Interrelationships Among Circulating Interleukin-6, C-Reactive Protein, and Traditional Cardiovascular Risk Factors in Women

Edmund A. Bermudez, Nader Rifai, Julie Buring, JoAnn E. Manson, Paul M. Ridker

Objective—Interleukin-6 (IL-6) and C-reactive protein (CRP) are markers of systemic vascular inflammation that herald atherothrombosis and may have important interrelationships with traditional cardiovascular risk factors. Methods and Results—We conducted a cross-sectional analysis among 340 apparently healthy women enrolled in the Women’s Health Study. In unadjusted analyses, higher levels of IL-6 and CRP were seen with increasing body mass index (BMI), systolic and diastolic blood pressure, and smoking exposure. IL-6 levels were related to the frequency of alcohol intake (P<0.002) and showed an inverse relationship with exercise frequency and hormone replacement therapy (P<0.0001 for both). CRP levels increased with hormone replacement therapy (P=0.0002). Associations among IL-6, CRP, and lipid levels were minimal. Overall, mean levels of IL-6 and CRP increased with increasing numbers of clinical risk factors (P<0.0001). In multivariate analyses, independent relationships were seen between levels of IL-6 and age, BMI, smoking, systolic blood pressure, alcohol use, presence of diabetes, and frequency of exercise. CRP was associated with age, BMI, systolic blood pressure, high density lipoprotein, smoking, and hormone replacement therapy in adjusted analyses. Conclusions—Plasma levels of IL-6 and CRP are independently related to several clinical cardiovascular risk factors in women. (Arterioscler Thromb Vasc Biol. 2002;22:1668-1673.)

Key Words: interleukin-6 ■ C-reactive protein ■ risk factors ■ inflammation ■ atherosclerosis

A ccumulating evidence suggests that inflammatory processes, in part, mediate the development and progression of atherosclerosis. In this regard, several circulating proinflammatory molecules have been associated with thrombotic cardiovascular events. Included among these are acute-phase proteins, cellular adhesion molecules, selectins, and cytokines. The stimuli activating or inciting the thrombotic process are protean but may be associated with various clinical cardiovascular risk factors.

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The plasma cytokine interleukin-6 (IL-6) plays an important role in mediating inflammation and is a central stimulus for the acute-phase response. In particular, IL-6 induces the hepatic synthesis of C-reactive protein (CRP), a known proinflammatory marker of atherothrombotic vascular disease. Although circulating levels of IL-6 and CRP are therefore physiologically linked, it remains unclear whether these markers of systemic vascular inflammation track with one another in respect to various traditional risk factors in healthy individuals. However, IL-6 and CRP have independently demonstrated associations with cardiovascular events and mortality among apparently healthy men and women. The interplay between the inflammatory process, cardiovascular risk factors, and atherothrombosis is complex. Despite evolving theories regarding IL-6 and CRP in the central mechanisms of inflammation and atherogenesis, there exists a paucity of data coherently relating clinical cardiovascular risk factors with circulating levels of systemic vascular inflammatory markers in healthy individuals.

Given this, we sought to determine the relationship among circulating baseline IL-6, CRP, and clinical cardiovascular risk factors in a cohort of apparently healthy women. We further sought to determine any additive effects of these clinical risk factors on circulating concentrations of these inflammatory markers.

Methods

A cross-sectional analysis was performed in apparently healthy women enrolled in the prospective Women’s Health Study, a randomized primary prevention trial of aspirin and vitamin E, the details of which are described elsewhere. Detailed self-reported questionnaires containing information on the history of hypertension,
TABLE 1. Clinical Characteristics of Study Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>n=340</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>60.1±8.6</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.2±5.1</td>
</tr>
<tr>
<td>Systolic blood pressure ≥140 mm Hg, %</td>
<td>20.6</td>
</tr>
<tr>
<td>Diastolic blood pressure ≥90 mm Hg, %</td>
<td>10.3</td>
</tr>
<tr>
<td>Parental history of MI before age 60, %</td>
<td>15.8</td>
</tr>
<tr>
<td>History of hypercholesterolemia, %</td>
<td>34.1</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>4.4</td>
</tr>
<tr>
<td>Exercise frequency, %</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>44.1</td>
</tr>
<tr>
<td>&lt;1 time/week</td>
<td>21.2</td>
</tr>
<tr>
<td>≥1 time/week</td>
<td>34.7</td>
</tr>
<tr>
<td>Frequency of alcohol use, %</td>
<td></td>
</tr>
<tr>
<td>Rarely or never</td>
<td>45.6</td>
</tr>
<tr>
<td>1–3 drinks/month</td>
<td>13.9</td>
</tr>
<tr>
<td>1–6 drinks/week</td>
<td>31.5</td>
</tr>
<tr>
<td>1+ drinks/day</td>
<td>9.1</td>
</tr>
<tr>
<td>Smoking status, %</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>28.0</td>
</tr>
<tr>
<td>Former</td>
<td>28.6</td>
</tr>
<tr>
<td>Never</td>
<td>44.5</td>
</tr>
<tr>
<td>Current use of hormone replacement therapy, %</td>
<td>43.5</td>
</tr>
<tr>
<td>Total cholesterol, (mg/dL)</td>
<td>222.7±38.4</td>
</tr>
<tr>
<td>HDL, mg/dL</td>
<td>49.5±15.5</td>
</tr>
</tbody>
</table>

