Risk Factor Analysis of Plasma Cytokines in Patients With Coronary Artery Disease by a Multiplexed Fluorescent Immunoassay

Thomas B. Martins,1 Jeffrey L. Anderson, MD,2,5 Joseph B. Muhlestein, MD,2,5 Benjamin D. Horne, PhD, MPH,2 John F. Carlquist, PhD,2,5 William L. Roberts, MD, PhD,1,3 and Harry R. Hill, MD1,4,5

Key Words: Inflammation; Atherosclerosis; Myocardial infarction; Cytokines; Multiplexed assay

Abstract

Coronary artery disease (CAD) is the leading cause of death in the United States. Increasing evidence suggests involvement of inflammation in the atherosclerotic process. We examined cytokines and other inflammatory markers in 865 patients with chest pain in whom coronary angiography revealed no evidence of CAD or CAD with or without concomitant myocardial infarction (MI). We developed a multiplexed immunoassay to simultaneously assess the plasma concentrations of 8 cytokines (interferon γ, interleukin [IL]-2, IL-4, IL-6, IL-8, IL-10, IL-12, and tumor necrosis factor α), IL-2r, and soluble CD40 ligand in the patient groups. Concentrations of C-reactive protein (CRP) and IL-18 also were determined. Significant differences (P < .05) between no CAD and combined CAD groups were found for IL-2, IL-4, IL-6, IL-12, and IL-18. When the no CAD group was compared with the group with CAD with subsequent MI, significant differences were found for proinflammatory markers IL-6 (P ≤ .001), IL-8 (P = .017), and CRP (P ≤ .001). Cytokine profiles may have a role in differentiating patients with CAD with MI from those with chest pain due to other disorders and in deciphering the role of inflammation in the pathogenesis of CAD.

Coronary artery disease (CAD) is the end result of atherosclerosis in which the inflammatory process likely has a central role.1 The role of inflammation in the process of atherosclerosis is complex but usually is initiated by the expression of adhesion molecules and selectins in the vascular endothelium.2 The adhesion molecules then cause selective adherence of circulating inflammatory cells, including macrophages and T cells, which in turn produce a variety of cytokines and other mediators of inflammation, leading to the initial formation and eventual rupture of the atherosclerotic plaque.

A better understanding of the inflammatory process in atherogenesis has led to the study of inflammatory markers as potential predictors of CAD and myocardial infarction (MI). One of the most studied markers of cardiovascular risk is C-reactive protein (CRP), which is the only inflammatory marker recommended for clinical application.3-6 although not studied as extensively as CRP, cytokines also have been studied as potential markers for the prediction of future cardiovascular events. The proinflammatory cytokine, interleukin (IL)-6, secreted by macrophages, is largely responsible for CRP production by the liver and also has been shown to be an independent risk factor for future MI.7,8 and is elevated in patients with acute coronary syndromes.9-12 Additional investigators have reported on tumor necrosis factor (TNF)-α,13,14 IL-2 and IL-10,15 IL-18,16,17 and soluble CD40 ligand18-20 as potential markers for predicting cardiovascular events.

Although many markers have been studied individually or in small groups, there is a paucity of information about the use of extensive arrays of cytokines and other inflammatory markers in a well-defined, relatively large sample of patients who have undergone coronary angiography to precisely define coronary artery phenotype. By using a multiplexed fluorescent assay...
microsphere immunoassay system (Luminex 100, Luminex, Austin, TX), we developed a sandwich capture assay to simultaneously assess the concentration of 8 cytokines (interferon [IFN]-γ, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, and TNF-α), 1 cytokine receptor (IL-2r), and soluble CD 40 ligand in only 75 µL of plasma sample. We used this multiplexed assay, as well as traditional assays for CRP and IL-18, to determine cytokine profiles in 885 patients admitted to LDS Hospital, Salt Lake City, UT, with chest pain to determine whether cytokines are involved in or predictive of CAD occurring with or without acute MI.

