Inflammatory Markers and Risk of Heart Failure in Elderly Subjects Without Prior Myocardial Infarction

The Framingham Heart Study

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Methods and Results

—We investigated the relations of serum interleukin-6 (IL-6), C-reactive protein (CRP), and peripheral blood mononuclear cell (PBMC) production of tumor necrosis factor-α (TNFα) to CHF incidence among 732 elderly Framingham Study subjects (mean age 78 years, 67% women) free of prior myocardial infarction and CHF. On follow-up (mean 5.2 years), 56 subjects (35 women) developed CHF. After adjustment for established risk factors, including the occurrence of myocardial infarction on follow-up, there was a 60 (PBMC TNFα) to 68% (serum IL-6) increase in the occurrence of CHF per tertile increment in cytokine concentration (P=0.04, and 0.03, respectively, for trend). A serum CRP level ≥5 mg/dL was associated with a 2.8-fold increased risk of CHF (P=0.02).

Conclusions

—In our elderly, community-based sample, a single determination of serum inflammatory markers, particularly elevated IL-6, was associated with increased risk of CHF in people without prior myocardial infarction. Additional epidemiological investigations are warranted to confirm the contribution of inflammation to the pathogenesis of CHF in the general population. (Circulation. 2003;107:1486-1491.)

Key Words: cytokines • interleukin-6 • tumor necrosis factor-α • C-reactive protein • heart failure

Several clinical studies have suggested that serum levels of tumor necrosis factor-α (TNFα), interleukin-6 (IL-6), and C-reactive protein (CRP) are elevated in patients with congestive heart failure (CHF) regardless of the etiology of the condition.1–5 Furthermore, elevated blood levels of these inflammatory markers correlate with worsening functional class, increased hospitalization rates, and poorer survival.6–9

Experimental studies indicate that proinflammatory cytokines (TNFα and IL-6) are associated with certain molecular, clinical, and physiological aspects of heart failure, including but not limited to progressive left ventricular (LV) dysfunction, pulmonary edema, LV remodeling, fetal gene expression, myocyte hypertrophy, and myocyte apoptosis.10 Experimental evidence suggests that myocardial infiltration by peripheral blood mononuclear cells (PBMC) contributes to LV remodeling.11,12 PBMC production capacity of cytokines is enhanced in CHF,13,14 and may be related to an increased expression of genes for the TNFα superfamily.15

Thus, it is currently believed that proinflammatory cytokines may represent a class of biological mediators that are activated in CHF, akin to but distinct from neurohormones and the natriuretic peptide pathways. It is unclear whether elevated levels of inflammatory markers antedate the development of CHF. We hypothesized that individuals with increased serum levels of proinflammatory cytokines and/or elevated PBMC production of TNFα would be at

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increased risk of developing CHF. Accordingly, we examined prospectively the relations of serum IL-6, CRP, and PBMC production of TNFα obtained at a routine examination to the incidence of CHF on follow-up in a community-based sample.

Methods
Study Sample
The design and selection criteria of the Framingham Heart Study have been described previously.16 Participants who attended the 22nd biennial examination cycle (1992 to 1994) were eligible for the present investigation because blood specimens for cytokine analyses were obtained at this examination. Of 1167 subjects alive at the time of the baseline examination, we excluded 435 individuals (37.3%) for the following reasons: off-site examination (n = 226), prevalent CHF (n = 56) or myocardial infarction (MI, n = 79), and insufficient blood specimen for cytokine analyses (n = 74). Subjects with an MI were excluded because plasma cytokines may be elevated for a variable duration after myocardial injury. Further, prior MI is a powerful trigger for cytokine activation, progressive LV remodeling, and CHF.11,18 After exclusions, 732 participants remained eligible.

At the baseline examination, participants underwent a medical history, a physical examination, and assessment of cardiovascular disease risk factors.

Measurement of Cytokines and CRP
At the baseline examination, attendees underwent phlebotomy in the early afternoon. Specimens were immediately transported via courier from Framingham to the USDA Human Nutrition Research Center on Aging in Boston, where PBMC were isolated and cultured, and serum stored at −80°C.19 Serum IL-6 was measured with an ELISA (R&D Systems). CRP was measured with an immunoprecipitation assay (IncStar).

