Fluctuating Inflammatory Markers in Patients With Stable Ischemic Heart Disease

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Background: C-reactive protein (CRP), a marker of inflammation, is increasingly measured to stratify coronary artery disease risk and guide clinical management. However, little is known about how inflammatory markers fluctuate over time in patients with stable ischemic heart disease.

Methods: We examined serial serum CRP values in 159 patients with histories spanning the clinical spectrum of ischemic heart disease. Two to 8 CRP measurements were made at intervals varying from 15 days to 6 years. Successive interleukin (IL)-6 values were examined in 1 subgroup. Blood samples were always taken when patients were clinically stable, in the absence of any potentially confounding inflammatory condition.

Results: C-reactive protein values in individual patients fluctuated considerably when examined in the following ranges: less than 1 mg/L, 1 to 3 mg/L, and greater than 3 mg/L, proposed to indicate low, average, and high risk. Sixty-four patients (40.3%) changed risk category between the first and the second measurement. Within-patient variances of CRP and IL-6 levels were 1.79 mg/L (95% confidence interval, 1.60-2.00) and 2.69 pg/mL (95% confidence interval, 2.29-3.18), respectively. The variability of CRP was consistent over different times and across clinical groups, and independent of body mass index, smoking status, medication, and clinical events.

Conclusions: Relatively important fluctuations in CRP levels in patients with stable ischemic heart disease may be problematic for risk stratification and treatment monitoring. A similar IL-6 variability suggests that these patients have a dynamic inflammatory status whose kinetics may modulate acute coronary risk.

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Inflammation is now recognized as an essential component of atherosclerotic disease.1,2 C-reactive protein (CRP), a marker of inflammation, is associated with cardiovascular risk in healthy individuals as well as in patients with stable ischemic heart disease.3-10 Based on these findings, the use of CRP as a prognostic marker that may guide clinical management is increasingly advocated.11,12 And yet, virtually all the long-term studies that have shown CRP to have a prognostic value rested on a single baseline CRP measurement. Considerable intrindividual variability in high-sensitivity CRP values has been noted in healthy individuals13-17 but, surprisingly, this has received little attention. It remains to be determined whether such variability also extends to patients with ischemic heart disease. Marked intrindividual changes in CRP levels in these patients could render problematic the clinical use of CRP as a routine risk factor marker. Importantly, if such changes reflected a fluctuating inflammatory state, they would raise the possibility that the susceptibility to acute coronary episodes is not static, but might vary as a function of an individual's dynamic inflammatory biologic status. We undertook this study to ascertain whether fluctuations in CRP levels occurred over different intervals in various groups of clinically stable patients with ischemic heart disease.

METHODS

The present report is a composite study, partly retrospective and partly prospective, of successive serum measurements of high-sensitivity CRP levels in 159 clinically stable patients with ischemic heart disease who belonged to 1 of 5 groups constituted for clinical studies. Serum levels of CRP were measured (1) to relate the results to different manifestations of ischemic heart disease; or (2) to investigate the effect of placebo on CRP in a randomized study; or (3) prospectively, for the purpose of the present study. For all patients, the diagnosis of ischemic heart disease required a documented myocardial infarction and/or angiographically documented stenosis (≥70%) of at least 1 major coronary vessel. Clinical stability was defined as the absence of an acute coronary event (unstable angina or myocardial infarction) in the 6 months prior to any CRP measurement. Patients were eligible for study recruitment and CRP assessment if a struc-
tured questionnaire ascertained the absence of any chronic or recent (≤1 month) clinically significant infectious or inflammatory condition including asthma, trauma, vaccination, any invasive medical/surgical (≤3 months) or dental (≤1 month) procedure, or any recent use (≤1 month) of anti-inflammatory or anti-biotic drugs. All studies were approved by the hospital ethics committee and all patients had given written informed consent.

