Variability and Classification Accuracy of Serial High-Sensitivity C-Reactive Protein Measurements in Healthy Adults

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Background: Increased concentrations of high-sensitivity C-reactive protein (hs-CRP), a marker of systemic inflammation, are associated with increased risk for coronary heart disease. Because of its relationship to inflammation, hs-CRP has considerable biologic variation. This study was carried out to characterize CRP variation and to compare it to another risk factor, total serum cholesterol.

Methods: One hundred thirteen individuals were scheduled to have five measurements each of hs-CRP and total cholesterol carried out at quarterly intervals over a 1-year period. Variations of hs-CRP and total cholesterol were characterized, and classification accuracy was described and compared for both.

Results: The relative variation was comparable for hs-CRP and total cholesterol. When classified by quartile, 63% of first and second hs-CRP measurements were in agreement; for total cholesterol it was 60%. Ninety percent of hs-CRP measurements were within one quartile of each other. This relationship was not altered by the use of log-transformed hs-CRP data.

Conclusion: hs-CRP has a degree of measurement stability that is similar to that of total cholesterol.

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Recent prospective studies have reported that modest increases in markers of low-level systemic inflammation are associated with increased risk for coronary heart disease (CHD)7 (1). In a prospective study of apparently healthy middle-aged men, the risk for first myocardial infarction was increased 2.9-fold among those with baseline concentrations of high-sensitivity C-reactive protein (hs-CRP) in the highest compared with the lowest quartile (2). Similar findings have been reported in women (3, 4) and the elderly (5, 6). Thompson et al. (7) found a direct correlation between the incidence of coronary events and CRP concentrations, but adjustment for fibrinogen explained much of the association.

hs-CRP has been noted to add to the prediction of first myocardial infarction when combined with blood lipid measures (8). Given these findings, hs-CRP has been suggested as a potential risk factor for CHD (9, 10), and investigators have begun to seek therapies that may lower hs-CRP concentrations and, presumably, CHD risk (9, 11, 12).

CRP differs from other markers for CHD risk obtained from blood (e.g., lipoproteins) because it is an acute phase reactant (13). Increased hs-CRP concentrations reflect the presence and intensity of inflammation and, in response to injury or acute infection, can rapidly increase as much as 1000-fold from basal concentrations, declining to baseline over a period of 7–12 days (13). Studies of the distribution of hs-CRP that have excluded individuals with acutely increased values have found median values of ∼1–2 mg/L with the upper end of the reference interval (i.e., 97.5th percentile) between 3.2 and 5.5 mg/L (14, 15). Higher hs-CRP concentrations have been noted in smokers (16), individuals with osteoarthritis (17), and...
in the obese (18). For measurement of hs-CRP to have clinical utility as a CHD risk factor, the biologic variability of hs-CRP must be quantified and be low enough to enable reliable risk stratification with one or two blood samples. In a recent study of 214 postinfarction patients, the correlation coefficient between baseline hs-CRP concentrations (obtained at least 6 months post infarction) and values obtained 5 years later was 0.60 (P < 0.001), a degree of correlation that compares favorably to that for total cholesterol (r = 0.37) (19). Most other longitudinal studies of hs-CRP variability have been conducted in small (n < 30) homogeneous cohorts over relatively short periods of follow-up (6 months) (14, 20) and were limited by lack of gender- and age-specific comparisons. Moreover, the reliability of risk classification using serial hs-CRP measurements has not been formally examined. The purpose of the present investigation was to quantify the biologic variability of hs-CRP and to examine the risk classification accuracy of serial hs-CRP samples within the reference interval in a large heterogeneous cohort of healthy adults who were each followed for a 1-year period.

**Materials and Methods**

**PARTICIPANT RECRUITMENT AND STUDY DESIGN**

The Seasonal Variation of Blood Cholesterol Study (SEASON) was an observational longitudinal study of 641 healthy adults designed to quantify the magnitude and timing of seasonal changes in blood lipids and to identify the major factors contributing to this variation (21). These factors included dietary intake, physical activity, exposure to light, psychological variables, weather patterns, and changes in body mass. A subgroup of SEASON participants (n = 113) were recruited to participate in an ancillary study examining seasonal variation in additional blood indices, including hemostatic factors, apolipoproteins, antioxidants, and hs-CRP. Participants in this subgroup of the SEASON study form the cohort of the present investigation.

