Variability of C-Reactive Protein Levels among Patients with Stable Coronary Artery Disease and on Statin Therapy

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ABSTRACT: **Background:** High sensitivity C-reactive protein, a marker of inflammation, has been proposed to stratify coronary artery disease risk and is lowered by HMG-CoA reductase (statin) therapy. However, the reproducibility of persistently elevated hs-CRP levels and association with other markers of inflammation in patients with stable CAD on aggressive statin therapy is unknown.

Objectives: To determine the reproducibility of hs-CRP levels measured within 2 weeks in patients with documented CAD with stable symptoms and to identify associations with other markers of inflammation.

Methods: Levels of hs-CRP were measured twice within 14 days (7 ± 4) in 23 patients (22 males and 1 female, average age 66 ± 10 years) with stable CAD and hs-CRP ≥ 2.0 mg/L but ≤ 10 mg/L at visit 1. All patients had received statins for cholesterol management (low density lipoprotein-cholesterol 84 ± 25 mg/dl) with no dose change for › 3 months. None had a history or evidence of malignancy, chronic infection or inflammation, or recent trauma. There was no change in medications between visits 1 and 2, and no patient reported a change in symptoms or general health during this interval. White blood cell count and pro- and anti-inflammatory cytokines were measured at both visits.

Results: hs-CRP levels tended to be lower at visit 2 (median 2.4 mg/L, range 0.8–11 mg/L) than at visit 1 (median 3.3 mg/L, range 2.0–9.7; $P = 0.1793$). However, between the two visits hs-CRP levels decreased by more than 1.0 mg/L in 10 patients and increased by more than 1.0 mg/L in 4 patients. Changes in hs-CRP levels were unrelated to changes in levels of white blood cells ($P = 0.4353$). Of the cytokines tested, only the anti-inflammatory cytokine interleukin-1 receptor antagonist and the pro-inflammatory cytokine interleukin-8 were above lower limits of detection, but there were no correlations between changes in these values and changes in hs-CRP (both P › 0.5).

Conclusions: In stable CAD patients on aggressive statin therapy, hs-CRP levels may fluctuate over brief periods in the absence of changes in health, cardiac symptom status and medications, and without corroboration with other measures of inflammation. Accordingly, elevated hs-CRP levels should be interpreted with caution in this setting.

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KEY WORDS: C-reactive protein, coronary artery disease, statin therapy, vascular inflammation, risk stratification

C-reactive protein measured by high sensitivity assays is widely accepted as an independent marker of cardiovascular risk in otherwise healthy populations, based on multiple observational studies and randomized clinical trials [1-12]. The American Heart Association/Centers of Disease Control Task Force considers hs-CRP values ≤ 1 mg/L as low risk, 1–3 mg/L as intermediate risk, and ≥ 3 mg/L as high risk for future cardiovascular events and death in apparently healthy populations [13]. The use of hs-CRP for risk stratification in patients with established coronary artery disease was not recommended in the AHA/CDC guidelines but is supported by clinical trials, including a large trial that was conducted in patients with stable symptoms [14]. Although HMG-CoA reductase inhibitors (statins) have been shown to reduce the risk of recurrent cardiovascular events in CAD patients, persistent elevation of hs-CRP despite statin therapy may indicate continued high risk [15-20].

Concern has been raised by several groups that levels of hs-CRP may fluctuate over time, which may limit its clinical usefulness in individual patients as compared to large cohorts [21,22]. Studies raising these concerns, however, were performed in the absence of statin therapy. The purpose of our study was twofold: to determine the reproducibility of hs-CRP levels measured within 2 weeks in patients with documented CAD and stable symptoms, and to identify associations with other markers of inflammation.

PATIENTS AND METHODS

Patients with documented CAD who were on medical management and who reported no cardiac symptoms (Canadian

Hs-CRP = high sensitivity C-reactive protein
CAD = coronary artery disease
AHA/CDC = American Heart Association/Centers of Disease Control

Cardiovascular Society Functional Class I) consented to undergo testing for hs-CRP. Those found to have hs-CRP levels ≥ 2 and ≤ 10 mg/L returned for repeat testing within 2 weeks. All patients received statins with no dose change for > 3 months. None had a history or evidence of malignancy, chronic infection or inflammation, or recent trauma. There was no change in medications between visits 1 and 2, and no patient reported a change in cardiac status or general health during this interval. Vital signs and complete blood count were measured on both visits. Levels of hs-CRP were measured twice within 14 days (7 ± 4 days) using a high sensitivity (0.1 mg/L), solid-phase chemiluminescent immunometric assay. A multiplex assay was performed to quantify the following cytokines: tumor necrosis factor-alpha, interferon-gamma, interleukin-1 alpha, interleukin-1 beta, IL-1 receptor antagonist, interleukin-2, interleukin-6, and interleukin-8. The protocol was approved by the Institutional Review Board of the U.S. National Heart Lung and Blood Institute.