The patients in group 1 (n=15) had a history of 2 or more acute coronary events, at least 1 of which was myocardial infarction. They were selected for a clinical trial designed to evaluate the impact of a medication on inflammatory markers, after a screening process that required that 2 of 2 CRP values be greater than 2 mg/L within 14 to 21 days. These patients represented the arm of the study randomized to placebo and had 6 further CRP measurements (at time 0 and at 1, 3, 6, 7, and 9 months). The inflammatory cytokine interleukin (IL)-6 was also measured at these 6 times.

The patients in group 2 (n=43) came from the same screening process as those in group 1. They belonged to group 2 either because their second CRP value was 2 mg/L or less or because, although it was greater than 2 mg/L, they declined to participate in the group 1 study. Their 2 screening CRP values are the object of this analysis.

The patients in group 3 (n=62) came from the same screening process that yielded groups 1 and 2. In contrast to the patients in these groups, their first CRP value was 2 mg/L or less. They had a second CRP measurement 2 years later for the prospective investigation of the present study.

The patients in group 4 (n=27) had a history of 3 or more years of stable angina with no previous acute coronary event. They had a second CRP measurement if they did not experience an intervening acute coronary event.

The patients in group 5 (n=12) had stable angina, and an electrocardiographic exercise test demonstrated ischemia. They participated in a study of successive exercise tests, and 3 CRP measurements were prospectively performed at 3-week intervals.

LABORATORY MEASUREMENTS

Blood sampling was performed in the nonfasting state for groups 1, 2, and 3 and in the fasting state for groups 4 and 5, always in the same conditions within each group. C-reactive protein levels were either immediately measured (the first values of groups 2 and 3) or CRP was frozen at −80°C to be later measured in batch (for groups 1, 4, and 5, and for the second CRP values of groups 2 and 3). The N-Latex CRP monoaosay was used (interassay reproducibility, 3.6%-4.4%; assay range, 0.18-1100 mg/L; sensitivity, 0.18 mg/L) with the Behring Nephelometer 100 Analyzer (Dade Behring, Mississauga, Ontario). C-reactive protein from group 4 was also measured at another research facility to compare interlaboratory values using the same technique. Interleukin 6 (in group 1) was measured by quantitative sandwich enzyme immunoassay (Roche Diagnostics GmbH, Mannheim, Germany) (intraassay variance <8%).

STATISTICAL ANALYSIS

C-reactive protein levels were examined using the categories of low risk (<1 mg/L), average risk (1-3 mg/L), and high risk (>3 mg/L) proposed by the American Heart Association and the Centers for Disease Control and Prevention. To measure individual variability in CRP values over time, we created a 2-level hierarchical model. At the first level, the CRP value for each patient at each time point is assumed to follow a normal distribution, with each patient assigned his/her own mean parameter and (given these patient-specific mean parameters) constant variance across patients. At the second level, the individual means from the first level are assumed to follow a normal distribution with overall mean and variance parameters. We also tried gamma and log-normal models at the second level but found that the normal distribution fits better, and we report the results from the latter. The variance from the first level is of primary interest, as it represents a summary of within-individual variance of CRP. The second-level variance parameter measures the degree to which individual mean CRP levels vary from one subject to another. This model was run within each of our 5 groups, for all patients together, and for IL-6 values within group 1. In some analyses, only the first 2 CRP and IL-6 values were used for each patient, while in others, all data at all times were used. In all cases, the first CRP value of a patient from group 1, which was inexplicably very elevated, was excluded. The McNemar and paired t tests were used to analyze differences in the clinical characteristics of groups 3 and 4 between their 2-year and 6-year CRP blood sampling, respectively. The relation between the 2 CRP values per patient in groups 2, 3, and 4 was analyzed using Pearson correlation coefficients. To evaluate the effect of confounding variables, 2 statistical models were used, the first with the confounding variable in the model and the second with the variable excluded. The estimate of the variable of main interest, with its associated variance in the first model, was compared with the estimate obtained from the second model. Comparisons of first and second CRP values for all patients and for the 70 patients with 2 measurements within 1 month were made after log transformation because of the skewed distribution of CRP values. The relation between interlaboratory CRP values was examined by the method of Bland and Altman. The statistical package program SAS (SAS Institute Inc, Cary, NC) was used and the hierarchical model was fit using WinBUGS, version 1.4 (Medical Research Council Biostatistics Unit, Cambridge, England).