*Means±SD or percentages are shown. Not all percentages total 100 because of rounding.

Results

Baseline clinical characteristics of the study participants are shown in Table 1. Women with an average age of 60 years had a BMI of 26. Approximately 20% of the women had systolic hypertension (systolic blood pressure ≥140 mm Hg), 4% had diabetes, and 28% were current smokers. Mean levels for total and HDL cholesterol were 223 and 49 mg/dL, respectively.

Mean levels of IL-6 and CRP were significantly higher in women with several clinical cardiovascular risk factors. As shown in Table 2, compared with women without a history of hypertension, women with a history of hypertension had significantly higher levels of IL-6 and CRP. Similarly, IL-6 and CRP significantly increased with an increase in smoking exposure, and increasing IL-6 levels were associated with increasing exercise and the presence of diabetes. CRP levels were somewhat higher in women with a history of hypercholesterolemia. Last, the frequency of alcohol use was associated with a U-shaped relationship with plasma IL-6 levels (not shown).

IL-6 and CRP levels diverged in their association with HRT (Figure 1). IL-6 levels significantly increased among women who currently used, previously used, and never used HRT (P=0.01). In contrast, CRP levels significantly decreased among women who currently used, previously used, and never used HRT (P=0.0002).

Circulating levels of plasma inflammatory markers were analyzed in association with lipid parameters. Logarithmically normalized IL-6 and CRP minimally decreased with the HDL cholesterol level (parameter estimate −0.01, P<0.0001 for both analyses). IL-6 levels were not associated with total cholesterol levels (P=0.86), and CRP levels were only minimally associated with total cholesterol levels (P=0.03) in unadjusted analyses. However, the magnitude of these effects was quite small. Results were similar when examined among women not on HRT, with the exception that CRP was no longer associated with total cholesterol levels (P=0.21).

Circulating levels of IL-6 and CRP were evaluated in association with age and obesity. Levels of IL-6 linearly increased in association with age and BMI (P=0.0002 and P<0.0001, respectively). CRP, in a similar fashion, increased with increasing BMI (P<0.0001) but was not associated with age in univariate analyses (P=0.12).

Positive relationships were demonstrated between parameters of blood pressure, IL-6, and CRP levels (Figure 2). Systolic blood pressure was categorized into seven 10 mm Hg increments. Diastolic blood pressure was divided into the following categories: <65, 65 to 74, 75 to 84, 85 to 89, and ≥90 mm Hg. Increasing systolic blood pressure was positively associated with increasing levels of IL-6 and CRP (P<0.0001 and P=0.0004, respectively). Similarly, increasing diastolic blood pressure was associated with increasing IL-6 and CRP levels (P=0.005 for both). Results were virtually identical when analyses were performed among women without hypertension and not currently on HRT.

In an effort to examine the incremental relationships between these risk factors and vascular proinflammatory markers, concentrations of IL-6 and CRP were analyzed with increasing numbers of clinical factors (Figure 3). Circulating levels of IL-6 significantly increased with an increasing number of clinical cardiovascular risk factors (P<0.0001). In a similar fashion, CRP levels increased with an increasing number of clinical cardiovascular risk factors (P<0.0001).
Finally, to examine independent correlates of plasma inflammatory markers, adjusted analyses were performed by using linear regression models to account for the contributions of multiple cardiovascular risk factors (Table 3). Age and BMI remained positively associated with increasing IL-6 ($P<0.0001$ for both analyses). In addition, other independent relationships were seen between IL-6 and smoking status, systolic blood pressure, alcohol use, diabetes, and exercise frequency. Significantly higher plasma concentrations of IL-6 were found in women who currently smoked and in women with diabetes compared with women who never smoked and with nondiabetic women ($P=0.008$ and $P=0.02$, respectively). Lower levels of IL-6 were found in women who exercised or used alcohol at least weekly compared with women who never exercised or did not use alcohol ($P=0.008$ and $P=0.02$, respectively). These analyses were adjusted for all other covariates, including lipid levels. Among this cohort of women, the regression model accounted for 34% of the variance in logarithmically normalized IL-6, with BMI alone accounting for 15% of the variance in concentrations of IL-6.