Although PBMC production of several cytokines was evaluated,21 we specified TNFα as the PBMC cytokine of interest a priori for the present investigation. Briefly, PBMC were separated by Ficoll-Hypaque centrifugation, washed, and cultured for 24 hours in 96-well flat-bottom plates with ultrafiltered,20 pyrogen-free RPMI 1640 medium (Sigma) that was supplemented with 100-μg/mL streptomycin and 100-U/mL penicillin, with 1% autologous heat-inactivated pooled serum and 1% l-glutamine. After incubation, plates were then frozen at −80°C until assay. The average time from phlebotomy to isolation of PBMC was 75 minutes (range 35 to 120 minutes). Total PBMC production of TNFα was measured in unstimulated cells (spontaneous production) as well as after stimulation with 1 or 100 ng/mL of lipopolysaccharide (E. coli LPS, Sigma), as previously described.19 The interassay variability was <10%, and the intra-assay variability was <5% for all three inflammatory markers.19

Outcome
All study subjects were under continuous surveillance for the development of cardiovascular disease events. A panel of three investigators reviewed all suspected cardiovascular disease events by examining hospital records, information from physicians, and pathology reports as described previously.21 In the present investigation, the primary outcome of interest was the first occurrence of CHF as defined by the Framingham Heart Study criteria.22 These criteria require the simultaneous presence of at least two major criteria, or one major criterion in conjunction with two minor criteria, to establish a diagnosis of CHF, and have been validated previously.23

Statistical Methods
We used separate multivariable Cox proportional hazards regression models24 to examine the association of each inflammatory marker with the incidence of CHF. Because there was no effect modification by gender, sex-pooled analyses were performed. The inflammatory markers were analyzed both as continuous (natural logarithmic-transformed IL-6 and TNFα) and as categorical variables (tertiles for IL-6 and TNFα). In the case of CRP, we chose 5 mg/L as a cut point for dichotomizing subjects a priori because 68% of subjects had undetectable levels (because of a low-sensitivity assay), and this threshold has been reported to be of prognostic significance.25,26

We adjusted for the following baseline covariates21: age, sex, ratio of serum total cholesterol to HDL cholesterol, diabetes, systolic and diastolic blood pressure, antihypertensive treatment, smoking status, body mass index (BMI), valve disease, atrial fibrillation, prevalent cardiovascular disease (other than MI), and electrocardiographic LV hypertrophy.16 We examined the following multivariable models: model A, all covariates defined at baseline and ignoring the occurrence of MI on follow-up; model B, adjusting for covariates at baseline, and additionally for the occurrence of an MI on follow-up as a time-dependent covariate. The latter adjustment is important because elevated plasma cytokines can predispose to CHF by promoting MI.27,28 Models using serum IL-6 and PBMC TNFα categories examined trends across the tertiles and compared risk of CHF in each of the two upper tertiles with the first tertile serving as a referent.

Ancillary Analyses
We examined regression models with stepwise backward elimination of the 3 inflammatory markers to investigate which of the markers remained in a model that incorporated established CHF risk factors. We also evaluated the prognostic significance of combinations of the 3 biomarkers by comparing CHF risk in individuals with “elevated” levels of all 3 markers (empirically defined as values above the median for serum IL-6 and PBMC TNFα, and ≥5 mg/dL for serum CRP) with that in the rest of the subjects.

We performed analyses incorporating several interaction terms (inflammatory markers x covariate in Model B) to examine for any variation in the effect of inflammatory markers on CHF risk (effect modification) according to age, sex, systolic blood pressure, and BMI. We performed supplementary analyses in which we modeled death as a competing outcome to CHF, given the elderly study sample. For primary analyses, we defined a priori spontaneous production of TNFα by PBMC as the variable of interest. However, we performed supplementary analyses in which levels of TNFα produced by stimulated PBMC (with 1 and 100 ng/mL of lipopolysaccharide) were related to the risk of CHF. A two-sided P value of 0.05 was considered significant.

