Cytokines and Cytokine Receptors in Advanced Heart Failure
An Analysis of the Cytokine Database from the Vesnarinone Trial (VEST)

Anita Deswal, MD; Nancy J. Petersen, PhD; Arthur M. Feldman, MD, PhD; James B. Young, MD; Bill G. White, PhD; Douglas L. Mann, MD

**Background**—Previous reports have shown that elevated circulating levels of cytokines and/or cytokine receptors predict adverse outcomes in patients with heart failure. However, these studies were limited by small numbers of patients and/or they were performed in a single center. In addition, these studies did not have sufficient size to address the influence of age, race, sex, and cause of heart failure on the circulating levels of these inflammatory mediators in patients with heart failure.

**Methods and Results**—We analyzed circulating levels of cytokines (tumor necrosis factor [TNF] and interleukin-6) and their cognate receptors in 1200 consecutive patients who were enrolled in a multicenter clinical trial of patients with advanced heart failure. This analysis constitutes the largest analysis of cytokines and cytokine receptors to date. Analysis of the patients receiving placebo showed that increasing circulating levels of TNF, interleukin-6, and the soluble TNF receptors were associated with increased mortality. In men, there was a linear increase in circulating levels of TNF with advancing age. Women ≤50 years of age had relatively low levels of TNF, but TNF levels were disproportionately higher in women ≥50 years of age. No differences existed in cytokines and/or cytokine receptors in whites versus nonwhites, and circulating levels of cytokines and cytokine receptors were significantly greater in patients with ischemic heart disease.

**Conclusions**—Cytokines and cytokine receptors are independent predictors of mortality in patients with advanced heart failure. Moreover, circulating levels of cytokines are modified by age, sex, and cause of heart failure. (**Circulation. 2001;103:2055-2059.)**

**Key Words:** heart failure ★ cytokines ★ tumor necrosis factor

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Since the original observation that circulating levels of tumor necrosis factor (TNF) were elevated in patients with heart failure, there have been countless reports in which elevated levels of proinflammatory cytokines and/or their cytokine receptors have been observed in patients with heart failure.1 Thus far, prior studies have shown that circulating levels of TNF and interleukin-6 (IL-6) are elevated in direct relation to deteriorating functional class of heart failure.2-4 Moreover, previous reports have suggested that elevated levels of cytokines and cytokine receptors predict a worse clinical outcome in patients with heart failure.5-7,8 However, the clinical significance of these studies is uncertain for 2 reasons. (1) They were performed in small numbers of patients, and (2) many of the studies were performed in a single center, in which systematic referral bias is perhaps unavoidable. In addition to the above limitations, no previous study has had sufficient size to address a number of important issues relating to the elaboration of cytokines and cytokine receptors in heart failure, including the influence of age, sex, race, and cause of heart failure on cytokines and cytokine receptors.

To address the limitations of the above studies, we performed a systematic analysis of cytokines and their cognate cytokine receptors in a large-scale, multicenter, clinical trial in patients with advanced heart failure: the Vesnarinone trial (VEST).9 The present analysis, which was performed in 1200 consecutive patients with advanced heart failure, constitutes the largest analysis of cytokines and cytokine receptors that has been performed to date.

**Methods**

**Patient Population**
The patient population consisted of the first consecutive 1200 patients enrolled in VEST.9 The patient population was drawn from 189 different clinical sites in North America. A total of 31 patients were excluded from the final data analysis because of protocol violations (18 patients were in New York Heart Association [NYHA] class II at the time of enrollment, and 13 had inadequate baseline...
ences were said to exist at
hazards model (SAS for Windows, version 6.12). Significant differ-
and cytokine receptor levels was examined using a Cox proportional-
using the log-rank test. The prognostic value of increased cytokine
the Kaplan-Meier method, and survival among groups was compared
for differences in baseline characteristics and to evaluate
using Pearson’s product-moment correlations. ANCOVA was used
be compared with results from other studies, the cytokine data

blood samples). Thus, the final patient cohort consisted of 1169
patients.

