Circulating Levels of Tumor Necrosis Factor Correlate With Indexes of Depressed Heart Rate Variability*

A Study in Patients With Mild-to-Moderate Heart Failure

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Objectives: Patients with heart failure have increased circulating levels of tumor necrosis factor (TNF) and TNF receptors. It is not known whether TNF, which is known to blunt β-adrenergic responsiveness in experimental models, contributes to the loss of heart rate variability in patients with heart failure. Therefore, we examined heart rate variability in relation to circulating levels of TNF, TNF receptors, and norepinephrine in patients with heart failure and in control subjects.

Methods: Heart rate variability was obtained from 24-h ambulatory ECG recordings in age-matched control subjects (n = 10) and patients with mild (n = 15) to moderate (n = 14) heart failure. Plasma levels of TNF and soluble type 1 and 2 TNF receptors were measured by enzyme-linked immunoassay; plasma norepinephrine levels were measured by high-performance liquid chromatography.

Results: There was a significant inverse linear correlation between increased circulating levels of TNF, TNF receptors, and norepinephrine for time-domain and frequency-domain indexes of heart rate variability among patients with heart failure and control subjects. Multiple stepwise linear regression analysis showed that TNF was a stronger independent predictor of frequency-domain indexes of heart rate variability than norepinephrine.

Conclusions: TNF is an independent predictor of depressed heart rate variability in patients with heart failure. Insofar as TNF blunts β-adrenergic signaling, this study suggests the possibility that overexpression of TNF and subsequent loss of β-adrenergic responsiveness contributes to the decrease in heart rate variability observed in heart failure. (CHEST 2003; 123:716–724)

Key words: cytokine; heart failure; heart rate variability; norepinephrine; tumor necrosis factor

Abbreviations: ANOVA = analysis of variance; HF = high frequency; LF = low frequency; NYHA = New York Heart Association; SDANN = SD of the means of NN intervals in all 5-min segments; SDNN = SD of NN intervals; sTNFR1 = soluble type 1 tumor necrosis factor receptor; sTNFR2 = soluble type 2 tumor necrosis factor receptor; TNF = tumor necrosis factor

Previous studies have shown that indexes of depressed heart rate variability not only correlate with the extent of disease progression in heart failure, but also appear to predict adverse outcomes in patients with heart failure.1–6 The mechanisms that have been proposed for decreased heart rate variability include an imbalance in sympathovagal activation, changes in β-adrenergic receptor number and function, abnormal baroreflex function, and central abnormalities of autonomic regulatory function.1,7–11 While a large body of clinical and experimental evidence supports the importance of each one of these mechanisms, given the enormous com-

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Correspondence to: Douglas L. Mann, MD, FCCP, Winters Center for Heart Failure Research, 6565 Fannin, MS 524 Houston, TX 77030; e-mail: dmann@bcm.tmc.edu
plexity of worsening heart failure, it is extremely likely that other factors may be involved as well.

For editorial comment see page 664

Relevant to the above discussion is the relatively recent observation that proinflammatory cytokines such as tumor necrosis factor (TNF) are elevated in patients with heart failure.12–15 We and others have suggested that the overexpression of TNF may contribute to disease progression in heart failure, by virtue of the direct toxic effects that this cytokine exerts on the heart and the peripheral circulation.16,17 In addition to the well-recognized deleterious effects of TNF, including left ventricular dysfunction,18 left ventricular remodeling, cardiac myocyte apoptosis,19 and endothelial cell dysfunction,20 there were several intriguing lines of experimental evidence that suggested that TNF might contribute to depressed heart rate variability in patients with heart failure. For example TNF had been shown to alter β-adrenergic signaling in isolated cell culture models.21,22 Moreover, mice harboring cardiac restricted overexpression of TNF were shown to have blunted responsiveness to isoproterenol.23 Accordingly, in the present study, we sought to examine changes in heart rate variability in relation to peripheral circulating levels of TNF and norepinephrine in patients with mild-to-moderate heart failure, in order to determine whether circulating levels of these biological mediators might contribute to the decrease in heart rate variability that is observed in worsening heart failure.