For the analysis and presentation of our data, the cytokines included in our study were divided into 3 categories: (1) thymus helper (TH)-1–type proinflammatory cytokines IL-2, IL-12, IL-18, and IFN-γ, which generally are secreted by lymphocytes and macrophages involved in the proinflammatory, neutrophil activating, granulomatous response; (2) TH-2–type cytokines, including IL-4 and IL-10, associated with a humoral immune response that also may inhibit proinflammatory cytokines; and (3) other proinflammatory cytokines, including TNF-α, IL-6, and IL-8, which are released by macrophages and monocytes and primarily activate other cells, including T lymphocytes and neutrophils.

Materials and Methods

Study Population

Inflammatory marker and cytokine profiles were generated retrospectively for 885 blinded samples obtained at the time that patients were admitted to LDS hospital with chest pain or other indications for cardiac catheterization; patients subsequently underwent coronary angiography for suspected CAD.

Sample selection comprised a nested case-control cohort matched 1:1:1 by age (± 5 years) and sex based on baseline patient glucose status (<110 mg/dL <6.1 mmol/L), 110-125 mg/dL [6.1-6.9 mmol/L], or ≥126 mg/dL [≥7.0 mmol/L]. Patient demographics are given in Table II. For this analysis, cases were divided into 2 groups for comparison. CAD was defined as demonstrable atherosclerotic plaques identified on angiography. One group (n = 199) had no CAD demonstrable by angiography, and the second group (n = 686) comprised patients who showed evidence of CAD and who could be divided further into those who did not or did (n = 186) experience a cardiovascular event, including acute MI or death.

All patient samples were obtained from the cardiac catheterization registry of the Intermountain Heart Collaborative Study,21 The study was approved by the hospital’s institutional review board, which allowed obtaining unrestricted age and sex blood samples from patients who gave written and informed consent at the time of angiography. Plasma was separated and stored at −70°C until testing was performed. The individuals performing the laboratory analysis were completely blinded as to individual medical information relating to samples tested.

Multiplexed Cytokine Assay

The Luminex Multi-Analyte Profiling system (Luminex) is a flow cytometry–based instrument that allows multiple analytes to be assayed simultaneously in a single sample.22 The technology is based on the process of internally labeling 5.6-µm polystyrene microspheres with 2 fluorescent fluorophores. As the microsphere passes through the flow cell, it is interrogated by 2 lasers. One laser identifies the microsphere based on the ratio of the 2 fluorophores contained within the microsphere, whereas the other laser quantitates the amount of analyte bound to the microsphere by the intensity

### Table II
Demographics of Study Patients by Presence or Absence of Coronary Artery Disease*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall (N = 885)</th>
<th>Yes (n = 686)</th>
<th>No (n = 199)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD age (y)</td>
<td>62 ± 10</td>
<td>62 ± 10</td>
<td>59 ± 9</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Male</td>
<td>73</td>
<td>76</td>
<td>59</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>62</td>
<td>65</td>
<td>54</td>
<td>.007</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>59</td>
<td>64</td>
<td>42</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>24</td>
<td>27</td>
<td>16</td>
<td>.002</td>
</tr>
<tr>
<td>Smoker</td>
<td>26</td>
<td>29</td>
<td>16</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Family history</td>
<td>39</td>
<td>42</td>
<td>31</td>
<td>.01</td>
</tr>
<tr>
<td>Previous myocardial infarction</td>
<td>25</td>
<td>29</td>
<td>8</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Manifestations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stable angina pectoris or noncoronary</td>
<td>47</td>
<td>40</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Unstable angina pectoris or noncoronary</td>
<td>31</td>
<td>33</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>22</td>
<td>27</td>
<td>6†</td>
<td></td>
</tr>
</tbody>
</table>

* Data are given as percentages unless otherwise indicated.
† Myocardial infarction in the absence of coronary artery disease may be due to embolic or thrombotic events that resolved by the time of catheterization.
of reporter fluorescence. The surface of each microsphere contains multiple carboxyl groups that function as sites for covalent ligand attachment. The amount of analyte bound to the microspheres is determined by the median fluorescence intensity of the reporter molecule, phycoerythrin, which usually is conjugated to a secondary or “detection” antibody.