Circulating Levels of Cytokines and
Cytokine Receptors
Circulating levels of TNF, IL-6, soluble TNF receptor 1 (sTNFR1),
soluble TNF receptor 2 (sTNFR2), and soluble IL-6 receptor
(sIL-6R) were measured at baseline before randomization into
VEST. If the patient had a recent infection, the cytokine and cytokine
receptors were drawn 2 weeks after the resolution of the most recent
infection. All patients were on stable doses of ACE inhibitors,
diuretics, digoxin, and/or vasodilators for 30 days before obtaining
baseline measurements. Circulating levels of cytokines and cytokine
receptors were measured using an ELISA (R&D Systems) that
measures “total” TNF and IL-6 (ie, free [unbound] cytokine and
cytokine bound to receptors; see Data Supplement for details). After
completing the cytokine analysis, the relevant demographic and
clinical data corresponding to these samples were obtained from the
VEST Data Coordinating Center at the University of Wisconsin
in Madison, Wisconsin.

Statistical Analysis
All data are presented as mean±SEM. Because the cytokine data
were not normally distributed, data were subjected to logarithmic
transformation before all statistical analyses. However, to permit
comparison with results from other studies, the cytokine data are
presented as the mean±SEM of the untransformed data. Student’s t
was used to test for differences in continuous variables, and the
χ² test was used for categorical variables. Tests for differences in
cytokine levels by race were performed using ANOVA. Correlations
of cytokine levels with demographic characteristics were performed
using Pearson’s product-moment correlations. ANCOVA was used
to correct for differences in baseline characteristics and to evaluate
differences in levels of cytokines and receptors between certain
subgroups. Survival curves for cytokine groups were calculated by
the Kaplan-Meier method, and survival among groups was compared
using the log-rank test. The prognostic value of increased cytokine
cytokine and cytokine receptor levels was examined using a Cox proportional-
hazards model (SAS for Windows, version 6.12). Significant differ-
ences were said to exist at P<0.05 for all parameters except for
correlations, which were considered to be significant at P<0.01 due
to multiple comparisons.

Results
Baseline Characteristics of the Study Population
As shown in Table 1 the age, left ventricular ejection fraction,
serum sodium, body weight, and Minnesota living with heart
failure scores were significantly different in the patients with
NYHA class III and IV heart failure, reflecting the greater
degree of disease severity in the patients with class IV heart

Table 1. Patient Demographics

<table>
<thead>
<tr>
<th></th>
<th>NYHA III (n=1036)</th>
<th>NYHA IV (n=133)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>61.6±0.4</td>
<td>66.1±1.0</td>
<td>0.0001</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>77.3</td>
<td>79.0</td>
<td>0.74</td>
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<tr>
<td>Ischemic cause of heart failure, %</td>
<td>58.5</td>
<td>66.9</td>
<td>0.07</td>
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<tr>
<td>Weight, kg</td>
<td>82.8±0.6</td>
<td>78.1±1.6</td>
<td>0.01</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>20.8±0.2</td>
<td>19.1±0.5</td>
<td>0.0009</td>
</tr>
<tr>
<td>Serum sodium, mEq/L</td>
<td>138.6±0.1</td>
<td>137.4±0.4</td>
<td>0.002</td>
</tr>
<tr>
<td>MLWHF</td>
<td>50.9±0.8</td>
<td>64.9±1.6</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Values are mean±SEM. MLWHF indicates Minnesota Living With Heart
Failure score for quality of life assessment. Higher scores indicate a poorer
quality of life.

Figure 1. Circulating levels of TNF and IL-6. Circulating levels
of TNF and IL-6 were grouped into 2 to 3 pg/mL increments for
patients with NYHA class III and IV heart failure, and degree of
overlap of circulating cytokine levels was examined for each
NYHA class. Compared with values corresponding to upper limit
of normal in our laboratory (>2 SDs beyond mean for age-
matched subjects free of cardiovascular disease), ∼80% of
patients with NYHA class III and IV heart failure had elevated
levels of TNF (upper limit of normal, 3.6 pg/mL), whereas ∼30% of
patients with NYHA class III and IV heart failure had elevated
levels of IL-6 (upper limit of normal, 5.7 pg/mL).