Materials and Methods

Patient Demographics, Clinical Characteristics, and Study Protocol

The study cohort consisted of 29 patients with New York Heart Association (NYHA) classes I through IIIa heart failure and 10 age-matched healthy subjects who were free of cardiovascular disease or other comorbid conditions. The diagnosis of heart failure was established according to standard guidelines, and was based on the presence of a depressed left ventricular ejection fraction ≤ 40% (measured by radionuclide ventriculography or two-dimensional echocardiography) at the time of initial screening, and signs and symptoms compatible with the diagnosis of heart failure, namely shortness of breath and/or fatigue. For the purpose of this study, we classified patients with NYHA class I or II heart failure as “mild,” whereas patients with NYHA class IIIa heart failure were classified as “moderate.” The rationale for studying patients with mild-to-moderate heart failure, as opposed to patients with more severe end-stage heart failure was twofold: first, we were interested in studying the activation of the adrenergic and cytokine systems relatively early in the disease process, rather than at the end of the disease process, when both systems were fully activated; second, we were interested in studying a stable population of patients with heart failure who could easily tolerate being withdrawn from their medications for a short period of time.

At the time they were enrolled in the study, all of the patients were in sinus rhythm and were receiving stable medical therapy for at least 2 weeks prior to enrollment. None of the patients had been hospitalized for or with heart failure during the preceding 6 months. Both the patients with heart failure and the control subjects were recruited from the ambulatory care clinics at the Houston Veterans Affairs Medical Center. Exclusion criteria included age ≥ 80 years, acute febrile or infectious illnesses within a 2-week period before the study, and neoplastic, infectious, inflammatory diseases or any other condition that could increase circulating levels of proinflammatory cytokines. Subjects were also excluded if they were receiving antiarrhythmic therapy, had implantable defibrillators or pacemakers, had a recent myocardial infarction (within 3 months of enrollment), or had a recent episode of unstable angina (within 28 days of enrollment), insofar as these conditions might independently affect heart rate variability. In addition, we excluded patients with diabetes mellitus, chronic renal failure, or peripheral or CNS conditions that involved the autonomic nervous system. The research protocol was approved by the Institutional Board for Human Subject Research at Baylor College of Medicine.

All heart failure medications were discontinued for at least 24 h prior to admission to the hospital. The patients with heart failure were admitted to an observation unit in the Houston Veterans Affairs Medical Center during the study period, in order to monitor for clinical decompensation while they were not receiving their usual heart failure medications. All patients were instructed to maintain their usual activity level while in the hospital. ECG monitors were placed at the time of hospital admission and were continued for a total of 24 h. Twenty-four hours after placement of the ambulatory monitor, the monitor was removed and blood samples were collected for the assessment of circulating levels of TNF, TNF receptors, and norepinephrine, as described below. Control subjects went home following placement of the ECG monitor and returned to the hospital 24 h later for removal of the monitor and collection of blood samples.

Circulating Levels of TNF and Soluble TNF Receptors

Blood samples for cytokine measurements were obtained from an antecubital vein, placed in prechilled tubes containing ethylenediamine tetra-acetic acid, and the plasma separated by centrifugation within 15 min of collection. Aliquots were arranged in duplicate and stored at –70°C until the time of analysis. The measurements of circulating levels of TNF and soluble type 1 TNF receptor (sTNFR1) and soluble type 2 TNF receptor (sTNFR2) were performed in the Cardiac Cytokine Laboratory at the Houston Veterans Affairs Medical Center, using commercially available enzyme-linked immunosorbent assay kits (Quantikine HS; R&D Systems; Minneapolis, MN), exactly as described previously.15,24 The laboratory personnel performing the assays were blinded with respect to the clinical data.