The multiplexed cytokine assay we used was developed in the ARUP Institute for Clinical and Experimental Pathology, University of Utah, Salt Lake City, using a standard sandwich capture format and has been described previously. Briefly, monoclonal antibodies to human IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12 p70, TNF-α, IFN-γ, and IL-2 soluble receptor α were used as capture antibodies and coupled to carboxylated Luminex microspheres (Luminex) using a 2-step carbodiimide reaction.

A standard curve for each cytokine was made using known concentrations of recombinant human cytokine. The patient plasma sample was diluted 1:2 (75 µL of sample diluent plus 75 µL of patient plasma) and incubated for 10 minutes on a rotator before the addition of the capture antibody–coupled microspheres to allow for absorption of heterophil antibodies. The sample diluent contained 10% vol/vol fetal bovine serum, 5% vol/vol mouse serum, and 2.5% vol/vol rat serum (Sigma, St Louis, MO) diluted in 10 mmol/L of phosphate-buffered saline plus 0.02% Tween 20 (PBST) containing 0.05% Proclin (Sigma). Previous studies found this formulation of sample diluent to significantly reduce or eliminate falsely elevated cytokine values caused by samples known to contain heterophil antibodies.23

The cytokine standards, control samples, plasma samples, and microspheres were incubated for 2 hours at room temperature on a rotator using a 96-well filter bottom microtiter plate (Millipore, Bedford, MA) to allow for subsequent washing. This step was followed by the addition of 100 µL of a mixture of 10 different biotinylated secondary monoclonal antibodies to complete the sandwich capture assay. Following a second 30-minute incubation on the rotator and washing, 100 µL of 10 µg/mL of streptavidin-conjugated R-phycoerythrin (Molecular Probes, Eugene, OR) was added to each well. After a 15-minute incubation and final wash, the microspheres were resuspended in 100 µL of PBST, and the 96-well microplate was placed in a Luminex 100 instrument with an XY platform (automated microtiter plate handler). The median fluorescence intensity of the unknown patient plasma sample then was converted into a picograms per milliliter value based on the known cytokine concentrations of the standard curve using a 5-parameter regression formula. Because the analyte specificity and position of each microsphere classification in the array is known, a single fluorescent reporter molecule can be used to measure all 10 cytokine concentrations.

CRP concentrations were determined by using a high-sensitivity immunoturbidimetric method (Roche Diagnostics, Indianapolis, IN). A standard enzyme-linked immunosorbent assay (ELISA) (Biosource, Camarillo, CA) was used for IL-18 quantification because we could not obtain commercial IL-18 monoclonal antibody of sufficient specificity to use in the multianalyte assay.

Statistical Analysis

Statistical analyses of cytokine results were performed by analysis of variance (ANOVA) using the Tukey HSD to adjust for multiple pairwise comparisons. Non–normally distributed biomarkers were transformed by using the natural logarithm before analysis. In this study, samples from 885 patients were tested for 12 different biomarkers, resulting in 10,620 individual results. Initial comparisons were performed using 1-way ANOVA to evaluate univariate differences in mean cytokine concentrations between patients with and without CAD. Further analyses used multivariate ANOVA with inclusion of groupings of cytokine variables or all cytokines in the analysis. Determination of multicollinearity was performed, and the best fitting variables were used in final modeling.

Further modeling of the predictive ability of the cytokines for CAD was performed after categorization of the cytokine values into quartiles of equal sample size. Multiple variable logistic regression was used to adjust for cytokine variables and for traditional demographic (age and sex) and cardiac risk factor variables (hypertension, hyperlipidemia, smoking, diabetes, family history of early CAD, and high-sensitivity CRP [hsCRP]). Final models included all traditional variables and the important and independent cytokine variables. Because prehospital use of medications such as statins, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, beta blockers, diabetic medications, and diuretics was not known, no data are given regarding medication use.

Comparison of traditional covariables with CAD initially was performed by using the Student t test or the χ² test. Statistical significance in each analysis was set at a P value of .05 or less.

