Original Article

The Value of Plasma Levels of Tumor Necrosis Factor-α and Interleukin-6 in Predicting the Severity and Prognosis in Patients with Congestive Heart Failure

Background. High plasma levels of pro-inflammatory cytokines play an important role in the pathophysiology of congestive heart failure (CHF). Therefore, we conducted a case-control study to determine the correlations between plasma levels of cytokines, i.e., tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-6, and the severity and mortality in patients with CHF.

Methods. One-hundred and 18 cases (62 ± 15 years old) were classified into 3 groups: group 1 comprised 44 control cases with normal coronary arteriogram and left ventriculography and without valvular disorders or cardiomyopathy; group 2 comprised of 37 cases with mild CHF in New York Heart Association (NYHA) functional class (FC) II; group 3 had 37 cases with moderate/severe CHF in NYHA FC III or IV. Pre-catheterization plasma levels of TNF-α and IL-6 along with clinical and hemodynamic variables and follow-up data of cardiac death were assessed.

Results. Patients of group 3 had smaller body mass index, lower systolic and diastolic blood pressures, faster heart rates, higher left ventricular end-diastolic pressure and lowered triglyceride levels than the patients of groups 1 and 2. The plasma levels of TNF-α and IL-6 increased significantly in patients of group 3 in comparison with patients of groups 1 and 2 (both \( p < 0.001 \)). Over the following 1.5 years, 13 patients died. Univariate analysis identified the following variables to be associated with poor prognosis: NYHA FC (\( p < 0.001 \)), plasma TNF-α (\( p = 0.013 \)), plasma IL-6 (\( p < 0.001 \)), systolic blood pressure (\( p = 0.001 \)), heart rate (\( p = 0.045 \)) and left ventricular end-diastolic pressure (\( p = 0.021 \)). Multivariate Cox regression analysis identified the independent predictors of cardiac death as FC (\( p = 0.007 \)) and plasma IL-6 (\( p = 0.021 \)).

Conclusions. Our findings indicate that the plasma levels of IL-6 and TNF-α and especially the former, is a useful marker to correlate the progression of severity and late cardiac death in patients with CHF.

Studies took great interests in the connections between the inflammatory process and the pathophysiology of congestive heart failure (CHF). Pro-inflammatory cytokines, especially tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-6, have been shown to elevate in CHF, suggesting that they probably play important roles in the underlying pathophysiology of CHF. TNF-α and IL-6 are polypeptide cytokines synthesized and released locally in response to endotoxin infection and tissue injury. TNF-α is a predominantly leukocyte-derived cytokine, and hypoxia increases the production of TNF-α by human mononuclear cells. TNF-α production can be induced in cardiac myocytes of the failing human heart, whereas IL-6 is produced from leukocytes, endothelial cells and vascular smooth muscle cells when stimulated with a variety of inflammatory mediators. TNF-α and IL-6 are reported to be potentially negative inotropes, able to produce cardiac cachexia, muscle wasting and deterioration of ventricular performance. Despite assay sensi-
The circulating levels of TNF-α and IL-6 in patients with CHF were shown to be increased with the severity of symptoms.

In the present study, we analyzed the clinical variables and hemodynamic parameters of cardiac catheterization in patients with CHF to determine (1) if any of these variables were in association with the increment of plasma levels of TNF-α and IL-6 and (2) to clarify the independent predictors of fatal outcome in patients with CHF.

METHODS

From August 23, 1997 to July 11, 2000, 118 cases were enrolled consecutively into the present study. The study cohort was comprised of 74 patients with documented CHF and 44 subjects who complained of chest pain and had positive treadmill exercise test or thallium-201 scintigraphy, all of whom underwent cardiac catheterization for clinical indications. A written informed consent was obtained from each patient before participating in the study. All patients with CHF were classified according to the standards of New York Heart Association (NYHA) functional class (FC) II to IV for at least 2 weeks prior to admission. Patients with acute or chronic inflammatory or infectious diseases, hepatic, renal, gastrointestinal or connective tissue diseases, malignancies or myocardial infarction within the previous 3 months were excluded. In the documented cases of CHF, 42 patients had ischemic heart disease, 22 patients had dilated cardiomyopathy, and 10 patients had valvular heart disease. All patients were clinically stable with standard anti-heart failure treatment: 74 patients were treated with diuretics, 67 with digoxin, 66 with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, 47 with vasodilators and 33 with β-adrenergic blockers. All study cohorts were classified into 3 groups: group 1, comprised of 44 age-matched subjects with normal coronary angiography and left ventriculography, and without valvular disorders or cardiomyopathy; group 2, comprised of 37 patients with symptoms of CHF in NYHA FC II; group 3, comprised of 37 patients with moderate/severe symptoms of CHF in NYHA functional class III or IV.