Statistical analysis was done using the paired *t*-test for all variables, except for hs-CRP, which was analyzed with the Wilcoxon non-parametric t-test.

Table 1. Clinical, hematological and biochemical parameters of study patients

	Visit 1	Visit 2	*P* value
Systolic blood pressure (mmHg)	131 ± 16	131 ± 15	0.972
Diastolic blood pressure (mmHg)	74 ± 8	75 ± 9	0.85
Heart rate (beats/min)	65 ± 10	60 ± 9	0.014
Hemoglobin (g/dl)	14.4 ± 1.3		
White blood cells (/µl)	9517 ± 1473	6409 ± 1580	0.3180
Platelets (/µ/l)	227,400 ± 61,904	230,260 ± 55,245	0.8735
Creatinine (mg/dl)	1.22 ± 0.23		
Bilirubin (mg/dl)	0.88 ± 0.25		
Aspartate aminotransferase (U/L)	27.5 ± 7.4		
Alanine aminotransferase (U/L)	25.1 ± 6.7		
Cholesterol (mg/dl)	147 ± 29		
LDL-C (mg/dl)	84 ± 25		
HDL-C (mg/dl)	48 ± 12		
Trigycerides (mg/dl)	125 ± 80		

LDL-C = low density lipoprotein-cholesterol, HDL-C = high density lipoprotein-cholesterol

RESULTS

Of the 63 patients (58 men and 5 women, age 66 ± 10 years old) who participated in the screening protocol, 23 were found to have hs-CRP levels (22 men and 1 woman, age 66 ± 10). The levels were ≥ 2 and ≤ 10 mg/L at visit 1, and the patients were told to return for a repeat measurement within 14 days (7 ± 4 days).

One patient had diabetes mellitus type 2 (well controlled), and 12 patients were smoking. Nineteen had high blood pressure, but it was well controlled [Table 1]. The patient group comprised 17 Caucasians, 4 African-Americans and 2 Asians. Average body mass index was 29 ± 4 kg/m^2 (range 20–40 kg/m^2). Hs-CRP levels at visit 1 were 3.3 mg/L (median value, range 0.8–11 mg/L) and were not correlated with BMI (P = -0.084), white blood cells (P = 0.081), total cholesterol (P = -0.07), high density lipoprotein-cholesterol (P = -0.119), and triglycerides (P = -0.166). LDL-cholesterol was inversely correlated with hs-CRP (P = -0.022) [Table 1].

Hs-CRP levels tended to be lower at visit 2 (median 2.4 mg/L, range 0.8–11) compared with visit 1 (median 3.3 mg/L, range 2.04–9.65; P = 0.1793). Between the visits hs-CRP level decreased by more than 1 mg/L in 10 patients and increased by more than 1 mg/L in 4 patients. More than that, of the 15 patients whose hs-CRP decreased between the two visits, 5 patients changed their risk status from "high risk" (> 3 mg/L) to "intermediate risk" (1–3 mg/L), and 6 patients changed their risk status from "intermediate risk" to "low risk" (< 1 mg/L). Among the eight patients whose hs-CRP increased, two changed their risk status from "intermediate risk" to "high risk" [Figure 1].

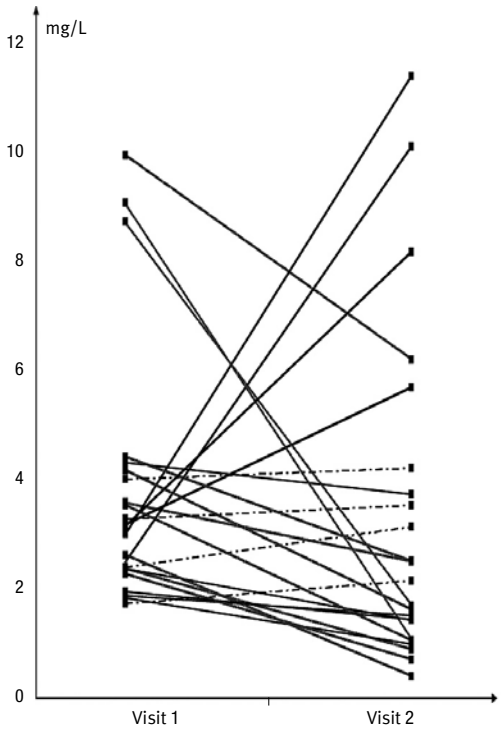
No correlation was found between the change in hs-CRP and the change in WBC between visits (r = 0.1962, P = 0.4353). Among the pro-inflammatory cytokines only IL-8 was above the lower limit of detectability at visits 1 and 2 (22.9 ± 5.9 vs. 21.4 ± 4.7 pg/ml, P = 0.18). Among the anti-inflammatory cytokines that were measured only IL-1 receptor antagonist was detectable and there was no difference between the two visits (1284.4 ± 629.1 vs. 1302.1 ± 1413.3 pg/ml, P = 0.95). There was no correlation between the change in hs-CRP and the change in IL-8 (r = -0.1266, P = 0.5648) or between the change in hs-CRP and the change in IL-1ra (r = -0.06017, P = 0.7851).