Results
Clinical Characteristics
In our elderly sample, about 70% of subjects were hypertensive (Table 1). Serum IL-6 levels were modestly related to CRP levels in both sexes and to spontaneous PBMC production of TNFα in women (Table 2). CRP was not related to TNFα in either sex. Nine percent of the subjects had CRP levels ≥5 mg/dL.

Inflammatory Markers and CHF Risk
On follow-up (mean 5.2 years), 56 participants (35 women) developed CHF. The cumulative incidence of CHF was greater with increasing levels of the inflammatory markers (Figure 1, Panels A-C). Table 3 displays the results of multivariable analyses examining the relations of serum IL-6 and PBMC spontaneous production of TNFα to the risk of CHF. There was a 36% (IL-6) to 46% (TNFα) increase in CHF risk for every standard deviation increment in log cytokine concentration (Table 3, model B1). Risk of CHF increased about 1.6-fold per tertile increment in cytokine level (Table 3, model B2). Participants with serum CRP levels ≥5 mg/dL experienced an increased risk of CHF (model A: hazards ratio 2.18 [95%
CI 1.00 to 4.79, \( P=0.05 \); model B: 2.81 [95\% CI 1.22 to 6.50, \( P=0.02 \)].

**Additional Analyses**

In stepwise backward elimination models, only IL-6 remained in the models after adjustment for the established CHF risk factors; the other two inflammatory markers did not.

In our sample, 11.5\% of women and 7\% of men had elevated levels of all three markers (elevation defined as above the median for serum IL-6 [3.52 pg/mL] and PBMC spontaneous production of TNF-\( \alpha \) [3.80 ng/mL] and CRP \( \geq 5 \) mg/dL). However, this group accounted for 21\% of all CHF cases. Individuals with elevated levels of all 3 markers had a markedly increased risk of CHF (model A: hazards ratio 3.00 [95\% CI 1.13 to 7.95, \( P=0.03 \)]; model B: 4.07 [95\% CI 1.34 to 12.37, \( P=0.01 \)]).

**TABLE 1. Characteristics of Study Subjects**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men (n=244)</th>
<th>Women (n=488)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>78.0±4.3</td>
<td>78.6±4.6</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>27.0±3.9</td>
<td>26.7±5.1</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>144±21</td>
<td>143±20</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>74±11</td>
<td>72±11</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>68.4</td>
<td>73.6</td>
</tr>
<tr>
<td>Hypertension treatment, %</td>
<td>42.9</td>
<td>50.0</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>194±31</td>
<td>214±36</td>
</tr>
<tr>
<td>HDL, mg/dL</td>
<td>42.8±14.0</td>
<td>54.0±16.0</td>
</tr>
<tr>
<td>Total cholesterol/HDL</td>
<td>4.9±1.5</td>
<td>4.3±1.4</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>16.8</td>
<td>8.6</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>6.6</td>
<td>9.8</td>
</tr>
<tr>
<td>Clinical valve disease, %*</td>
<td>7.8</td>
<td>5.3</td>
</tr>
<tr>
<td>ECG left ventricular hypertrophy, %†</td>
<td>3.4</td>
<td>3.0</td>
</tr>
<tr>
<td>Prevalent atrial fibrillation, %</td>
<td>10.3</td>
<td>5.1</td>
</tr>
<tr>
<td>Prevalent cardiovascular disease, %‡</td>
<td>28.3</td>
<td>22.1</td>
</tr>
<tr>
<td>Myocardial infarction on follow-up, %</td>
<td>8.6</td>
<td>4.3</td>
</tr>
</tbody>
</table>

*Values are expressed as mean±SD or percentages.

*Systolic murmur of grade 3 or more (out of 6) or any diastolic murmur.
†Voltage criteria plus repolarization abnormalities.
‡Does not include recognized myocardial infarction, but does include angina and cerebrovascular or peripheral vascular disease. Hypertension was defined as a systolic BP \( \geq 140 \) mm Hg, a diastolic BP \( \geq 90 \) mm Hg, or use of antihypertensive agents.