Circulating Levels of Cytokines and Cytokine
Receptors in Heart Failure
The baseline levels of sTNFR1, sTNFR2, and IL-6 were
significantly greater in the patients with class IV heart failure
when compared with class III heart failure patients, but no
significant differences existed in the plasma TNF levels in
patients with NYHA class III and IV heart failure (Data
Supplement). Figure 1 illustrates the distribution of circulat-
ing TNF and IL-6 levels as a function of NYHA functional
class. The important finding illustrated by these figures is that
there was a great deal of overlap of both TNF and IL-6 levels in
patients with NYHA class III and IV heart failure, suggesting that
the elaboration of proinflammatory cytokines
is not strictly confined to patients with end-stage heart failure.

Relationship Between Cytokines, Cytokine
Receptors, and Patient Characteristics
As shown in Table 2, a significant positive correlation existed
between patient age and circulating levels of TNF, IL-6,
sTNFR1, and sTNFR2. Table 3 compares and contrasts the
circulating levels of TNF and IL-6 in men and women.
Although the mean TNF level was not significantly different
in men and women, a small but significant increase existed in
the mean level of IL-6 in men. To determine whether there

Table 2. Correlation of Age With Circulating Levels of
Cytokines and Cytokine Receptor Levels

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>TNF</td>
<td>0.22</td>
<td>0.0001</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.15</td>
<td>0.0001</td>
</tr>
<tr>
<td>sTNFR1</td>
<td>0.38</td>
<td>0.0001</td>
</tr>
<tr>
<td>sTNFR2</td>
<td>0.38</td>
<td>0.0001</td>
</tr>
<tr>
<td>sIL-6R</td>
<td>0.07</td>
<td>0.02</td>
</tr>
</tbody>
</table>
was an interaction between age and sex, we examined the relationship between age and circulating levels of TNF in men and women. The salient finding shown by Figure 2 is that TNF levels increased linearly with age in men, whereas women had a dichotomous relationship: TNF levels remained relatively flat in women until ~50 years of age, and after 50 years of age, TNF levels sharply increased. Moreover, the levels of TNF in women after age 50 were relatively greater than the levels observed in men up until 70 years of age, at which time the circulating levels of TNF became similar in the 2 groups. This significant interaction \( P < 0.05 \) between patient sex and age was not observed for IL-6, sTNFR1, or sIL-6R (data not shown).

Table 3 shows that the levels of TNF and IL-6 were significantly increased in patients with ischemic heart disease. Similarly, levels of sTNFR1, sTNFR2, and sIL-6R were also significantly higher in patients with ischemic heart disease when compared with those in patients with dilated cardiomyopathy (data not shown). These differences persisted even after correcting for baseline characteristics including age, sex, NYHA class of heart failure, ejection fraction, and serum sodium. We also examined circulating levels of TNF and IL-6 as a function of race (white, black, and Hispanic), smoking status, and history of alcohol consumption. Table 3 shows that no significant difference existed in either TNF or IL-6 levels for any of these subgroups of patients.

**Relationship Between Cytokines, Cytokine Receptors, and Mortality**

To determine whether circulating levels of cytokines and/or cytokine receptors predicted adverse outcomes, we examined the relationship between cytokines and/or cytokine receptors and patient mortality in the group of patients who received placebo (NYHA class III, 352 patients; NYHA class IV, 32 patients). The baseline clinical characteristics of the placebo group were similar to the patients who received active therapy (data not shown). At the end of the follow-up period (mean duration, 55 weeks; maximum duration, 78 weeks), 65 patients (16.9%) died and 319 patients (83.1%) were alive. As shown in Table 4, the baseline levels of TNF, IL-6, sTNFR1, and sTNFR2 were significantly higher in the group of patients who died during follow-up, whereas the circulating levels of sIL-6R were not significantly different in survivors and nonsurvivors.