RESULTS

The clinical characteristics of groups 1 through 5 are shown in the Table, including the characteristics of the patients in groups 3 and 4 at the second CRP measurement 2 and 6 years, respectively, after the first measurement. The CRP values of patients in groups 1 through 5 at all sampling times and IL-6 values of the patients in group 1 are plotted in Figure 1. Within-patient variance of CRP values for the 159 patients was considerable (1.79 mg/L [95% confidence interval (CI), 1.60-2.00] for the first 2 measures in all patients and 1.73 mg/L [95% CI, 1.59-1.89] for all values in all patients). Within-patient variance of IL-6 was also marked (2.69 pg/mL [95% CI, 2.29-3.18]). In group 1, when the 8 CRP values, taken over 10 months, were examined in the low-risk (<1 mg/L), the average-risk (1-3 mg/L), and the high-risk (>3 mg/L) ranges, the values of 6 patients (40%) remained in the same range for all measurements, those of 7 patients (47%) were in 2 risk ranges, and those of 2 patients (13%) were spread over the 3 risk ranges. Similarly, in group 5, over 9 weeks, 5 patients (42%) had their 3 values in the same range, those of 6 patients (50%) were in 2 ranges, and 1 patient had a value in each of the 3 ranges. The 2 CRP values of groups 2, 3, and 4 had moderate to low correlations, with 36%, 29%, and 9%, respectively, of the second CRP measurement accounted for by the first. In groups 3 and 4, adjustment for changes over time in body mass index, low-density lipoprotein cholesterol levels, smoking status, and medications that could affect CRP (Table), and adjustment for the occurrence of acute coronary events that occurred between the CRP sampling times.
We compared the first and second CRP values in all 159 subjects in relation to risk range (Figure 2). Patients were fairly evenly distributed among the 3 ranges. Sixty-four patients (40%) changed risk category at the second measurement. Similar percentages of patients in each initial range remained in the low-, average-, and high-risk groups, respectively, changed risk categories at the second CRP measurement. The mean and median values of the first CRP measurement were 2.93 ± 3.64 mg/L and 1.70 mg/L, while the corresponding values of the second CRP measurement were 2.72 ± 2.71 mg/L and 1.60 mg/L. (P = .7).

We examined the 70 patients who had 2 CRP measurements within 1 month (Figure 3). Although the interval was short, 50%, 45.5%, and 38.1% of the patients in the low-, average-, and high-risk groups, respectively, changed risk categories at the second CRP measurement. The mean and median values of the first CRP measurement were 4.98 ± 4.42 mg/L and 3.45 mg/L, while the corresponding values of the second CRP measurement were 3.77 ± 2.98 mg/L and 2.70 mg/L (P < .001).

We analyzed groups 1 and 5 combined because they had at least 3 CRP values recorded over a 2- to 3-month interval. In this subset, 34.6% of patients changed risk category from the first to the second CRP measurement. We then compared the risk category of the mean of the first 2 CRP values with the third CRP value and found that 38.5% of these patients again changed risk category at the third measurement.

CRP measurements performed in 2 laboratories at a single time point were evaluated for group 3. Although the control laboratory had slightly higher values than the study laboratory, suggesting a more sensitive measurement, reproducibility was consistent and satisfactory (Figure 4). The coefficient of variation of CRP values based on fresh serum was similar to that for frozen samples.