**TABLE 2. Mean Values and Spearman Rank Correlations Among Inflammatory Markers**

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP, mg/L</td>
<td>1.60</td>
<td>3.93</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>6.69</td>
<td>15.90</td>
</tr>
<tr>
<td>TNF-( \alpha ), ng/mL</td>
<td>4.68</td>
<td>3.66</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>2.66</td>
<td>8.47</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>7.68</td>
<td>17.22</td>
</tr>
<tr>
<td>TNF-( \alpha ), ng/mL</td>
<td>5.33</td>
<td>4.34</td>
</tr>
</tbody>
</table>

Values of CRP and IL-6 indicate serum levels, whereas TNF-\( \alpha \) levels refer to spontaneous production by PBMC.

*Spearman rank correlations.
†\( P<0.001 \).

Panel A, Kaplan-Meier plots showing the crude cumulative incidence of CHF in subjects according to tertiles of serum IL-6. Panel B, spontaneous production of TNF-\( \alpha \) by PBMC, and Panel C, for individuals with serum CRP values above and below 5 mg/dL. T1, T2, and T3 represent the tertiles of the marker (T3 being the top tertile). No. of CHF cases/No. at risk for each tertile: Serum IL-6 (pg/mL): T1 =7/220 (3.2\%); T2 =11/167 (6.6\%); T3 =24/165 (14.5\%). PBMC spontaneous production of TNF-\( \alpha \) (ng/mL): T1 =19/217 (4.1\%); T2 =18/220 (8.2\%); T3 =19/119 (9.5\%). For serum CRP: <5 mg/dL, 48/657 (7.3\%); \( \geq 5 \) mg/dL, 8/66 (12.1\%).
TABLE 3. Serum IL-6, Spontaneous Production of TNFα by PBMC, and Risk of CHF

<table>
<thead>
<tr>
<th>Model</th>
<th>Serum IL-6</th>
<th>Spontaneous Production of TNFα by PBMC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>A: Multivariable models adjusting for covariates at baseline*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1: Log marker as continuous variable</td>
<td>1.36 (1.09 to 1.71)</td>
<td>0.01</td>
</tr>
<tr>
<td>A2: Tertiles of marker†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1 Referent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>1.62 (0.57 to 4.56)</td>
<td>0.37</td>
</tr>
<tr>
<td>T3</td>
<td>3.07 (1.26 to 7.47)</td>
<td>0.01</td>
</tr>
<tr>
<td>Trend across tertiles</td>
<td>1.77 (1.15 to 2.74)</td>
<td>0.01</td>
</tr>
<tr>
<td>B: Models adjusting for covariates at baseline* and for myocardial infarction on follow-up (time-dependent covariates)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1: Log marker as continuous variable</td>
<td>1.36 (1.06 to 1.74)</td>
<td>0.02</td>
</tr>
<tr>
<td>B2: Tertiles of marker†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1 Referent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>1.77 (0.59 to 5.33)</td>
<td>0.31</td>
</tr>
<tr>
<td>T3</td>
<td>2.85 (1.09 to 7.44)</td>
<td>0.03</td>
</tr>
<tr>
<td>Trend across tertiles</td>
<td>1.68 (1.05 to 2.67)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

HR indicates hazards ratio per 1 SD increment of marker. For log IL-6, 1 SD = 0.64; for log TNFα, 1 SD = 0.81.
*All models adjust for age, sex, diabetes, systolic blood pressure, hypertension treatment, smoking status, BMI, total cholesterol/HDL, valvular disease, prevalent atrial fibrillation, prevalent cardiovascular disease (other than myocardial infarction), and ECG-LVH.
†Marker tertiles: for serum IL-6 (pg/ml), T1 = <3.12, T2 = 3.13 to 5.27, and T3 = 5.28 to 275; for PBMC spontaneous production of TNFα (ng/ml): T1 = 0.04 to 2.60, T2 = 2.61 to 5.00, and T3 = 5.01 to 40.

Discussion

For many centuries, physicians have recognized that patients with CHF may have clinical features observed in chronic inflammatory conditions. More recently, there has been a resurgence of interest in the inflammatory origins of CHF because of the role of proinflammatory cytokines in mediating the cachexia of severe CHF. The role of inflammation in the pathogenesis of CHF has been strengthened further, with the implication of several cytokines (notably IL-6) in the disease process in experimental studies, and the demonstration that inflammatory markers are elevated in patients with milder degrees of CHF, including those with asymptomatic LV systolic dysfunction.