Circulating Levels of Norepinephrine

Blood samples for norepinephrine were obtained through an IV cannula inserted into an antecubital vein. All study subjects rested in the supine position in a quiet, dark room for 30 min before blood samples were collected. All blood samples were placed in prechilled tubes containing ethylenediamine tetra-acetic acid and were centrifuged within 15 min. The plasma samples were aliquoted into polypropylene tubes containing...
Assessment of Heart Rate Variability

In order to assess heart rate variability, 24-h ECG recordings were obtained on patients and control subjects, using two-channel 8500 Marquette recorders (Marquette Medical Systems; Milwaukee, WI). Heart rate variability was analyzed using commercially available software incorporated into the MARS 8000 ECG Analysis and Editing System (Marquette Medical Systems). Automatic labeling of beats was performed, with additional visual inspection and manual editing by experienced technicians in order to categorize QRS complexes as normal or premature. The technicians performing the analysis were unaware of the clinical characteristics of the patients.

For the studies presented herein, we examined several different indexes of heart rate variability. First, in order to obtain a measure of overall heart rate variability, we measured long-term time-domain indexes, including the SD of NN intervals (SDNN) and the SD of the means of NN intervals in all 5-min segments (SDANN). Second, we used so-called frequency-domain measures in order to determine the components of heart rate variability that were attributable to altered sympathetic and/or parasympathetic activity. The frequency-domain indexes were evaluated by spectral analysis, using a fast Fourier transform algorithm. Spectral power was determined over two regions of interest: a low-frequency (LF) band (0.040 to 0.150 Hz), which is considered to reflect sympathetic activity/modulation, and a high-frequency (HF) band (0.150 to 0.40 Hz), which is thought to reflect increased parasympathetic activity/modulation.7,26–28

Table 1—Demographic, Hemodynamic, Clinical, and Biochemical Characteristics of Patients With Heart Failure and Control Subjects*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control Subjects (n = 10)</th>
<th>Mild Heart Failure (NYHA I, n = 5; NYHA II, n = 10)</th>
<th>Moderate Heart Failure (NYHA IIIA, n = 14)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>59.50 ± 2.92</td>
<td>63.13 ± 2.11</td>
<td>62.43 ± 2.80</td>
<td>NS (0.62)†</td>
</tr>
<tr>
<td>Male/female gender, No.</td>
<td>10/0</td>
<td>15/0</td>
<td>14/0</td>
<td>NA</td>
</tr>
<tr>
<td>Etiology, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic</td>
<td>NA</td>
<td>92.86</td>
<td>93.33</td>
<td>NS (1.00)†</td>
</tr>
<tr>
<td>Nonischemic</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEF, %</td>
<td>60.30 ± 2.07</td>
<td>31.20 ± 2.08</td>
<td>26.79 ± 2.18</td>
<td>&lt; 0.0001†</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>89.67 ± 1.89</td>
<td>91.53 ± 2.78</td>
<td>94.93 ± 4.14</td>
<td>NS (0.54)†</td>
</tr>
<tr>
<td>Mean heart rate, beats/min</td>
<td>75.10 ± 2.99</td>
<td>74.53 ± 2.80</td>
<td>79.64 ± 2.66</td>
<td>NS (0.36)†</td>
</tr>
<tr>
<td>Sodium, mEq/L</td>
<td>138.00 ± 0.45</td>
<td>138.54 ± 0.49</td>
<td>138.21 ± 0.91</td>
<td>NS (0.86)†</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.01 ± 0.067</td>
<td>0.99 ± 0.042</td>
<td>1.10 ± 0.058</td>
<td>NS (0.32)†</td>
</tr>
<tr>
<td>Therapy, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-blockers</td>
<td>NA</td>
<td>20</td>
<td>7.14</td>
<td>NS (0.60)†</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>NA</td>
<td>100</td>
<td>100</td>
<td>NS (1.00)†</td>
</tr>
<tr>
<td>Diuretics</td>
<td>NA</td>
<td>53.33</td>
<td>92.86</td>
<td>0.035†</td>
</tr>
<tr>
<td>Digoxin</td>
<td>NA</td>
<td>26.67</td>
<td>57.14</td>
<td>NS (0.14)†</td>
</tr>
<tr>
<td>Long-acting nitrates</td>
<td>NA</td>
<td>33.33</td>
<td>25.57</td>
<td>NS (1.00)†</td>
</tr>
</tbody>
</table>

*Data are presented as mean value ± SEM. LVEF = left ventricular ejection fraction; MAP = mean arterial pressure; ACE = angiotensin-converting enzyme; NA = not applicable; NS = not significant.
†One-way ANOVA.
‡Fisher exact test.