During a median follow-up of 2 years from the last CRP measurement, 9.4% of patients had a cardiovascular event (there were 2 deaths, 5 myocardial infarctions, and 8 unstable anginas) at a median time of 13.0 months (interquartile range, 2.4-18.2 months). There was no significant difference between the last CRP values of those who had an adverse event compared with those who remained event free. A higher CRP value was not associated with an earlier time to event.

in 6 patients of group 3, did not modify the fundamental pattern of variability.

We compared the first and second CRP values in all 159 subjects in relation to risk range (Figure 2). Patients were fairly evenly distributed among the 3 ranges. Sixty-four patients (40%) changed risk category at the second measurement. Similar percentages of patients in each initial range changed range at the second CRP measurement, and similar percentages of patients went into a higher (22%) and a lower (18%) risk range at the second measurement. Despite the considerable fluctuations in CRP values in the aggregate and within each group, there was no significant difference between the first and second values. The mean ± SD and median values of the first CRP measurement were 2.93 ± 3.64 mg/L and 1.70 mg/L, while the corresponding values of the second CRP measurement were 2.72 ± 2.71 mg/L and 1.60 mg/L (P = .7).

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**Abbreviations:** ACE, angiotensin-converting-enzyme; BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); CABG, coronary artery bypass graft surgery; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; PCI, percutaneous coronary intervention.

*Values are given as number (percentage) unless otherwise indicated.

†Visit 2 of group 4 was 6 years later.
‡Visit 2 of group 3 was 2 years later.
§Visit 2 of group 3 was 2 years later.
¶P < .01 vs visit 1.
¥P = .04 vs visit 1.
¥¥P = .03 vs visit 1.
#P = .007 vs visit 1.
Our findings reveal considerable, apparently spontaneous, fluctuation of CRP values in patients with stable ischemic heart disease. Previous small studies have noted significant variability in high-sensitivity CRP values over 4- to 6-month sampling periods in mostly healthy volunteers, with intraindividual variabilities of 42% to 63%. We extend this observation, over varying sampling intervals, to a larger number of patients who spanned a broad clinical spectrum of ischemic heart disease. There were patients with a history of repetitive acute coronary events, patients with ischemia on exercise electrocardiography, some of whom with a history of myocardial infarction, and patients with long-standing angina without evidence of previous acute coronary events. Levels of CRP fluctuated irrespective of clinical profile, sampling interval, and number of measurements. While women and patients with higher low-density lipoprotein cholesterol levels and greater left ventricular dysfunction were less represented in this study, there are no a priori grounds to assume that they would manifest less variability.

The variability that we found could not be attributed to clinically apparent inflammation or infection because the patients were expressly screened to exclude such conditions at each sampling. Nor could it be attributed to changes over time in patients’ clinical condition, body mass index, smoking status, or medication use. While...
uncontrolled changes in dosages of some medications may have contributed to CRP variability, this effect is probably negligible because variability was similar whether the sampling interval was 2 to 9 weeks or 2 to 6 years, when changes in dosages would be more likely to have occurred. Finally, although our laboratory has several years’ experience with the same highly sensitive CRP measuring technique, we assured ourselves of the reproducibility of its technique with another laboratory.