Similarly, CRP was independently associated with several traditional cardiovascular risk factors in adjusted analyses. BMI, systolic blood pressure, and smoking remained positively associated with CRP ($P<0.0001$, $P=0.04$, and $P=0.01$, respectively). In contrast to IL-6, CRP remained associated with HRT such that women currently on HRT had higher levels of CRP compared with other women after adjustment for other covariates ($P<0.0001$). Furthermore, HDL cholesterol remained inversely related to CRP levels ($P=0.004$). Finally, in contrast to the univariate analyses, age was now significantly associated with CRP levels in a manner similar to IL-6, which was likely the result of negative confounding of other covariates. The frequency of alcohol use and exercise, total cholesterol level, and the presence of diabetes were no longer associated with CRP levels after multivariate adjustment. The full regression model accounted for 34% of the variance in logarithmically normalized CRP, with BMI alone accounting for 14% of the variance in concentrations of CRP.

**Discussion**

The present study demonstrates that several clinical cardiovascular risk factors are related to circulating plasma levels of IL-6 and CRP in women. Specifically, age, BMI, smoking status, systolic blood pressure, alcohol use, presence of diabetes, and exercise frequency are all independent correlates of circulating plasma concentrations of IL-6. We observed that age, HRT, BMI, systolic blood pressure, smoking status, and, to a lesser extent, HDL cholesterol are independently associated with circulating CRP levels. When exam-
ined in combination, these factors account for incremental levels of plasma IL-6 and CRP among women free of symptomatic cardiovascular disease.

These results are consistent with current evidence supporting the importance of inflammation in atherogenesis. Similarly, these data suggest important relationships between inflammation and traditional cardiovascular risk factors; the data also suggest that plasma vascular inflammatory molecules may play an integral role in either the biological mechanisms of these cardiovascular risk factors or provide a marker of smoldering systemic vascular disease. Similar data from healthy males have previously suggested that CRP levels increase in proportion to the prevalence of risk factors.

Circulating IL-6 concentrations may be the result of a variety of stimuli, including various clinical risk factors, from which several cell types may be induced to release IL-6, including smooth muscle cells and macrophage foam cells found in atheromatous plaques. For example, cigarette smoking may be related to elevated levels of IL-6, CRP, and other systemic markers of vascular inflammation. In theory, circulating IL-6 levels may mediate a biological interplay between various immune responses and exert autocrine, paracrine, and endocrine responses to link clinical risk

Figure 1. Association between IL-6 and CRP with HRT in apparently healthy women.

Figure 2. Plasma concentration of IL-6 and CRP in relation to systolic and diastolic blood pressure.

Figure 3. Plasma concentration of IL-6 and CRP with increasing number of cardiovascular risk factors.
factors with plasma-based markers of inflammation, such as CRP, in a pathway toward atherothrombosis. For example, IL-6 can be released from adipocytes and may stimulate a response from the hypothalamic-pituitary-adrenal axis, resulting in hypertension, obesity, and/or an insulin resistance. Recent data from our group have demonstrated associations between parameters of blood pressure and IL-6 levels in healthy males. Furthermore, laboratory data suggest that IL-6 may mediate essential pathways in type 2 diabetes. This concept is echoed in our data by the significant contribution of BMI to plasma concentrations of IL-6 and CRP. Moreover, we have recently shown that elevated levels of IL-6 and CRP are associated with an increased risk of developing type 2 diabetes.

IL-6 is a primary stimulant for the hepatic acute-phase response, with which other known proinflammatory risk factors (primarily CRP and fibrinogen) are associated. In fact, IL-6 is the only known cytokine capable of inducing all acute-phase proteins involved in the inflammatory response. As such, the induction of CRP by IL-6 may be one step in the pathobiology of atherothrombosis. Given this, it is plausible that other proinflammatory molecules are actually surrogates for circulating IL-6 activity.

In fact, elevations in IL-6 itself have independent associations with cardiovascular events, even after control for CRP. For example, in the Physician’s Health Study, elevated levels of IL-6 in apparently healthy males predicted up to a 2.3-fold increase in myocardial infarction over 6 years. Among these men, the number of risk factors at baseline was correlated with the plasma concentration of IL-6. Baseline IL-6 levels have also been found to predict cardiovascular events in the Women’s Health Study. Furthermore, in another study, IL-6 levels had been shown to be a better predictor of mortality than CRP.