Statistical Analysis

All data are presented as mean value ± SEM. The Fisher exact test or χ² test were used to test for differences in nominal variables (eg, drug treatment and etiology of heart failure). A one-way analysis of variance (ANOVA) was used to test for differences in continuous variables (eg, age, TNF, TNF receptors, norepinephrine levels, and indexes of heart rate variability). Where appropriate, post hoc analysis of multiple comparison testing was performed to test for differences from control values (Dunnett), or to test for differences between groups of patients (Newman-Keuls). A correlation analysis (Pearson) was used to test for significant linear relationships between the various measures of heart rate variability and TNF, TNF receptors, and norepinephrine levels. All data were tested for normality of distribution; data that were not normally distributed were subjected to log-normalization before performing parametric analyses. In addition, in order to be certain that the analysis of the log-normalized data were robust, we repeated the analysis of the nonnormally distributed data using the Spearman rank-order correlation analysis. Finally, we used a linear regression and multiple stepwise linear regression analyses to evaluate the relative contributions of circulating levels of TNF, TNF receptors, and norepinephrine levels to changes in heart rate variability. Statistical analyses were performed using Sigma Stat Statistical Software (version 2.0; SPSS; Chicago, IL). Significant difference was said to exist at the p < 0.05 level.

Results

Patient Demographics and Clinical Characteristics

The study cohort consisted of 15 patients with mild heart failure (NYHA class I/II), 14 patients with mod-
erate heart failure (NYHA class IIIA), and 10 age-matched control subjects who were free of cardiovascular disease or other comorbid conditions. As shown in Table 1, all subjects in the study were male, reflecting the demographics of the patient population at the Houston Veterans Affairs Medical Center. There were no significant differences in age, mean arterial BP, heart rate, sodium levels, and creatinine levels among the different groups of patients. However, as expected, there were significant overall differences in left ventricular ejection fraction among patients with heart failure and control subjects (ANOVA p < 0.001); post hoc ANOVA revealed no significant differences in left ventricular ejection fraction between patients with mild-to-moderate heart failure. The use of β-blockers (metoprolol), angiotensin-converting enzyme inhibitors (lisinopril), digitalis (digoxin), and long-acting nitrates (isosorbide dinitrate) was similar in both groups of patients with heart failure. However, the use of diuretics was significantly greater in the patients with moderate heart failure when compared to patients with mild heart failure (p < 0.05). Figure 1 shows two important findings with respect to the levels of TNF, soluble TNF receptors, and norepinephrine. First, when compared with age-matched control subjects, there was a progressive increase in circulating levels of TNF with worsening NYHA class, as we and others have reported previously.15,29,30 In contrast, the levels of sTNFR1 and sTNFR2 remained relatively unchanged in patients with mild heart failure compared to control subjects, and only increased significantly in patients with moderate heart failure. Second, the values of plasma norepinephrine were similar in patients with mild heart failure and control subjects, consistent with previous reports31; and although there was a trend toward increased norepinephrine levels in patients with moderate heart failure, the increase was not statistically significant. One-way ANOVA indicated that there were significant overall differences in the levels of TNF (p < 0.001),

![Figure 1](http://publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21990/)
sTNFR2 (p < 0.005), and sTNFR1 (p < 0.05) between the different groups. Post hoc analysis of variance testing (Dunnett) indicated that there were significant differences in TNF levels between control subjects and both groups of patients with heart failure, as well differences in sTNFR1 and sTNFR2 between control subjects and patients with moderate heart failure (p < 0.05).