Cytokine measurements

Blood samples were collected during catheterization from femoral arteries and stored in test tubes containing ethylenedehmaminetetraacetic acid (EDTA), and plasma was separated with centrifugation and frozen at -80 °C. Plasma levels of TNF-α and IL-6 were measured using a commercially available, solid-phase, high sensitivity enzyme-linked immunosorbent assay (ELISA, Quantikine HS, R & D Systems Inc., Minneapolis MN, USA). The differences in TNF-α and IL-6 between the 2 assays using plasma taken at the same time were 5.0 ± 2.2% and 4.3 ± 2.0%, respectively.

Statistical Analysis

All numeric data are summarized as mean ± SD. In the case of asymmetrical distribution, data are presented as median and range. ANOVA was used for comparison in more than 2 groups. Categorical variables were tested by a contingency chi-square test. Kaplan-Meier analysis and log-rank test was performed on the cumulative rates of survival in patients, with CHF stratified on the basis of NYHA functional class. Finally, we used Cox’s hazard proportional regression analysis for both univariate and stepwise multivariate to identify the independent prognostic predictors of the prognosis of CHF patients. All cut-off values were determined by Receiver Operator Characteristic curve analyses with utmost sensitivity and specificity. All tests were 2-tailed, and a p value of less
than 0.05 was considered statistically significant.

RESULTS

Demographic characteristics of the study groups

One-hundred and 18 cases (94 males/24 females; mean age 62 ± 15 years) were recruited. Forty-four cases (mean age 63 ± 14 years) were classified into group 1; 37 patients (mean age 64 ± 15 years) were group 2; 37 patients (mean age 58 ± 17 years) were group 3. The clinical and hemodynamic characteristics of these 3 groups are shown in Table 1. Among the 3 groups, there were no significant differences in age and gender distribution, as well as in history of hypertension, diabetes, smoking and alcohol drinking. Patients with moderate/severe CHF (group 3) had smaller BMI, lower SBP and DBP, faster HR, relatively higher LVEDP, and lower plasma triglyceride levels than the patients of groups 1 and 2.

Plasma levels of cytokines

Comparisons of plasma levels of TNF-α and IL-6 among the study groups are shown in Table 1 and Fig. 1. Plasma levels of TNF-α were elevated significantly in group 3 than in groups 1 and 2 ($p < 0.001$). Plasma levels of IL-6 were also elevated significantly in group 3 than in groups 1 and 2 patients ($p < 0.001$).

Mortality analysis

During the follow-up period (from 12 to 1074 days, median 544 days), 13 patients died (11 died of refractory heart failure and 2 of sudden death). Among the deaths, 3 were group 2 patients and 10 were group 3 patients. The cumulative survival rate for all patients was 87% [95% confidence interval (CI), 79%-96%] at 6 months (8 deaths), 81% [95% CI, 71%-91%] at 12 months (3 deaths), 78% [95% CI, 68%-90%] at 18 months (1 death),

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Table 1. Demographic characteristics, hemodynamic and laboratory data of the study groups