Importantly, however, our data suggest that circulating concentrations of IL-6 and CRP may not always track one another. Our results echo the results from previous analyses suggesting that CRP is elevated in women on HRT irrespective of HRT preparation and other cardiovascular risk factors. However, we observed diverging concentrations of IL-6 and CRP in association with HRT, suggesting that IL-6–independent pathways may exist to upregulate circulating concentrations of CRP. Laboratory data suggest that estrogens do not influence IL-6 production in rat vascular smooth muscle cells, further supporting alternative pathways for CRP production. In fact, recent clinical data have shown the discordant effects of HRT on IL-6, CRP, and tumor necrosis factor-α and further suggest the possibility of direct hepatic stimulation of CRP by HRT. Furthermore, despite data indicating that circulating IL-6 levels are similar to CRP levels, we also observed that several other clinical risk factors were discordantly related with either IL-6 or CRP, such as alcohol use and exercise. Therefore, although CRP and IL-6 are pathobiologically linked, our data indicate that divergent levels may be seen under certain conditions among apparently healthy women and suggest that IL-6–independent pathways may exist in the regulation of circulating levels of CRP.

Taken together, IL-6 and CRP herald systemic inflammation associated atherothrombosis. Previous data have demonstrated the importance of vascular inflammation in determining vascular risk over and above that of clinical hyperlipidemia. In the present study, IL-6 and CRP were associated with several traditional risk factors, independent of lipid levels, supporting a separate or concurrent biological pathway toward atherothrombosis. This nonlipid association with traditional risk factors provides support that IL-6 and CRP may have direct vascular effects on plaque stability. Therefore, it is plausible that therapies aimed at modulating IL-6 and CRP activity, such as peroxisome proliferator–activated receptor-γ agonists, ACE inhibitors, or 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, may afford cardioprotection in the future.

Several limitations of these data should be taken into account. Because of the cross-sectional design, relationships between IL-6, CRP, and risk factors cannot be deemed causal in nature. Additionally, it is possible that unmeasured variables may account for the relationships observed. Despite these limitations, inherent with any cross-sectional analysis, our observations are consistent with known previous reports and are supported by biological plausibility. Last, because our analysis consisted of a relatively older volunteer population of women, many of whom were postmenopausal, caution

### TABLE 3. Independent Correlates of Baseline IL-6 and CRP in Regression Analyses

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Parameter Estimates per Unit/Category of Variable (95% CI)</th>
<th>IL-6</th>
<th>CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>0.02 (0.01, 0.03) P&lt;0.000</td>
<td>0.02 (0.01, 0.04) P=0.001</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>0.04 (0.02, 0.05) P&lt;0.0001</td>
<td>0.08 (0.05, 0.10) P&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Smoking status (current vs never)</td>
<td>0.23 (0.06, 0.39) P=0.008</td>
<td>0.30 (0.01, 0.60) P=0.04</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (per 10 mm Hg)</td>
<td>0.05 (0.01, 0.10) P&lt;0.02</td>
<td>0.10 (0.02, 0.18) P=0.1</td>
<td></td>
</tr>
<tr>
<td>Alcohol use (weekly vs other)</td>
<td>-0.16 (-0.30, -0.02) P=0.02</td>
<td>0.10 (-0.14, 0.34) P=0.39</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.32 (0.004, 0.64) P=0.0</td>
<td>0.02 (-0.53, 0.57) P=0.93</td>
<td></td>
</tr>
<tr>
<td>Exercise frequency (≥1 time/week vs never)</td>
<td>-0.25 (-0.41, -0.11) P=0.0008</td>
<td>-0.15 (-0.41, 0.11) P=0.27</td>
<td></td>
</tr>
<tr>
<td>Hormone replacement (current vs none)</td>
<td>-0.04 (-0.17, 0.10) P=0.57</td>
<td>0.81 (0.58, 1.04) P&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>-0.003 (-0.008, 0.002) P=0.22</td>
<td>-0.01 (-0.02, -0.004) P=0.004</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>-0.001 (-0.003, 0.0005) P=0.16</td>
<td>0.0009 (-0.002, 0.004) P=0.57</td>
<td></td>
</tr>
</tbody>
</table>
should be exercised when generalizing these data to other populations.

In summary, IL-6 and CRP are independently related to several clinical cardiovascular risk factors among apparently healthy women. Furthermore, although physiologically related, changes in circulating levels of CRP and IL-6 may not always track with one another regarding several traditional cardiovascular risk factors. Finally, although the relationship between inflammation, cardiovascular disease, and traditional risk factors is complex, IL-6 and CRP may be important mediators in the pathway toward atherothrombotic events.

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References

30. van der Wal AC, Becker AE, van der Loos CM, Das PK. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. Circulation. 1994;89:36–44.
32. Albert M, Danielson E, Rifai N, Ridker PM. Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. JAMA. 2001;286:64–70.
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