Heart Rate Variability

Table 2 shows that both time-domain and frequency-domain indexes of heart rate variability decreased with increased severity of heart failure, as has been reported previously.\(^1\)\(^2\)\(^6\) One-way ANOVA indicated significant overall differences in all time-domain and frequency-domain indexes of heart rate variability (p < 0.05). Post hoc ANOVA testing (Dunnett) showed that patients with moderate heart failure had a significant (p < 0.05) decline in all measures of heart rate variability when compared to control subjects. When post hoc comparisons (Newman-Keuls) were made between patient groups with mild-to-moderate heart failure, significant differences were found only for SDNN (p < 0.05). Additionally, patients with mild heart failure had a significant decline in frequency-domain measures of heart rate variability (LF and HF, p < 0.05 [Newman-Keuls, p < 0.05]), but not in time-domain measures (SDNN and SDANN).

Relationship Between TNF and Measures of Heart Rate Variability

Figure 2 shows the relationship between TNF levels and both time-domain and frequency-domain measures of heart rate variability in normal subjects and patients with heart failure. As shown, increasing levels of TNF were significantly inversely correlated with the decrease in the time-domain indexes SDNN (r = 0.52, p < 0.001) and SDANN (r = 0.51, p < 0.001), as well as decreased power in the LF band (r log-LF = 0.51, p < 0.001) and HF band (r log-HF = 0.32, p < 0.05). Similar findings were obtained when the nonnormally distributed data were examined using a nonparametric test (Spearman), suggesting that the analysis of the log-normalized data were robust. There were significant inverse correlations between increasing levels of sTNFR1 and the time-domain measures of heart rate variability (SDNN [r = 0.39, p = 0.01], SDANN [r = 0.37, p = 0.02]), but not with the frequency-domain measures of heart rate variability (LF [r = 0.28, p = 0.09], HF [r = 0.14, p = 0.39]). Similarly, there were significant inverse correlations between the increasing levels of sTNFR2 and the time-domain measures of heart rate variability (SDNN [r = 0.36, p = 0.03], SDANN [r = 0.33, p = 0.04]), as well as with the power in the LF band (r = 0.32, p = 0.046), but not with power in the HF band (r = 0.25, p = 0.12).

Relationship Between Norepinephrine Levels and Measures of Heart Rate Variability

Table 2 shows that both time-domain and frequency-domain indexes of heart rate variability decreased with increased severity of heart failure, as has been reported previously.\(^1\)\(^2\)\(^6\) One-way ANOVA indicated significant overall differences in all time-domain and frequency-domain indexes of heart rate variability (p < 0.05). Post hoc ANOVA testing (Dunnett) showed that patients with moderate heart failure had a significant (p < 0.05) decline in all measures of heart rate variability when compared to control subjects. When post hoc comparisons (Newman-Keuls) were made between patient groups with mild-to-moderate heart failure, significant differences were found only for SDNN (p < 0.05). Additionally, patients with mild heart failure had a significant decline in frequency-domain measures of heart rate variability (LF and HF, p < 0.05 [Newman-Keuls, p < 0.05]), but not in time-domain measures (SDNN and SDANN).

Table 2—Heart Rate Variability in Patients With Heart Failure and Control Subjects*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (n = 10)</th>
<th>Mild (NYHA I, n = 5; NYHA II, n = 10)</th>
<th>Moderate (NYHA IIIA, n = 14)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDNN</td>
<td>116.00 ± 11.45</td>
<td>96.33 ± 7.18</td>
<td>71.93 ± 7.56</td>
<td>0.0043*</td>
</tr>
<tr>
<td>SDANN</td>
<td>100.90 ± 11.61</td>
<td>79.60 ± 6.11</td>
<td>58.50 ± 7.17</td>
<td>0.0039*</td>
</tr>
<tr>
<td>LF</td>
<td>704.64 ± 164.31</td>
<td>291.83 ± 32.55</td>
<td>211.46 ± 45.77</td>
<td>0.0024*</td>
</tr>
<tr>
<td>HF</td>
<td>216.51 ± 32.80</td>
<td>113.45 ± 25.06</td>
<td>102.79 ± 23.94</td>
<td>0.016*</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SEM.
†One-way ANOVA.
‡One-way ANOVA on log-transformed values.
Multivariate Analysis of TNF, TNF Receptors, and Norepinephrine Levels with Heart Rate Variability

