have a data-driven explanation for this finding. It may be that this is because our study population was one of chronic stable HF rather than that of more acutely decompensated syndromes. It is also clear that fragments of BNP elaborated may be critical.

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal to elevated BNP (≥95th percentile for age, gender in normal population)</td>
<td>2.90</td>
<td>0.88 to 9.55</td>
<td>0.080</td>
</tr>
<tr>
<td>Elevated BNP to normal value</td>
<td>4.94</td>
<td>1.82 to 13.40</td>
<td>0.002</td>
</tr>
<tr>
<td>Elevated BNP to &lt;30% further change in BNP (increase or decrease but none back to normal value)</td>
<td>5.09</td>
<td>2.02 to 12.80</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Elevated BNP to ≥30% further increase in BNP</td>
<td>5.78</td>
<td>2.09 to 16.0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

All values are relative to normal value (≥95th percentile) that remained normal.

**Figure 3.** Risk of death/cardiac transplantation. Time-dependent multivariate model with serial follow-up troponin T and BNP values. + indicates elevated BNP or troponin T levels and ≥0.03 ng/mL or >0.01 ng/mL or ≥0.01 ng/mL; –, not elevated (HR, 1.0 for elevation of neither BNP or troponin T).

**Figure 4.** A, Kaplan-Meier curves of time-dependent events of hospitalization for decompensated HF, death, or cardiac transplantation for troponin T levels <0.01 ng/mL, 0.01 to 0.03 ng/mL, and >0.03 ng/mL in patients with chronic HF. B, Kaplan-Meier curves of time-dependent events of hospitalization for decompensated HF, death, or cardiac transplantation for elevated BNP levels (above the 95th percentile of normal adjusted for age and gender) and nonelevated BNP levels in patients with chronic HF.
The increased risk in these patients is similar to the increased risk associated with elevations of biomarkers seen during acute decompensations that lead to hospitalizations. In that setting, it is clear that prognosis is markedly influenced by the acute event of hospitalization. Most of the elevations we observed were not associated with hospitalizations. However, it may be that these elevations are similar in prognostic importance to those associated with hospitalizations. If so, these elevations may represent similar pathophysiology but without overt clinical decompensation and thus only detectable by monitoring of biomarkers such as cTnT and BNP. These data suggest that both clinically detectable and subclinical myocardial injury and left ventricular volume overload may occur in a stepwise fashion based on discrete events rather than gradually over time and can be detected in otherwise clinically stable patients. Such events, which may in part reflect ongoing myocardial apoptosis, are consistent with the concept of small “HF myocardial infarcts” that contribute to progressively decompensating myocardial function. Elevations in cTnT above 0.03 ng/mL measured at any time during follow-up conveyed a 4- to 8-fold increased risk of death or cardiac transplantation, whereas an elevated BNP also measured at any time during follow-up supported a 4-fold increase in risk of a poor outcome. The combined elevation of both biomarkers contributed an 8-fold increase in risk. A decrease in cTnT to normal value was associated with a decrease in risk, whereas a decrease in BNP to normal level did not alter risk.

The mechanisms for these elevations in biochemical biomarkers and especially cardiac cTnT cannot be determined from the present study. The elevations in cardiac cTnT are consistent with myocyte injury/necrosis and/or apoptosis. There is a large number of putative mechanisms that occur in patients with HF that could cause myocardial injury, such as subendocardial ischemia/necrosis, apoptosis induced by myocardial stretch, or toxic cytokines. Abnormal rates of cardiomyocyte apoptosis have been described in patients with HF, and the intermittent elevations in cTnT may reflect this accelerated process of cell death. We also cannot exclude the participation of coronary artery disease in patients whose HF was related to underlying ischemic heart disease or endothelial dysfunction. However, we did not observe major differences in the prognostic effects of the biomarkers over time on the basis of the HF etiology. We attempted to take into account most other relevant covariants. Renal function (GFR) was the most important and worsened as the extent of myocardial injury increased as reflected in the association of decreasing GFR and progressive increases in cTnT levels.