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 39 or 44)</th>
<th>Group 2 (n = 33 or 37)</th>
<th>Group 3 (n = 35 or 37)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>63.0 ± 13.6</td>
<td>64.3 ± 14.7</td>
<td>62.0 ± 15.1</td>
<td>NS</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>37/7</td>
<td>28/9</td>
<td>29/8</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension (yes/no)</td>
<td>15/29</td>
<td>16/21</td>
<td>11/26</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetic (yes/no)</td>
<td>3/41</td>
<td>6/31</td>
<td>8/29</td>
<td>NS</td>
</tr>
<tr>
<td>Smoker (yes/no)</td>
<td>12/32</td>
<td>10/27</td>
<td>16/21</td>
<td>NS</td>
</tr>
<tr>
<td>Drink (yes/no)</td>
<td>13/31</td>
<td>6/31</td>
<td>4/33</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.2 ± 3.4</td>
<td>25.4 ± 3.9</td>
<td>23.5 ± 3.2</td>
<td>0.004</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>73.8 ± 17.1</td>
<td>89.2 ± 19.8</td>
<td>90.9 ± 16.2</td>
<td>&lt;0.001</td>
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<tr>
<td>SBP (mmHg)</td>
<td>149.5 ± 22.2</td>
<td>129.0 ± 23.2</td>
<td>112.7 ± 20.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>79.9 ± 9.7</td>
<td>76.2 ± 14.1</td>
<td>69.2 ± 11.8</td>
<td>0.001</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>19.8 ± 6.9</td>
<td>18.8 ± 11.7</td>
<td>24.0 ± 9.2</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>170.5 ± 35.6</td>
<td>174.4 ± 40.1</td>
<td>158.4 ± 44.9</td>
<td>NS</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>133.6 ± 68.9</td>
<td>161.9 ± 157.6</td>
<td>87.2 ± 39.5</td>
<td>0.008</td>
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<tr>
<td>TNF-α(pg/mL)</td>
<td>6.04 ± 0.52</td>
<td>6.88 ± 1.60</td>
<td>7.16 ± 1.57</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>2.11 ± 0.56</td>
<td>3.54 ± 1.73</td>
<td>5.61 ± 4.34</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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$^a$ Group 1 = patients without congestive heart failure (CHF); Group 2 = patients with mild CHF; Group 3 = patients with moderate-to-severe CHF. NS = not significant; BMI = body mass index; HR = heart rate; SBP = systolic blood pressure; DBP = diastolic blood pressure; LVEDP = left ventricular end-diastolic pressure; TNF-α = tumor necrosis factor-alpha; IL = interleukin.
and 72% [95% CI, 56%-88%] at 24 months (1 death). To determine whether the plasma IL-6 and TNF-α levels were independent prognostic factors, variables were entered into a Cox proportional analysis. The univariate analyses of related factors of cardiac death including clinical variables, hemodynamic parameters and plasma levels of TNF-α and IL-6 are listed in Table 2. The analyses showed that NYHA FC above II, plasma TNF-α level > 8.1 pg/mL, plasma IL-6 level > 3.2 pg/mL, SBP < 110 mmHg, HR > 100 bpm and LVEDP > 24 mmHg were significant predictors for mortality. In the multivariate prognosis analyses listed in Table 3, only the NYHA FC (RR = 27.06, 95% CI, 2.44-300.68, p = 0.007) and IL-6 level (RR = 7.72, 95% CI, 1.36-43.67, p = 0.021) were identified as significant independent predictors of cardiac death in CHF patients.

### DISCUSSION

In the present study, plasma levels of TNF-α and IL-6 elevated along with the deterioration of the functional status of the heart, and predicted the prognosis in patients with severe CHF. Mann et al.\(^{18}\) suggested that the short-term expression of stress-activated cytokines (TNF-α, IL-1 and IL-6) within the heart may be an adaptive response to stress, whereas long-term expression of these molecules may be frankly maladaptive by producing cardiac decompensation. Sharma et al.\(^{19}\) reported that adult patients with congenital heart disease had elevated levels of bacterial endotoxin and TNF-α and IL-6, which relate to functional status. They found that plasma TNF-α levels were significantly higher in patients with moderate-to-severe symptoms (NYHA FC III/IV) compared with patients who were asymptomatic or had mild symptoms (NYHA FC I/II). The activities of TNF-α and IL-6 are modulated by corresponding soluble cytokine receptors and cytokines with anti-inflammatory activities, such as transforming growth factor β-1 and IL-10.\(^{20}\)

Analyzing the complexity of the cytokine network in CHF, Aukrust et al.\(^{21}\) concluded that profound disturbances in the levels of both inflammatory and anti-inflammatory mediators with a marked imbalance took part in the pathophysiology of CHF. Dibbs et al.\(^{22}\) re-
ported that there was a time-dependent increasing natural variability in the circulating levels of both TNF-α and IL-6, but more obviously for IL-6. They also suggested that plasma concentrations of soluble TNF receptors vary less than those of TNF-α and IL-6 and may therefore more closely relate to the patient’s clinical condition.