We correlated the changes in hs-CRP levels (between visit 1 and 2) with the changes in BMI (r = 0.0438, P = 0.8502), systolic blood pressure (r = -0.3765, P = 0.1245), diastolic blood pressure (r = -0.3765, P = 0.1018), heart rate (r = 0.1275, P = 0.5922), hemoglobin (r = 0.2304, P = 0.3285), platelets (r = 0.2384, P = 0.3408), creatinine (r = 0.1039, P = 0.7019), creatine phosphokinase (r = 0.4681, P = 0.0914), bilirubin (r = -0.0480, P = 0.8649), aspartate aminotransferase (r = 0.2606, P = 0.3682) and alanine aminotransferase (r = 0.3484, P = 0.2222).

BMI = body mass index
LDL = low density lipoprotein

WBC = white blood cells
IL-8 = interleukin-8
IL-1ra = interleukin-1 receptor antagonist

Figure 1. High sensitivity C-reactive protein levels on visit 1 and visit 2



DISCUSSION

THE VARIABILITY OF HS-CRP REPETITIVE MEASUREMENTS

We found that hs-CRP levels in patients with stable CAD on statin therapy were not consistent and may change significantly within days – changes that may affect the risk assessment of patients at risk to develop future cardiovascular events. There were no correlations between changes in hs-CRP levels and vital signs or biochemical and hematological markers that were measured, or with the cytokines that were measured at both visits.

When AHA/CDC cutoff points for risk were extended to CAD patients, the risk status changed from high risk (> 3 mg/L) at visit 1 to intermediate risk (1-3 mg/L) at the second visit in five patients, from intermediate risk in the first visit to low risk (< 1 mg/L) in the second visit in six patients, and from intermediate risk in the first visit to high risk in the second visit in two patients.

A prospective study examined serial (2–8 measurements) serum CRP values in 159 patients with ischemic heart disease, at intervals varying from 15 days to 6 years [23]. Blood samples were taken when patients were clinically stable and without any potentially confounding inflammatory condition. In this study [23] the CRP values in individual patients fluctuated considerably when examined in the following ranges: < 1 mg/L, 1–3 mg/L, and > 3 mg/L. Sixty-four patients (40.3%) changed risk category between the first and the second measurement. The within-patient variance of CRP was 1.79 mg/L (95% confidence interval 1.60–2.00) and the variability of CRP was consistent over different times and across clinical groups, independent of body mass index, smoking, medication, and clinical events [23]. These findings reveal considerable, apparently spontaneous fluctuations of CRP values in patients with stable ischemic heart disease. Previous small studies have noted significant variability in hs-CRP values over 4 to 6 month sampling periods in mostly healthy volunteers, with intra-individual variability of 42–63% [24].

HS-CRP AND ANXIETY

We noticed among our patients that whenever they experienced a difficult time (such as death in the family) their hs-CRP levels increased. In the ATTICA study (a study conducted in the area of Attica in Greece), 453 men (age 19–89) and 300 women (18–84 years old) were enrolled to a prospective study where various inflammatory markers and coagulation factors were evaluated in relation to the anxious state (assessed by the Spielberger State Anxiety Inventory) after several adjustments made for potential confounders. The STAI score was positively correlated with CRP, tumor necrosis factor, IL-6, homocysteine and fibrinogen levels in men, and positively correlated with CRP, white blood cell counts, IL-6, homocysteine and fibrinogen levels in women [25]. Links between psychological factors and heart disease have been hypothesized for centuries, but during recent decades epidemiological evidence suggests that a relationship exists between emotional states, such as anxiety and depression, and the development of cardiovascular disease [25]. However, these studies provided little evidence regarding the relationship between anxiety and the pathogenesis of coronary heart disease.

CONCLUSIONS

Even in the absence of changes in health or medications, CRP levels may fluctuate over brief periods in CAD patients. Consequently, repeat measurements of CRP are needed to estimate the inflammatory status of these patients.

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STAI = Spielberger State Anxiety Inventory

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