Serum Level of the Antiinflammatory Cytokine Interleukin-10 Is an Important Prognostic Determinant in Patients With Acute Coronary Syndromes

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Background—Convincing evidence suggests that atherosclerosis is an inflammatory disease. The inflammatory response is an important determinant of atherosclerotic plaque instability. Therefore, we investigated the prognostic impact of key inflammatory players, namely the inflammatory marker C-reactive protein (CRP) and the antiinflammatory cytokine interleukin-10 (IL-10), in patients with acute coronary syndromes.

Methods and Results—IL-10, CRP, and troponin T were measured at baseline and before discharge in 547 patients enrolled in the placebo group of the c7E3 Anti Platelet Therapy in Unstable Refractory angina (CAPTURE) trial. Death and nonfatal myocardial infarction were recorded during 6-month follow-up. IL-10 levels did not correlate with troponin T concentrations but were inversely correlated with CRP levels ($P<0.001$). Patients with elevated IL-10 levels ($>3.5$ pg/mL; $n=276$) were at significantly lower risk compared with patients with elevated IL-10 levels (hazard ratio, 0.33; 95% confidence interval [CI], 0.25 to 0.76; $P=0.002$). The predictive value of IL-10 was independent of myocardial necrosis but significantly interacted with CRP levels. CRP-positive patients with IL-10 serum levels above the calculated threshold value of 3.5 pg/mL were protected from the increased cardiac risk of CRP-positive patients with low IL-10 levels (adjusted hazard ratio, 0.25; 95% CI, 0.10 to 0.63; $P=0.003$). Moreover, discharge IL-10 levels $>2.5$ pg/mL were associated with lower cardiac risk during 6-month follow-up (hazard ratio, 0.38; 95% CI, 0.19 to 0.83; $P=0.005$).

Conclusions—Elevated IL-10 serum levels are associated with a more favorable prognosis in patients with acute coronary syndromes and elevated CRP levels. These data demonstrate the importance of the balance between proinflammatory and antiinflammatory markers as a major determinant of patients’ outcome in acute coronary syndromes. (Circulation. 2003;107:2109-2114.)

Key Words: angina ■ prognosis ■ inflammation ■ C-reactive protein ■ troponin

Atherosclerosis is a complex multifactorial process resulting from an excessive inflammatory response to various forms of injurious stimuli to the arterial wall. The transition of a stable coronary atherosclerotic lesion into a ruptured and/or eroded plaque results in the clinical manifestation of an acute coronary syndrome. The understanding of the factors that induce such events is essential for the prevention and treatment of atherosclerosis. Mechanistically, atherosclerotic plaque instability is the consequence of a complex inflammatory response of the vessel wall ignited by activated macrophages and T-cells leading to proteolytic degradation of connective tissue matrix, excessive pro-inflammatory cytokine production, and apoptosis of vascular wall cells. Indeed, patients with acute coronary syndromes demonstrate elevated serum levels of C-reactive protein (CRP), serum amyloid A, or interleukin (IL)-6, indicative of a systemic inflammatory response. In contrast, serum levels of the potent antiinflammatory cytokine IL-10 have recently been shown to be decreased in patients with acute coronary syndromes, thus suggesting that reduced levels of IL-10 may favor plaque instability and the development of acute coronary syndromes.

Whereas elevation of systemic markers of inflammation is firmly established to predict an unfavorable outcome in patients with acute coronary syndromes, there are currently no data addressing the prognostic significance of antiinflammatory serum markers in patients with acute coronary syndromes. Given the multifaceted antiinflammatory...
properties of IL-10, we investigated whether IL-10 serum levels provide prognostic information in patients with acute coronary syndromes using the database from the CAPTURE study (c7E3 Anti Platelet Therapy in Unstable Refractory angina).13

Methods

Patients

The CAPTURE trial enrolled 1265 patients with acute coronary syndromes and angiographically documented coronary artery disease. All patients were scheduled for coronary interventions between 18 and 24 hours after randomization. Baseline angiograms were centrally assessed by an Angiographic Committee at Cardiology, Rotterdam (the Netherlands), with respect to characteristics of the culprit lesion according to the American Heart Association (AHA)/American College of Cardiology (ACC) grading system.13

Analytical Techniques

High-sensitivity IL-10 was measured by ELISA (R&D Systems).9 Detection limit was 0.5 pg/mL, and intra-assay variation among triplicates was 7.6%. For quantification of troponin T, a one-step enzyme immunoassay based on electrochemiluminescence technology (Elecsys 2010, Roche Diagnostics) was used.14 CRP was measured by N Latex CRP Mono tests performed on a Behring BN II Nephelometer (Behring Diagnostics).15 A calculated diagnostic threshold level of 10 mg/L was used.10,11