The notion that CRP values are sufficiently stable for prognostic stratification is largely based on findings from 2 studies. In one, a study of serial CRP measurements in 113 healthy adults, it was claimed that the stability of CRP values was similar to that of cholesterol values. However, to establish classification accuracy, CRP values were divided into 4 arbitrary and unequal groups (≤0.50, 0.50-0.99, 1.00-1.99, and ≥2.00 mg/L), while cholesterol was divided into quartiles. Since there was considerably less variability in the lower ranges, in which most CRP values were located, and since all CRP values of 2 mg/L or greater were classified in the same group, this would suggest a stability of CRP more apparent than real. In addition, if CRP tends to be more stable in healthy individuals, this might not be the case in patients with coronary artery disease; in these patients, CRP values are likely to be higher and could be subject to greater fluctuations. In the second study, a drug trial in patients with previous myocardial infarction, CRP was measured at baseline and 5 years later in a randomly selected subset of 214 placebo-arm patients. Subjects with any CRP value greater than 3 SDs above the mean were excluded. The median CRP hardly changed in the 5-year interval and the age-adjusted correlation coefficient of log-normalized CRP value at baseline and 5 years was 0.60 (P < .001). However, log-transformation considerably attenuates the variance of the data and the exclusion of subjects with outlying values may be questioned. Thirteen (8.2%) of our patients had CRP values greater than 3 SDs above the mean. Importantly, the considerable fluctuation between 2 measurements in the same individual can be obscured, if not cancelled out, when means or medians of a large group are examined. It is striking how different—and hazardous—inferences may be made in this way. Thus, when we compared the first with the second CRP value in all patients, irrespective of sampling interval, there was no significant difference despite considerable individual fluctuations, which conveyed an impression of stability of values. But when we performed another analysis restricted to the patients who had 2 measurements within 1 month, the second value was significantly decreased, a likely effect of regression to the mean since a large proportion of this subset was in an initially high CRP range owing to the CRP selection criteria of groups 1 and 2. Although such a directional effect may be expected, its degree is problematic since there was no reason for values to change so mark-

Figure 2. Change in risk category from first to second C-reactive protein (CRP) value obtained from all 159 patients in relation to low-, average-, and high-risk ranges.

Figure 3. Change in risk category from first to second C-reactive protein (CRP) value obtained from 70 patients (groups 1, 2, and 5 combined) whose second serum CRP concentration was measured within 1 month of the first.

Figure 4. Representation, following the method of Bland and Altman, of the relation between the interlaboratory C-reactive protein (CRP) values of the patients in group 4.
edly within such a short interval in patients who had been carefully screened to ensure clinical stability and the absence of potentially confounding factors. The magnitude of change was considerable since more than 40% of patients, whether in the short or longer term, changed risk categories on a second measurement irrespective of the risk category of their first measurement.

These analyses highlight the complexities and pitfalls of evaluating the clinical pertinence of isolated or even serial CRP values. Even when we performed the exercise of considering the mean of 2 CRP values taken at a 2- to 4-week interval, the risk category changed at a third short-term measurement in nearly 40% of the patients. This raises questions regarding the recommendation of a joint committee of the American Heart Association and the Centers for Disease Control and Prevention that the mean of 2 values separated by 2 weeks be obtained to provide a more stable estimate of an individual’s CRP risk status, or a recent analysis favoring 3 serial measurements. On the other hand, it is well accepted that CRP values can identify, in large long-term studies, cohorts of subjects at higher or lower risk of acute coronary disease, albeit with low positive predictive values. C-reactive protein level has also been found to be a powerful predictive tool in very selected subsets of patients with ischemic heart disease at particularly high risk for recurrent acute coronary events.

This attests to the strength of this biological marker and the pathophysiological importance of inflammation in the atherosclerotic disease process, despite the considerable potential for confounding “background noise” and for apparently spontaneous fluctuations in CRP values. Nevertheless, our findings suggest that extrapolating CRP data from epidemiological studies and small selective clinical studies to the broad clinical setting may be problematic when the aim is to stratify coronary risk in individual patients and monitor their status and response to medical interventions.

Because of important fluctuations in the inflammatory cytokine IL-6, these results go further; they suggest that it is inflammatory status, rather than CRP, that is the active variable. This raises the possibility that the inflammatory profile of individuals with apparently stable ischemic heart disease may be more dynamic than generally assumed, and more complex than a relatively static inflammatory state determined by the measurement, at 1 time point, of 1 inflammatory marker. It is intriguing to speculate that the risk of acute coronary disease could be modulated on a temporal vulnerability that is a function of an individual’s fluctuating inflammatory status. The determinants of these fluctuations of an individual’s inflammatory profile in the clinically quiescent state are unknown. Subclinical infectious or inflammatory conditions may be responsible. A promising avenue for future research is suggested that may provide clues to the triggers of acute vascular events.

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