Munger et al. found that plasma levels of cytokines (TNF-α, IL-6 and IL-1-α) did not differ by heart failure etiology. In the present study, despite coming from different etiologies, plasma levels of both TNF-α and IL-6 were significantly elevated in patients with severe CHF, consistent with other studies. In the stepwise multivariate analysis, our data showed that higher plasma level of IL-6 is more valuable than plasma level of TNF-α to provide prognostic information in patients with CHF. Immunohistochemistry study in pacing-induced CHF of animal model showed that in the heart, TNF-α was located mainly in the vascular endothelium. Torre-Amione et al. reported that overexpression of TNF-α may be responsible for the progressive cardiac decompensation that occurs in advanced heart failure. They also found that approximately 50% of their patients with elevated levels of TNF-α in their sera did not have immunodetectable TNF-α in their hearts. Thus, they suggested there may be multiple sites of TNF-α production in advanced heart failure. IL-6 is a multifunctional pro-inflammatory and vasodepressor cytokine that mediates both immune and inflammatory responses. The increase of IL-6 reflected a compensatory response against various vasoconstrictions such as endothelin-1, angiotensin II and norepinephrine in patients with CHF. In mice model, prevalence and severity of myocarditis were markedly reduced in the absence of IL-6. Tsutamoto et al. demonstrated that IL-6 spillover in the peripheral circulation increases with the severity of CHF. High plasma levels of IL-6 can provide prognostic information in patients with CHF, independent of left ventricular ejection fraction and plasma norepinephrine, suggesting an important role for IL-6 in the pathophysiology of CHF. Sato et al. found that in patients with acute left heart decompensation in the absence of infection or coronary events, the changes of IL-6, likewise with CRP, are dynamic and distinct between both compensated and decompensated states in patients with CHF.

The present study demonstrated that circulating TNF-α and IL-6 levels correlated well with the severity of CHF, favoring inflammatory process plays an important role in the pathophysiology of CHF. Traditional anti-CHF medications, such as angiotensin-converting enzyme inhibitors, inotropic agents and angiotensin II type 1 receptor antagonists decreased the plasma levels of the pro-inflammatory cytokines effectively in patients with CHF. There is controversy in using agents with anticytokine properties as adjunctive therapy to modulate pro-inflammatory cytokine levels in patients with heart failure. Deswall et al. found that TNF-α antagonist not only decreases TNF-α bioactivity but also improves cardiac performance significantly in patients with NYHA FC III. However, Chung et al. reported using a chimeric monoclonal antibody of TNF-α to treat patients with moderate-to-severe heart failure did not improve clinical condition in these patients. Although the exact clinical significance of elevated levels of TNF-α and IL-6 in advanced heart failure is still uncertain, what is clear is that elevated levels of TNF-α and IL-6 can produce classical features of CHF. In the present study, we observed that in patients with severe CHF, they all had smaller BMI and lowered plasma triglyceride levels along with faster HR rate, lower BP, and more elevated LVEDP, which indicate that both metabolic and cardiovascular reserves deteriorate as heart failure progresses. The concept and mechanisms of heart failure evolved from a cardiorenal disorder to a cardiocirculatory dysfunction and also a neurohumoral disorder. The syndrome of cardiac cachexia is characterized by a severe catabolic/anabolic imbalance in favor of catabolic metabolism. In cachectic CHF patients, catabolic factors that act to increase protein and fat tissue degradation and stimulate energy production are increased from the effects of catecholamines, cortisol, and TNF-α, whereas anabolic factors appear to develop a resistance effect from growth hormone and insulin.

There are 2 limitations in this prospective case-control study. First, we did not clarify the underlying pathophysiology of how pro-inflammatory cytokines are produced in CHF. Second, we did not compare the values of plasma cytokines according to which various anti-heart failure regimens were administered to individual patient with CHF. Although the pathophysiologic role is well recognized, a clear explanation of this relationship is still needed.
remains unclear, the circulating plasma levels of TNF-α and IL-6 are 2 useful markers, more obvious for IL-6, that not only correlate well with the status of cardiac decompensation but also can predict late cardiac death in patients with advanced CHF.

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