Statistical Methods

Patients were categorized in quartiles according to the IL-10 serum concentration. For each time point, the Cox proportional-hazards regression model was used to estimate the relative risk for death and myocardial infarction.14 The effect of baseline characteristics and other biochemical markers on any observed associations between IL-10 and cardiovascular events was analyzed using stepwise Cox proportional-hazards models. All results for continuous variables are expressed as mean±SD. Comparisons between groups were analyzed by t test (2-sided). Comparison of categorical variables was generated by the Pearson χ² test. Probability values <0.05 were considered statistically significant. All analyses were performed with SPSS 11.0 software (SPSS).

Results

Interaction Between IL-10 Serum Levels and Cardiac Risk

The present study is based on patients with available serum samples treated with placebo (n=547; 86% of the control patients). Baseline IL-10 serum levels showed a mean serum level of 4.28±2.72 pg/mL. Overall, IL-10 concentrations did not correlate with troponin T levels (Spearman rank correlation coefficient, r=0.020; P=0.49). Bivariate correlation analysis revealed an inverse correlation between IL-10 and CRP (r=-0.31; P<0.001). Patients were stratified into quartiles according to their measured IL-10 levels (<2.5 pg/mL, n=136 [first quartile]; 2.5 to 3.5 pg/mL, n=135 [second quartile]; 3.6 to 5.1 pg/mL, n=136 [third quartile]; and >5.1 pg/mL, n=140 [fourth quartile]). For the initial 24-hour period, the combined end points of mortality and nonfatal myocardial infarction tended to be lower in the third and fourth quartiles compared with the first quartile (fourth quartile, P=0.060; third quartile, P=0.048; Figure 1). For the 72-hour follow-up including peri-interventional events, the difference in cardiac events did not increase significantly (fourth quartile, P=0.057; third quartile, P=0.067). During 30-day and 6-month follow-up, however, the event rate curves markedly diverged. At 6 months of follow-up, differences in event rates were significant for the upper 2 quartiles (fourth quartile, P=0.007; third quartile, P=0.021). Consistent with these results, receiver-operating characteristic curve analysis indicated a threshold level of 3.5 pg/mL of IL-10 for maximized predictive value (Figure 2).

Stratification According to IL-10 Status

The study population was dichotomized according to the calculated threshold level of 3.5 pg/mL, resulting in 276 patients with elevated IL-10 levels (50.5%). Except for a higher incidence of CRP elevation in patients with high IL-10 serum levels, baseline characteristics did not significantly differ between patients with high and low IL-10 serum levels (Table). The adjusted hazard ratios for death and myocardial infarction were 0.19 (95% CI, 0.04 to 0.88; P=0.021) at 24 hours, 0.49 (95% CI, 0.26 to 0.95; P=0.039) at 72 hours (Figure 3A), 0.44 (95% CI, 0.24 to 0.83; P=0.007) at 30 days, and 0.43 (95% CI, 0.25 to 0.74; P=0.002) at 6 months.
Six-month cumulative event rates in patients with low IL-10 levels were 16.5% versus 7.9% for patients with high IL-10 levels. This difference in event rates was not only driven by a higher rate of nonfatal myocardial infarction, but also by a higher mortality in patients with reduced IL-10 serum levels (3.3% versus 1.0%; \( P = 0.069 \)).

Urgent revascularization procedures, including percutaneous coronary intervention and coronary artery bypass grafting, were consistently and significantly higher in patients with low IL-10 serum levels (14.9% versus 7.2%; \( P = 0.009 \)). Nonurgent revascularization procedures during 6 months of follow-up showed a trend toward higher incidence in patients with low IL-10 serum levels (30.6% versus 23.5%; \( P = 0.12 \)).