The observation that elevations of cTnT above 0.01 ng/mL and especially >0.03 ng/mL and the trend toward a reduction in risk when values decrease also suggests that there may be vulnerable periods during which patients are at increased risk. If confirmed, this observation would suggest the need to develop preventative responses to such events as a way to improve outcomes. These observations support the value of cTnT monitoring in these patients. We expected similar results with BNP but did not observe them. Once elevated, changes in BNP did not seem to alter prognosis. This may be a result of the high variability in BNP or because high values in some patients may be prognostic whereas in some series low values are also prognostic.

The present study is unique in several ways. Most studies have identified patients when acutely decompensated in the hospital. In this situation, patients with elevated biomarkers who failed to respond over a usually modest period of time were deemed to be at greater risk. No studies to our knowledge have performed time-dependent analyses, and our patients were not decompensated when enrolled but rather were outpatients with stable HF. The elevations in biomarkers could not be correlated with overt clinical events, and the changes observed were modest but nonetheless carried potent prognostic information.

Limitations of the Study

Twenty-eight patients of the 200 initially enrolled patients did not complete follow-up for reasons other than death or cardiac transplantation. Ten patients had no study enrollment biomarker values for technical reasons, and 18 patients elected at various times during follow-up not to continue participation in the study (6 patients had completed at least 1 year of follow-up) and therefore not all patients have 2 years of follow-up. Eight patients died after the initial enrollment visit and before the first 3-month follow-up visit; therefore, only enrollment (baseline) data were available for these patients. Although the prospective analysis plan included the analyses reported, the use of multiple end points and values over time make it possible that some of our results were the result of chance alone. Because our study generates a hypothesis, we did not correct for multiple comparisons and chose to report probability values. Confidence intervals should be noted; some subgroups are small in number and hence more variable. The consistency of these data, however, argues that the principles put forth are not likely the result of statistical artifact. We would encourage other studies to undertake similar analyses.

Clinical Implications of the Study

It appears that intermittent myocardial injury is associated with progression of HF and can occur in apparently clinically stable patients. The monitoring of cTnT, which is a very sensitive and specific biomarker of myocardial injury, over time provides a readily available means to identify a subgroup of HF patients at substantial risk of poor short-term outcomes. An association between elevated troponin levels and the injury of ventricular remodeling has been suggested and our findings support a high risk of death or decompensated HF in patients with elevated cTnT levels, even with a low threshold of ≥0.01 ng/mL. It is likely but unproven that troponin I would provide similar information, but the cutoff values would be dramatically different and would be assay dependent. In this patient cohort BNP levels were less predictive.
In conclusion, the present data show that elevations of cTnT and BNP detected at any time during clinical follow-up by serial monitoring in ambulatory patients with chronic HF are highly associated with increased risk for short-term events such as death, and that the combined elevations of cTnT and BNP substantially adds to risk. This strategy may therefore play an important role in detection and, after appropriate studies to define the best response, management of high-risk chronic HF patients. Moreover, elevated cTnT, particularly >0.03 ng/mL, is highly and independently prognostic (8-fold increased risk).

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Disclosures
Drs Miller and Jaffe have received research grants from Dade-Behring, and Dr Jaffe has served as a consultant for Dade-Behring and Roche Diagnostics. The remaining authors report no conflicts.

References
CLINICAL PERSPECTIVE

It appears that intermittent myocardial injury is associated with progression of heart failure (HF) and can occur in apparently clinically stable patients. The monitoring of cardiac troponin T (cTnT), which is a very sensitive and specific biomarker of myocardial injury over time, provides a readily available means to identify a subgroup of HF patients at substantial risk of poor short-term outcomes. An association between elevated troponin levels and the injury of ventricular remodeling has been suggested, and our findings support a high risk of death or decompensated HF in patients with elevated cTnT levels, even when a low threshold of ≥0.01 ng/mL is used. It is likely, but unproven, that troponin I would provide similar information, but the cutoff values would be dramatically different and would be assay-dependent. In this patient cohort, B-type natriuretic peptide levels were less predictive. In conclusion, the present data show that elevations of cTnT and B-type natriuretic peptides detected at any time during clinical follow-up by serial monitoring in ambulatory patients with chronic HF are highly associated with increased risk for short-term events such as death, and that the combined elevations of cTnT and B-type natriuretic peptide substantially adds to risk. This strategy may therefore play an important role in the detection and, after appropriate studies to define the best response, management of high-risk chronic HF patients. Moreover, elevated cTnT, particularly >0.03 ng/mL, is highly and independently prognostic (8-fold increased risk).
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