We next asked whether mortality increased as a function of increasing levels of cytokines and/or cytokine receptors. Figure 3A shows that a significant overall difference existed in survival as a function of increasing TNF levels \( P < 0.007 \) by log-rank test), with the worst survival in patients who had TNF levels >75th percentile. Similar findings were observed with respect to the Kaplan-Meier analysis of circulating levels of IL-6 and sTNFR1, which showed that a significant overall difference existed in survival as a function of increasing levels of IL-6 (Figure 3B; \( P = 0.007 \)) and sTNFR1 (Figure 3C; \( P = 0.0001 \)), with the worst survival observed for circulating levels of IL-6 and sTNFR1 that were >75th percentile. As shown in Figure 3D, survival also decreased with increasing levels of sTNFR2 (Figure 3D; \( P = 0.0001 \)), with the worst survival for circulating levels of sTNFR2 >50th percentile. A univariate Cox analysis showed that TNF \( P = 0.01 \), IL-6 \( P = 0.003 \), sTNFR1 \( P = 0.001 \), and sTNFR2 \( P = 0.0001 \) were significant univariate predictors of mortality.

We then entered each cytokine and/or cytokine receptor separately into a multivariate Cox proportional hazards model that included age, sex, cause of heart failure, NYHA class,
not significantly different in the survivors and nonsurvivors. In contrast, circulating levels of sIL-6R levels were and sTNFR2 receptors: indeed, circulating levels of TNF, IL-6, sTNFR1, and sTNFR2 (Figure 3) showed that patient survival decreased as a function of increasing levels of cytokines and cytokine receptors: indeed, circulating levels of TNF, IL-6, sTNFR1, and sTNFR2 >75th percentile were associated with the worst survival. In contrast, circulating levels of sIL-6R levels were not significantly different in the survivors and nonsurvivors.

Discussion

The results of the present study, in which we examined the levels of cytokines and cytokine receptors in a well-characterized database obtained from a large multicenter clinical trial, provide answers to several of the important questions that were raised at the outset of this study. With respect to the first question of whether circulating levels of cytokines and/or cytokine receptors predict adverse outcomes in patients with heart failure, the data (placebo group) suggest that circulating levels of TNF, IL-6, sTNFR1, and sTNFR2 are independent predictors of increased mortality in patients with advanced heart failure. This statement is supported by the following information.

First, the baseline levels of TNF, IL-6, sTNFR1, and sTNFR2 were significantly higher in the nonsurvivors than in the survivors (Table 4). Interestingly, the circulating levels of TNF and IL-6 were not different in the patients who died suddenly compared with those patients who died from pump failure (Data Supplement). Second, Kaplan-Meier analyses (Figure 3) showed that patient survival decreased as a function of increasing levels of cytokines and/or cytokine receptors: indeed, circulating levels of TNF, IL-6, sTNFR1, and sTNFR2 >75th percentile were associated with the worst survival. In contrast, circulating levels of sIL-6R levels were not significantly different in the survivors and nonsurvivors.

Third, a multivariate Cox proportional hazards model showed that circulating levels of TNF, IL-6, sTNFR1 and sTNFR2 were significant independent predictors of mortality. Thus, these findings are consistent with several previous studies.2,6,8 Interestingly, circulating levels of TNFR2 were the best independent overall predictor of mortality when cytokines and cytokine receptors were simultaneously entered into the multivariate model. This latter finding is consistent with a smaller study by Ferrari et al,5 but differs from that of Rauchhaus et al,8 who reported that TNF1 was the single best predictor of mortality in patients with heart failure. Although the reasons for this discrepancy are unknown, they may relate to differences in patient demographics between the studies (eg, tertiary care referral center versus multicenter clinical trial population). Given that circulating soluble TNF receptors are thought to be biologically inert,10 it is not clear whether circulating levels of sTNFR2 represent an phenomenon that is associated with, but not causally related to, worsening disease severity. In this regard, it is interesting to note that circulating levels of sTNFR2 are thought to reflect increased activity of TNF-α-converting enzyme,11 a membrane-bound enzyme that cleaves both TNF and TNFR2 from cell surface membranes. Importantly, TNF-α-converting enzyme levels correlate with the degree of LV systolic dysfunction in patients with dilated cardiomyopathy.12 Thus, circulating levels of sTNFR2 may be a “surrogate marker” for worsening LV function and/or LV remodeling and may thus predict worsening outcomes. Alternatively, it is possible that circulating levels of sTNFR2 are causally linked to adverse outcomes in heart failure, insofar as sTNFR2 is thought to act as a “carrier protein” that is capable of binding to TNF and then slowly releasing this potentially toxic cytokine into the circulation.13 However, it should be noted that because patients enrolled in this study had advanced heart failure, the prognostic significance of circulating cytokines or their receptors in mild heart failure cannot be assessed from this database.