Most notably, the predictive value of IL-10 was restricted to patients with elevated CRP levels (Figure 4A). If CRP serum levels were elevated (>10 mg/L), patients with IL-10 serum levels above the calculated threshold value of 3.5 pg/mL were protected from an increased cardiac risk (adjusted hazard ratio, 0.93; 95% CI, 0.40 to 2.18; \( P = 1.00 \)). In patients with high CRP serum levels, the complexity of the coronary lesions differed consistently and significantly between patients with high and low IL-10 serum levels. Type B2 and C lesions were more frequently found in patients with low IL-10 serum levels compared with...
patients with high IL-10 serum levels (41.6% versus 27.7%; P=0.009). In contrast, the predictive value of IL-10 was completely independent of the evidence for myocardial necrosis. Patients with elevated IL-10 serum levels were at significantly lower risk in both patients with normal troponin T serum levels (adjusted hazard ratio, 0.32; 95% CI, 0.14 to 0.71; P=0.005) and patients with elevated troponin T serum levels (adjusted hazard ratio, 0.55; 95% CI, 0.22 to 0.99; P=0.041; Figure 4B).

To further delineate a potential independent prognostic significance of the 3 individual biochemical markers, a multivariate analysis was performed including troponin T, CRP, and IL-10, as well as baseline characteristics that revealed significant predictive value in a univariate model. For the end points of death and nonfatal myocardial infarction at 30-day and 6-month follow-up, none of the established risk factors was an independent predictor after the dichotomized biochemical markers were introduced into the model. Troponin T (P=0.004) and IL-10 (P=0.007) remained the only independent significant predictors of patient outcome; CRP lost significance after IL-10 was introduced into the model (P=0.015 without IL-10; P=0.21 after introduction of IL-10).

Discharge IL-10 Serum Levels Predict Long-Term Outcome

A second blood sample drawn before discharge (7.2±4.5 days after randomization) was available for 489 patients (89.4%). IL-10 serum levels decreased from a mean of 4.28±2.72 pg/mL at baseline to 2.38±7.34 pg/mL at discharge (P<0.001). More importantly, however, there was a heterogeneous response in IL-10 serum levels of individual patients from baseline to discharge during treatment of the acute coronary syndrome (Figure 5A). In 33.5% of the patients, discharge IL-10 serum levels were reduced by >25% relative to the respective baseline samples. In 21.4% of the patients, IL-10 serum levels were unchanged (±25%), and in 45.1% of the patients, discharge IL-10 serum levels increased by >25% relative to the baseline IL-10 serum levels. Patients with unchanged or increased IL-10 serum levels had a significantly better outcome at both 30 days of follow-up (3.5% versus 0.0%; P=0.001) and 6 months of follow-up (low IL-10 versus high IL-10, 8.8% versus 1.8%; P<0.001; Figure 5B).

Moreover, a single IL-10 serum level obtained at discharge was similarly predictive for patient long-term prognosis. For patients with IL-10 serum levels below the median of 2.5 pg/mL at discharge (49.7%), the incidence of mortality and nonfatal myocardial infarction was significantly higher compared with patients with IL-10 serum levels >2.5 pg/mL, both at 30-day (2.4% versus 0.0%; P=0.030) and at 6-month follow-up (6.7% versus 1.6%; P=0.003; Figure 6).

Discussion

The results of the present study demonstrate that elevated serum levels of the antiinflammatory cytokine IL-10 are associated with a significantly improved outcome of patients with acute coronary syndromes. The predictive value of IL-10 serum levels was independent of elevated troponin levels, which reflect the acute risk secondary to thrombotic complications during an acute coronary syndrome. Thus, a reduced IL-10 serum level is not only a marker of plaque instability favoring the development of acute coronary syndromes, but more importantly is indicative of a poor prognosis even after the occurrence of an acute ischemic event caused by plaque instability. In addition, the beneficial effect of elevated serum levels of IL-10 was restricted to patients with elevated CRP serum levels, indicative of an enhanced systemic inflammatory response. These data further support the concept that the balance between proinflammatory and antiinflammatory cytokines is a major determinant of patient outcome in acute coronary syndromes.

IL-10 is secreted by activated monocytes/macrophages and lymphocytes.17 It has multifaceted antiinflammatory properties, including inhibition of the prototypic pro-inflammatory transcription factor nuclear factor-κB, leading to suppressed cytokine production,18 inhibition of matrix degrading metalloproteinases,19 reduction of tissue factor expression,20 inhibition of apoptosis of macrophages and monocytes after infection,21,22 and promotion of the phenotypic switch of lymphocytes into the Th2 phenotype.23 All these inflammatory mechanisms have been shown to play a pivotal role for...
atherosclerotic lesion development and progression, suggesting a potential regulatory role of IL-10. Indeed, numerous recent experimental studies have shown that either systemic or local IL-10 gene transfer not only attenuates atherogenesis,\textsuperscript{17,24,25} but also affects processes associated with lesion progression.\textsuperscript{21}