These observations raise the possibility that levels of inflammatory markers may be elevated early in the course of LV dysfunction and could predict occurrence of subsequent CHF. The present investigation examined this hypothesis prospectively. We chose to evaluate individuals without baseline MI because the demonstration of any effect in this group would support the hypothesis that inflammatory markers promote LV remodeling and CHF even in the absence of clinically apparent preexisting myocardial injury. We chose three markers: serum IL-6, produced by diverse cells and a correlate of systemic inflammation; spontaneous production of TNFα by PBMC, indicative of in vivo responses of a population of cells that infiltrate the myocardium during LV remodeling; and serum CRP, an acute phase reactant produced by the hepatocytes.

Principal Findings

Serum IL-6 was associated with an increased risk of CHF in a continuous fashion without evidence of a threshold. Subjects with elevated CRP levels also had increased risk of CHF. The association of inflammatory markers with CHF risk was maintained on adjusting for the occurrence of an MI on follow-up. An association of increased spontaneous PBMC production of TNFα and CHF risk was also noted. Stepwise regression models identified serum IL-6 as the best predictor of CHF risk of the three markers evaluated. It is important to point out that the present investigation does not provide an adequate comparison of the relative predictive powers of IL-6 versus CRP because we used a low-sensitivity assay for the latter.

Mechanisms

The observed association between inflammatory markers and CHF risk should not necessarily be interpreted as indicating a
role for IL-6 and TNFα in the pathogenesis of heart failure. It is possible that elevated serum cytokine levels are simply indicative of a generalized inflammatory state antedating CHF. Elevation of markers may be a reaction to the pathophysiological processes driving progressive ventricular remodeling.

It is also possible, however, that the association between inflammatory markers and CHF is a causal one. The strength of the association (about 60% increased risk per tertile increment), the presence of a stepwise rise in CHF risk across increasing tertiles of the markers, the demonstration of a temporal sequence, and the consistency of results in multiple analyses and across markers all support this notion. Both IL-6 and TNFα can adversely affect myocyte contractility and can directly influence LV remodeling.33,34 An observation further confirmed by findings of LV dilatation and LV hypertrophy, respectively, in experiments on transgenic mice overexpressing TNFα35 or IL-6 and its receptor.36 The role of IL-6 in particular may be complex, as it has both proinflammatory and anti-inflammatory effects and can both promote myocyte hypertrophy as well as protect cardiomyocytes from apoptosis.37 Although elevated CRP has not been related directly to LV remodeling, it is likely that it reflects increased hepatic synthesis under the influence of elevated IL-6 levels. Conversely, CRP may promote LV remodeling by stimulating IL-6 production.39

Interestingly, although spontaneous production of TNFα by PBMC was related to CHF risk, levels obtained under stimulated conditions were not. It can be argued that unstimulated cells reflect in vivo conditions better, whereas ex-vivo assessment of stimulated cells represents their maximal response to stress.19

**Strengths and Limitations**
The strengths of our investigation include the community-based sample and the measurement of inflammatory markers blinded to clinical outcome. Nonetheless, it is important to acknowledge the limitations of our investigation. Echocardiographic evaluation was not performed at the baseline examination, and consequently we were unable to examine the relative contributions of subclinical LV dysfunction and inflammatory cytokines to CHF risk. The use of a low-sensitivity CRP assay and the exclusion of institutionalized individuals (who were likely sicker and more likely to have elevated cytokine levels) are additional limitations. Our study sample was almost exclusively white and elderly, reducing the generalizability of our results.

**Conclusions**
Our findings suggest that elevated inflammatory markers constitute important risk factors for CHF in elderly women and men. Although prior reports have emphasized the importance of inflammation in determining prognosis of people with established CHF, our data suggest that the contribution of inflammatory markers may antedate overt CHF. If confirmed, our observational data raises the possibility of using inflammatory markers to identify patients at high risk for CHF. They also suggest a possible role of anti-inflammatory therapy as an experimental means to reduce risk in patients at high risk for CHF.

**Acknowledgments**
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**References**
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