With respect to the second set of questions regarding the influence of age, sex, race, and cause of heart failure on cytokines and cytokine receptors, the aggregate data provide several new and important insights that are not available from smaller data sets. For example, we observed that there was an important interaction between age and sex in patients with heart failure. Although we observed that there was an overall increase in the level of circulating cytokines and cytokine receptors with advancing age, as has been reported in aging subjects who are free of cardiovascular disease,14 the age-related changes that were observed were different in men and women. Although TNF levels increased linearly with age in men with heart failure, TNF levels remained relatively flat in women until ≈50 years of age, after which TNF levels in women abruptly increased. Although the mechanism(s) for this finding is not known, it is tempting to speculate that increased levels of estrogen in the women ≈50 years of age (presumably premenopausal) may have suppressed circulating levels of TNF. Indeed, several studies have demonstrated that estrogens inhibit TNF production in a variety of different cell types, including monocytes and osteoblasts.15 Nonethe-
less, these studies should be regarded as provisional given the relatively small number of female patients in each age group.

A second finding that was not anticipated from the clinical literature was that circulating levels of cytokines and cytokine receptors were consistently higher in patients with ischemic cardiomyopathy than in patients with dilated cardiomyopathy (Table 3). Importantly, these differences persisted even after correcting for baseline characteristics between the 2 groups, including, age, sex, NYHA class, ejection fraction, and serum sodium. Because myocardial ischemia is a known trigger for myocardial biosynthesis of TNF and IL-6, these observations raise the interesting possibility that episodic bouts of myocardial ischemia may contribute to the total cytokine burden in heart failure and thus contribute to disease progression and worsening outcomes in patients with ischemic cardiomyopathy. Alternatively, the higher levels of cytokines may be reflective of the generalized inflammatory process that has been observed in atherosclerotic heart disease.

Given the recent interest in differences in heart failure outcomes on the basis of race, we were also interested in determining whether there were potential differences in cytokine and/or cytokine receptor levels in different racial and ethnic groups in VEST. There were no obvious differences in the levels of cytokines (Table 3) and/or cytokine receptors between whites, blacks, and Hispanics. However, the data on cytokine levels in Hispanics should be regarded as provisional, because levels were obtained in only a limited number of Hispanic patients. Insofar as there seem to be differences in the frequency of TNF polymorphisms and cytokine responses in blacks and whites, this question remains an important area of discovery for the future.

Conclusions

The current analysis of cytokines and cytokine receptors in this large multicenter clinical trial bears a striking resemblance to prior analyses of neurohormonal markers, which have also been shown to be independent predictors of increased mortality in patients with heart failure. Although elevated levels of circulating neurohormones were once considered epiphenomena that were not related to disease progression in heart failure, the results of numerous clinical trials, wherein neurohormonal systems have been systematically antagonized, suggest that circulating neurohormones may contribute to disease progression in heart failure by virtue of their toxic effects on the heart and the peripheral circulation. Thus, the results of the present study suggest that, analogous to the classical neurohormones, cytokines may also represent an important therapeutic target in patients with heart failure. Indeed, this question is currently being addressed in ongoing multicenter clinical trials.

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