Convincing evidence suggests that the quality (plaque composition) rather than the size of the atherosclerotic lesion is critical for plaque stability. Vulnerable plaques are rich in inflammatory cells, contain a thrombogenic lipid core, and are characterized by a thin fibrous cap with a substantial loss in extracellular matrix.\textsuperscript{26} IL-10 is expressed in advanced human atherosclerosis and is associated with low inducible nitric oxide synthase expression and low levels of apoptosis, again suggesting a protective role of this antiinflammatory cytokine.\textsuperscript{27} In contrast, atherosclerotic lesions from IL-10-deficient mice demonstrate increased infiltration of inflammatory cells, increased production of interferon-\ensuremath{\gamma}, and, interestingly, a very low percentage of collagen in comparison with lesions from wild-type mice.\textsuperscript{17} Taken together, these findings indicate that the absence of IL-10 favors the development of atheromatous lesions with signs characteristic for increased vulnerability. Thus, the beneficial effect of elevated IL-10 serum levels on patient outcome observed in the present study may be related to increased plaque stability. Indeed, patients with low IL-10 serum levels in the presence of high CRP serum levels had more complex lesions according to the ACC/AHA lesion score.

IL-10 serum levels did not correlate with cardiac troponin levels. Moreover, the numbers of patients taking lipid-lowering medication or aspirin, which can both exert antiinflammatory effects in patients with coronary artery disease, before the onset of the acute coronary syndrome did not differ between patients with decreased and elevated IL-10 serum levels. Thus, neither pretreatment nor extent of myocardial necrosis seems to have influenced the results of the present study. However, elevated levels of IL-10 at discharge were important predictors of a favorable 6-month outcome in our patient population. These data suggest that increased IL-10 serum levels in response to an acute ischemic coronary event might beneficially affect the clinical course after an acute coronary syndrome. Recent data provide direct evidence that the inflammatory process in acute coronary syndromes is not confined to the culprit lesion, but rather is widespread throughout the coronary circulation.\textsuperscript{28} Indeed, in patients surviving an episode of acute coronary syndrome, predischARGE CRP serum levels predict subsequent adverse cardiac events, indicating that ongoing inflammatory activation exposes patients to an increased risk.\textsuperscript{29} Therefore, it is tempting to speculate that therapeutic interventions that increase endogenous IL-10 serum levels or even exogenous administration of IL-10 may represent novel therapeutic strategies to improve clinical outcome after acute ischemic syndromes, specifically in patients with elevated ongoing inflammatory activity.

At first sight, studies measuring IL-10 serum levels in patients with acute coronary syndromes have generated conflicting results. It is important to note that the present study is the only large-scale study to date that investigated the prognostic impact of IL-10 in a very well characterized set of patients with acute coronary syndromes. All patients in the CAPTURE trial underwent angiography documenting significant coronary artery disease with a culprit lesion \ensuremath{\geq}70\% and, thus, the patients represent a homogeneous population of high-risk patients with acute coronary syndromes. The results from the present study are consistent with a previous, smaller study reporting reduced IL-10 serum levels in patients with unstable angina and coronary events during 3 months of follow-up.\textsuperscript{30} Another study reported increased IL-10 serum levels in patients with unstable angina compared with patients with stable angina.\textsuperscript{31} These results may also be reconciled by our observation that during treatment of patients with acute coronary syndromes, IL-10 serum levels significantly decreased in the overall population. Importantly, however, the response of IL-10 serum levels of individual patients was heterogeneous, and patients with unchanged or even increased IL-10 serum levels had the lowest cardiovascular event rate during 6 months of follow-up. Thus, IL-10 serum levels may indeed increase during acute coronary syndromes in the overall population, but an inadequate increase or even a decrease of IL-10 in some patients seems to identify a subgroup of high-risk patients. This heterogeneity of the IL-10 response may explain the results of Smith et al.,\textsuperscript{9} who found that IL-10 serum levels were significantly lower in patients with unstable angina compared with patients with stable angina.

In summary, IL-10 serum levels provide independent prognostic information in patients with acute coronary syndromes. Elevation of IL-10 serum levels seems to have protective effects in patients with increased CRP concentrations. These data provide further evidence for the prognostic importance of a counterbalance to pro-inflammatory stimuli in patients with unstable coronary heart disease. Thus, therapeutic interventions to increase IL-10 levels may represent a novel therapeutic strategy for stabilizing atherosclerotic plaques and improving clinical outcome after acute coronary syndromes.

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References


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