Results

Validation of the Multiplexed Assay

The specificity of the multiplexed assay was determined by spiking high concentrations (up to 10,000 pg/mL) of only 1 of the 10 recombinant cytokines into fetal bovine serum to create 10 different samples. These 10 samples were run in the multiplexed format to check for cross-reactivity of the assay. For most of the spiked samples, values of less than 2 pg/mL were obtained, indicating no cross-reactivity. Slight cross-reactivity was noted between the IFN-γ–spiked sample and 3 other cytokines—IL-2 (9 pg/mL), IL-12 (11 pg/mL), and
TNF-α (11 pg/mL)—and between IL-10 and IL-2 (7 pg/mL). These findings were not of great concern because the high concentrations of spiked cytokine that were found to cause limited cross-reactivity in our specificity studies are found rarely in plasma samples, which typically have reference intervals ranging from less than 5 to 30 pg/mL of cytokine.

Precision of the 10-cytokine multiplexed assay in serum was determined by intra-assay and interassay studies using 2 independent (medium and low) level control samples. The coefficient of variation was calculated from 5 replicates of each level of control sample. Intra-assay coefficients of variation were mostly less than 10%, with interassay results ranging from less than 15% for the medium control sample to up to 28% for the low control sample.

TH-1–Type Proinflammatory Cytokines

Initial comparisons were made between 199 patients with chest pain but no angiographic evidence of CAD and the combined group of 686 patients with CAD with or without acute MI. Multivariate analysis showed significant differences between the 2 groups (P < .05) for the TH-1–type inflammatory cytokines IL-2, IL-12, and IL-18. IL-2 had a mean value of 28.2 pg/mL for the no CAD group, whereas the combined CAD group had a mean value of 29.5 pg/mL (P = .011) and IL-18 (499 vs 444 pg/mL; P = .029) compared with those with no CAD. This seems paradoxical because these 2 TH-1–type cytokines, which would be expected to exert a proinflammatory effect, actually were depressed. In contrast, the mean concentration of the major TH-1–type cytokine IFN-γ and most important activator of macrophages and neutrophils was elevated in patients with CAD (21.1 pg/mL) compared with the no CAD control subjects (14.3 pg/mL), but the differences did not reach statistical significance. Mean concentrations also were elevated in the combined CAD group for IFN-γ compared with the no CAD group, but the difference was not significantly different (Figure 1).

TH-2–Type Cytokines

The mean cytokine concentrations were elevated in the combined CAD group for the TH-2 cytokines IL-4 and IL-10 compared with the no CAD group, but only IL-4 showed statistically significant differences. The mean concentration for IL-4 was 3.3 pg/mL in the combined CAD group vs 3.1 pg/mL in the control group (P = .008). IL-10, which down-regulates TNF-α and IL-1 production, was increased in patients with CAD compared with the control group, but the results were not statistically significant.

Proinflammatory Cytokines

TNF-α, a major proinflammatory cytokine that up-regulates adhesion molecules and activates T lymphocytes and neutrophils, was elevated in patients with CAD (34.3 pg/mL) compared with the no CAD control subjects (28.5 pg/mL), but the differences only approached significance (P = .07). In contrast, IL-6, which is increased by TNF-α but has a longer half-life and, therefore, remains elevated longer, was increased significantly in patients with CAD (9.6 pg/mL) vs the no CAD control subjects (7.8 pg/mL; P < .001). IL-8, which is a monocyte/macrophage product that activates and stimulates chemotaxis of neutrophils, also was elevated in patients with CAD (23.6 pg/mL), but the differences vs the no CAD subjects (16.6 pg/mL) again only approached significance (P = .07).

Mean concentrations also were elevated in the combined CAD group for hsCRP and IL-2r compared with the no CAD group, but the differences were not significantly different. Mean concentrations and P values are summarized in Table 2.

The data were reanalyzed by identifying a subset of 186 patients from the combined CAD group with an acute MI. When this CAD with MI group was compared with the no CAD group (n = 199), statistically significant increases were found for the inflammatory markers IL-6 (mean, 10.0 vs 7.8 pg/mL; P < .001), IL-8 (mean, 23.5 vs 16.6 pg/mL; P = .017), and hsCRP (mean, 15.9 mg/L vs 10.0 mg/L; P ≤ .001). Elevated, but not significantly different responses also were observed in the CAD with MI group for IFN-γ, IL-4, IL-10, and IL-2r compared with the no CAD group. Soluble...
CD40 ligand concentrations actually were decreased in the combined CAD and CAD with MI groups compared with the no CAD control group, but the differences were not significantly different (Table 2).