In order to determine the relative weighted contribution of circulating levels of TNF and norepinephrine to the decrease in time-domain and frequency-domain measures of heart rate variability, we performed a multiple linear regression analysis. For this analysis, we selected SDNN as a representative measure of heart rate variability in the time domain, and LF as a representative measure of sympathovagal modulation in the frequency domain. Table 3 summarizes the univariate analysis for SDNN and LF. As shown, each of the variables selected was significantly correlated with either SDNN or log LF, except for sTNFR1, which did not correlate with LF. When these variables were examined together using a stepwise linear regression analysis, only TNF and norepinephrine contributed significantly to the variation observed in both measures of heart rate variability (Table 4). The multiple linear regression model showed that SDNN can be predicted from a combination of the variables TNF and norepinephrine, where $SDNN = 291.93 - 8.38 \times \text{TNF} - 59.53 \times \log \text{norepinephrine}$. Similarly, the multiple stepwise linear regression model showed that LF can be predicted from a combination of the variables TNF and norepinephrine, where $\log LF = 4.49 - 0.09 \times \text{TNF} - 0.61 \times \log \text{norepinephrine}$. In both models, TNF was a stronger predictor for depressed heart rate variability than was norepinephrine.

**Discussion**

The major new finding shown by this study is that there is a significant inverse relationship between increased levels of circulating TNF and indexes of decreased heart rate variability in patients with mild-to-moderate heart failure. As shown in Figure 2, there were statistically significant inverse correlations between circulating levels of TNF and two
time-domain indexes of heart rate variability, namely SDNN and SDANN. Insofar as the physiologic components of long-term time-domain indexes are extremely complex and reflect not only changes in sympathovagal activity, but also LF cyclical changes that are influenced by thermoregulatory mechanisms, fluctuations in the activity of the renin-angiotensin system, and peripheral chemoreceptors,6,26,32 we also examined the relationship between

Table 3—Univariate Analysis*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Intercept</th>
<th>Slope</th>
<th>r</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDNN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF</td>
<td>146.68</td>
<td>−8.60</td>
<td>0.52</td>
<td>0.00076</td>
</tr>
<tr>
<td>Log sTNFR1</td>
<td>377.61</td>
<td>−96.74</td>
<td>0.39</td>
<td>0.013</td>
</tr>
<tr>
<td>Log sTNFR2</td>
<td>421.53</td>
<td>−97.42</td>
<td>0.36</td>
<td>0.026</td>
</tr>
<tr>
<td>Log norepinephrine</td>
<td>245.15</td>
<td>−61.93</td>
<td>0.42</td>
<td>0.0077</td>
</tr>
<tr>
<td>Log LF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF</td>
<td>2.99</td>
<td>−0.092</td>
<td>0.51</td>
<td>0.00008</td>
</tr>
<tr>
<td>Log sTNFR1</td>
<td>4.60</td>
<td>−0.74</td>
<td>0.28</td>
<td>NS (0.089)</td>
</tr>
<tr>
<td>Log sTNFR2</td>
<td>5.65</td>
<td>−0.96</td>
<td>0.32</td>
<td>0.046</td>
</tr>
<tr>
<td>Log norepinephrine</td>
<td>3.98</td>
<td>−0.64</td>
<td>0.40</td>
<td>0.012</td>
</tr>
</tbody>
</table>

*NS = not significant.