A risk model was fit for the combined endpoint of CAD with or without event, wherein IL-2 quartiles 2 (odds ratio [OR], 0.57; \( P = .037 \)), 3 (OR, 0.51; \( P = .010 \)), and 4 (OR, 0.48; \( P = .011 \)) and IL-6 quartiles 2 (OR, 1.71; \( P = .032 \)), 3 (OR, 1.83; \( P = .022 \)), and 4 (OR, 1.77; \( P = .042 \)) predicted CAD after full adjustment for traditional factors and hsCRP. Marginal significance was found for IL-8 (quartile 3: OR, 2.06; \( P = .010 \); and quartile 4: OR, 1.58; \( P = .12 \)) and IL-18 (quartile 3: OR, 0.58; \( P = .041 \); and quartile 4: OR, 0.68; \( P = .12 \)) with \( P \) values of .01 to 0.12.

Evaluation of the risk model including IL-2, IL-6, IL-8, and IL-18 showed a receiver operating characteristic curve area of 0.756. Compared with the area under the curve of 0.737 for a model including only traditional factors (ie, age, sex, hypertension, hyperlipidemia, diabetes, smoking, family history of CAD, and high hsCRP), this suggests an incremental value for the interleukin panel in the capability to predict CAD.

### Table 2

Mean Cytokine and Inflammatory Marker Concentrations in 885 Patients With Chest Pain

<table>
<thead>
<tr>
<th>Marker</th>
<th>No CAD (n = 199)</th>
<th>Combined CAD (n = 686)</th>
<th>CAD With Death or MI (n = 186)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
</tr>
<tr>
<td>hsCRP†</td>
<td>10.0</td>
<td>12.7</td>
<td>15.9</td>
</tr>
<tr>
<td>CD40 ligand</td>
<td>316.0</td>
<td>232.0</td>
<td>284.0</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>14.3</td>
<td>21.1</td>
<td>15.5</td>
</tr>
<tr>
<td>TNF-α</td>
<td>28.5</td>
<td>34.3</td>
<td>270.0</td>
</tr>
<tr>
<td>IL-2</td>
<td>28.2</td>
<td>29.5</td>
<td>24.2</td>
</tr>
<tr>
<td>IL-2r</td>
<td>320.0</td>
<td>324.0</td>
<td>379.0</td>
</tr>
<tr>
<td>IL-4</td>
<td>3.1</td>
<td>3.3</td>
<td>3.3</td>
</tr>
<tr>
<td>IL-6</td>
<td>7.8</td>
<td>9.6</td>
<td>10.0</td>
</tr>
<tr>
<td>IL-8</td>
<td>16.6</td>
<td>23.6</td>
<td>23.5</td>
</tr>
<tr>
<td>IL-10</td>
<td>5.4</td>
<td>8.1</td>
<td>7.1</td>
</tr>
<tr>
<td>IL-12</td>
<td>18.3</td>
<td>14.2</td>
<td>16.5</td>
</tr>
<tr>
<td>IL-18</td>
<td>499.0</td>
<td>444.0</td>
<td>412.0</td>
</tr>
</tbody>
</table>

CAD, coronary artery disease; hsCRP, high-sensitivity C-reactive protein; IL, interleukin; IFN, interferon; MI, myocardial infarction; TNF, tumor necrosis factor.

† Values reported in mg/L, all other markers reported in pg/mL.

* Compared with the No CAD group.
Discussion

The combined CAD patient group, with or without MI, was found to have significantly elevated concentrations of the inflammatory cytokine IL-6 compared with the no CAD control group. Although hsCRP also was elevated in this group, it was not significantly different between those with CAD and those with chest pain without angiographic evidence of CAD. When samples from the patients with CAD who had an MI were analyzed independently, however, IL-6 and hsCRP were significantly elevated compared with values for the control group. Because IL-6 is a known enhancer of hepatic production of CRP, it is appropriate that both inflammatory markers are elevated in patients undergoing acute coronary episodes.\(^8\)\(^-\)\(^10\) IL-6 and CRP have been identified previously as independent risk factors for future MI,\(^7\) which is supported by the results of our study in patients with CAD who had an acute MI.

Although mean TNF-\(\alpha\) concentrations were elevated in the combined CAD group (33.3 pg/mL) compared with the control group (28.5 pg/mL), the differences only approached statistical significance (\(P = .07\)). An increase in the level of TNF-\(\alpha\), which has a very short half-life, is known to precede increases in IL-6, which in turn stimulates the production of CRP, both of which were found to be significantly elevated in our study groups. It is likely, therefore, that TNF-\(\alpha\) concentrations were elevated initially in our study patients but had decreased by the time the blood samples were obtained from these patients who were admitted to the emergency department with suspected or documented acute coronary syndromes. In other studies demonstrating elevated concentrations of TNF-\(\alpha\) in patients with recent coronary events, the blood samples were obtained only after the occurrence of MI.\(^15\)\(^,\)\(^26\)

IL-2 was the only TH-1–type cytokine significantly elevated in our combined CAD group vs the no CAD group. IL-2 generally is classified as an immunoregulatory cytokine because it is derived from T cells and is involved in the activation, growth, and differentiation of several cell types, including T cells, monocytes, B cells, and natural killer cells. Its role in atherosclerosis is not entirely understood, and there are conflicting reports in the literature. In a study comparing 66 patients with stable angina with 24 patients with unstable angina, Simon et al\(^27\) found significantly higher mean IL-2 concentrations in patients with stable angina. In contrast, an independent study of 189 patients with CAD by Mizia-Stec et al\(^15\) found significantly elevated mean serum concentrations of IL-2 in patients with MI and unstable angina compared with patients with stable angina.

The TH-2–type cytokine IL-4 was statistically elevated (\(P = .008\)) in the combined CAD group compared with the no CAD control group. Besides having the ability to stimulate IgE antibody production, IL-4 also suppresses proinflammatory cytokines such as TNF-\(\alpha\) and IL-1. Although the role of IL-4 in atherosclerosis has been studied in animal models\(^28\) and IL-4 has been classified as a suppressor of other inflammatory cytokines in cardiovascular diseases,\(^29\) our results seem to be the first to describe elevated concentrations of IL-4 in a well-defined human study of CAD.

IL-8 was the only cytokine that was significantly elevated in the CAD group with MI that was not elevated in the combined CAD group compared with the no CAD control group. IL-8 is a proinflammatory cytokine that directly activates neutrophils. It is expressed by macrophages and released in response to the proinflammatory cytokines IL-1\(\beta\) and TNF-\(\alpha\). It is also a chemoattractant for neutrophils that, along with macrophages, accumulate at the sites of plaque rupture and likely have a major role in atherosclerosis.\(^30\)\(^,\)\(^31\) Evidence for IL-8 as a marker of coronary heart disease, however, remains limited. A study including 38 patients by Romuk et al\(^32\) found elevated concentrations of IL-8 in patients with unstable angina compared with patients with stable angina and control subjects. A study in a Chinese population by Zhou et al\(^33\) including 32 patients with acute MI found similar results to ours: serum concentrations were significantly higher for patients with acute MI compared with those for healthy control subjects.

Two other cytokines with statistically significant differences in our study by CAD status were IL-12 and IL-18. Concentrations of these cytokines, paradoxically, actually were decreased in the combined CAD group compared with the non CAD control group. IL-18 is a proinflammatory cytokine that in conjunction with IL-12 mediates a TH-1–type immune response. Both of these cytokines are known to elevate the TH-1–type cytokine IFN-\(\gamma\). IL-18 also can stimulate TH-2–cytokine production and has been shown to have a role in atherosclerotic plaque destabilization, leading to acute ischemic syndromes.\(^34\) Blankenberg et al\(^17\) also found IL-18 to be a strong predictor of cardiovascular death in patients with stable and unstable angina. Baseline concentrations of IL-18 were measured in patients already diagnosed with CAD and prospectively compared with those for patients who subsequently died of cardiovascular causes. This design differed from the design of our study in which we compared IL-18 concentrations in patients with CAD with concentrations in those without and actually found depressed IL-18 concentrations (\(P = .029\)) in patients with CAD compared with the control group.