Table 4—Multivariate Stepwise Linear Regression Analysis

<table>
<thead>
<tr>
<th>Variables Entered (Stepwise)</th>
<th>Improvement in $R^2$</th>
<th>$R^2$</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDNN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First: TNF</td>
<td>0.27</td>
<td>0.00050</td>
<td></td>
</tr>
<tr>
<td>Second: log norepinephrine</td>
<td>0.43</td>
<td>0.16</td>
<td>0.0028</td>
</tr>
<tr>
<td>Log LF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First: TNF</td>
<td>0.26</td>
<td>0.00048</td>
<td></td>
</tr>
<tr>
<td>Second: log norepinephrine</td>
<td>0.40</td>
<td>0.14</td>
<td>0.0056</td>
</tr>
</tbody>
</table>

*Significance level for the individual variables in the statistical model.
circulating TNF levels and two different spectral power components of heart rate variability. Figure 2 further shows that circulating levels of TNF were inversely correlated with the LF component of spectral power, which is thought to reflect changes in sympathetic activity, and were also significantly inversely correlated with the HF component of spectral power, which is thought to reflect changes in vagal activity.7,26–28 Interestingly, we observed that the LF component of spectral power was more closely correlated with circulating levels of TNF than was the HF component. These latter findings suggest that increased TNF levels correlate better with decreased sympathetic activity/modulation to the heart, rather than decreases in parasympathetic activity/modulation, although the difference in correlations was modest. The explanation for a relationship between TNF levels and decreased parasympathetic tone is not known.

Consistent with previous reports,11,33–35 we also observed that levels of norepinephrine were significantly and inversely correlated with time-domain and frequency-domain measures of heart rate variability (Fig 3). Similar to what we observed with circulating levels of TNF and norepinephrine, circulating levels of the sTNFR1 and sTNFR2, both correlated with time-domain indexes of heart rate variability. However, unlike TNF and norepinephrine, the soluble TNF receptors did not correlate well with spectral power indexes of heart rate variability. The reason for, and the clinical significance of, the lack of an overall significant relationship between the soluble TNF receptors and the spectral power indexes of heart rate variability is unclear.

A second important finding of this study was that the circulating level of TNF was a stronger independent predictor of depressed heart rate variability than was the circulating level of norepinephrine. That is, when we performed a multiple stepwise linear regression analysis, using both time-domain and spectral power indexes, we observed that TNF and norepinephrine were independent predictors of both SDNN and the LF component of spectral power (Table 4); however, TNF was a stronger predictor of depressed heart rate variability than was norepinephrine, which is entirely consistent with the modest degree of sympathetic activation in the group of patients with mild-to-moderate heart failure examined in this study. Importantly, however, the combination of TNF and norepinephrine was a better predictor than TNF alone, suggesting that both circulating TNF and norepinephrine levels contribute to the depressed heart rate variability observed in patients with mild-to-moderate heart failure.

**Conclusion**

In summary, in the present study, we observed that TNF was an independent predictor of depressed indexes of heart rate variability that are influenced by sympathetic activity. Nonetheless, it bears emphasis that a direct cause-and-effect relationship cannot be inferred from a clinical study of this nature. For example, the correlation of TNF with changes in heart rate variability might simply reflect an association with NYHA classification. However, based on what is known about the biology of TNF signaling, it is tempting to speculate that increased expression of TNF may be one of several different mechanisms that contribute to blunting of β-adrenergic responsiveness of the failing heart to sympathoadrenergic drive during the progression of heart failure. Indeed, previous experimental studies have shown that TNF is sufficient to inhibit β-adrenergic signal transduction through increased activation of inhibitory G proteins and/or an impairment of the action of stimulatory G proteins.21,22 From a teleologic point of view, TNF-induced blunting of β-adrenergic responsiveness in the early stages of heart failure may be viewed as an adaptive mechanism that protects the cardiac myocyte from the deleterious actions of catecholamines.36 However, in the more advanced stages of disease, the overexpression of TNF may become maladaptive, by contributing to depressed cardiac output and worsening neurohormonal activation. Further studies will be necessary to test these interesting possibilities.

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