In multivariate analyses examining the cytokines simultaneously and using quartiles to make comparisons, independent predictive ability was found for quartiles of IL-2 and IL-6, with borderline significance shown for IL-8 and IL-18. The failure of IL-4 and IL-12 to achieve significance in these analyses was primarily the result of their accounting for the same risk as the other 4 cytokines that did achieve independent significance.
By examining samples from a relatively large number of patients with chest pain in a single study, we were able to identify statistically significant differences in cytokine concentrations between patients with CAD and those without. Applying these findings to an individual patient to predict the presence or absence of CAD or determine the outcome in a clinical setting, such as in an emergency department, may be of limited use. For example, although the differences in IL-2 for the patient groups in our study were significant ($P = .026$; 28.2 pg/mL for no CAD vs 29.5 pg/mL for CAD), the difference of only 1.3 pg/mL would make it impossible to make decisions based on a laboratory value in individual patients, given the broadly overlapping distributions of values in diseased patients and control subjects. Our findings, however, are not inconsistent with other published studies of this nature. In a study by Ridker et al., baseline concentrations of IL-6 in 202 men who subsequently had an MI compared with those who did not were significantly different ($P = .002$), even though the difference in IL-6 concentration was only 0.35 pg/mL between the 2 groups.

A limitation of previous studies on cytokines and inflammatory markers in CAD is the inclusion of only 1 to 3 different cytokines. Multiple cytokines are involved in the inflammatory process, having overlapping, antagonistic, and synergetic effects on many cell types and up-regulating and down-regulating the production of other cytokines and inflammatory markers. The optimal manner, therefore, in which to correlate a specific disease process with changes in cytokine concentrations requires analyzing individual samples for multiple cytokines. The multianalyte technology that we based our assay on allowed us to generate full cytokine profiles for each individual patient while requiring less sample, time, expense, and labor than ELISA assays traditionally used in these types of studies. Circulating cytokines are known to have a short half-life, and cytokines involved in the earlier cascade of events of the inflammatory process may be down-regulated at later time points or at patient admission. For these reasons, it often is important to evaluate a full profile of TH-1 and TH-2 proinflammatory and anti-inflammatory cytokines and markers to obtain a more complete picture of the evolving inflammatory process.

The multiplexing capability of the Luminox instrument is well suited to the development of multiple analyte profiles, which require less patient sample, reagent, and expense than traditional ELISA methods. Previous studies in which this technology was used to develop a multiplexed cytokine assay demonstrated it to be an accurate and reliable system for simultaneously quantitating 21 cytokines in only 75 µL of plasma sample. 23

The studies reported herein suggest that analysis of cytokine profiles may have a role in differentiating patients with CAD and those with acute MI from those with chest pain due to other disorders. Because the range of expected values for any individual cytokine overlaps for diseased and control groups, simultaneously assessing multiple cytokines could increase diagnostic specificity. Our results also suggest that these differences can be related not only to the presence of angiographic CAD but also to its major complication, MI. When samples from patients with CAD and acute MI were compared with those from the no CAD group, statistically significant increases were found for IL-6, IL-8, and CRP, all of which are proposed indicators of vascular inflammation and plaque instability or rupture, the common feature of acute coronary syndromes. This profile of markers also may be important for deciphering the role of cytokines in the pathogenesis of CAD.

From 1Associated Regional and University Pathologists (ARUP) Institute for Clinical and Experimental Pathology; 2Cardiovascular Department, LDS Hospital; and the Departments of 3Pathology, 4Pediatrics, and 5Medicine, University of Utah School of Medicine, Salt Lake City.

Address reprint requests to Mr Martins: ARUP Institute for Clinical and Experimental Pathology, 500 Chipeta Way, Salt Lake City, UT 84108.

References


   *Coron Artery Dis.* 2003;14:431-438.


   *Clin Cardiol.* 2001;24:253-256.


29. Fisman EZ, Motro M, Tenenbaum A. Cardiovascular diabetology in the core of a novel interleukins classification: the bad, the good and the aloof. 


   *J Atheroscler Thromb.* 2001;8:30-32.