SEASON participants were recruited from the Fallon

<table>
<thead>
<tr>
<th>Variable</th>
<th>All a (n = 113)</th>
<th>Males a (n = 64)</th>
<th>Females a (n = 49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>49.0</td>
<td>49.8</td>
<td>48.1</td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>52</td>
<td>48.5</td>
</tr>
<tr>
<td></td>
<td>20–70</td>
<td>24–70</td>
<td>20–70</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.6</td>
<td>28.5</td>
<td>26.5</td>
</tr>
<tr>
<td></td>
<td>27.0</td>
<td>27.5</td>
<td>25.4</td>
</tr>
<tr>
<td></td>
<td>17.7–46.8</td>
<td>18.9–46.8</td>
<td>17.7–41.4</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L (mg/dL)</td>
<td>5.77 (223.1)</td>
<td>5.82 (224.9)</td>
<td>5.71 (220.8)</td>
</tr>
<tr>
<td></td>
<td>5.77 (223)</td>
<td>5.91 (228.6)</td>
<td>5.62 (217.2)</td>
</tr>
<tr>
<td>LDL, mmol/L (mg/dL)</td>
<td>3.82 (147.8)</td>
<td>3.86 (149.3)</td>
<td>3.77 (145.9)</td>
</tr>
<tr>
<td></td>
<td>3.79 (146.5)</td>
<td>3.85 (149)</td>
<td>3.73 (144.2)</td>
</tr>
<tr>
<td></td>
<td>1.09–6.53 (42–252.6)</td>
<td>42–221 (42–221)</td>
<td>92–252.6 (92–252.6)</td>
</tr>
<tr>
<td>HDL, mmol/L (mg/dL)</td>
<td>1.19 (46.0)</td>
<td>1.11 (43.0)</td>
<td>1.29 (49.9)</td>
</tr>
<tr>
<td></td>
<td>1.16 (44.8)</td>
<td>1.05 (40.6)</td>
<td>1.27 (49.2)</td>
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<tr>
<td></td>
<td>0.66–2.13 (25.7–82.4)</td>
<td>0.66–2.02 (25.7–78)</td>
<td>0.76–2.13 (29.2–82.4)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L (mg/dL)</td>
<td>1.71 (151.2)</td>
<td>1.92 (169.8)</td>
<td>1.43 (126.9)</td>
</tr>
<tr>
<td></td>
<td>1.50 (132.5)</td>
<td>1.65 (146.4)</td>
<td>1.18 (104.8)</td>
</tr>
<tr>
<td></td>
<td>0.46–7.23 (40.8–640.8)</td>
<td>0.46–7.23 (40.8–641)</td>
<td>0.53–4.22 (47.2–374)</td>
</tr>
<tr>
<td>Energy intake, kJ (kcal)</td>
<td>8301 (1886)</td>
<td>9288 (2222)</td>
<td>7014 (1678)</td>
</tr>
<tr>
<td></td>
<td>7854 (1879)</td>
<td>9196 (2200)</td>
<td>6801 (1627)</td>
</tr>
<tr>
<td>hs-CRP, mg/L</td>
<td>2.82</td>
<td>3.22</td>
<td>2.31</td>
</tr>
<tr>
<td></td>
<td>3.42</td>
<td>2.21</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>0.1–16.2</td>
<td>0.1–14.9</td>
<td>0.2–16.2</td>
</tr>
</tbody>
</table>

a Values given as mean, median, range.
b HS, high school; Assoc, Associate's; Bach, Bachelor's; Grad, graduate.
Healthcare System, a health maintenance organization serving the central Massachusetts area. Additional minority participants were recruited from the greater Worcester area. Individuals were eligible if they were residents of Worcester County, 20–70 years of age, had telephone service, and were not taking cholesterol-lowering medication. Recruitment was carried out between December 1994 and February 1997, and follow-up was completed in March 1998. The Institutional Review Boards of the Fallon Healthcare System and the University of Massachusetts Medical School approved all participant recruitment and data collection procedures. Each participant read and signed an approved informed consent.

At baseline and in each of four subsequent quarters of follow-up (at 90-day intervals), individuals came to the clinic to provide blood samples, have their body mass measured, and to return a series of self-administered questionnaires. Physical activity, diet, and light exposure data were collected using three 24-h recall interviews of each of the five data collection points.

CLINICAL MEASUREMENTS
Demographic data (e.g., age, gender, marital status, education, employment) were collected by self-administered questionnaire at study baseline. Anthropometric data, including body mass (kg), height (m), and waist and hip circumferences (cm) were measured during clinic visits by the SEASON staff.

Information regarding infections was collected by self-report of the number of cold or flu episodes in the previous 90 days. The mean (SD) annual number of upper-respiratory tract infections was 1.4 (1.5) events per year. The occurrence of upper-respiratory tract infections peaked in the winter months (~40%) and was lowest in the summer (~10%). These data closely resemble annual and seasonal incidence of upper-respiratory tract infections reported in other investigations using more intensive self-report methods (22, 23).

BLOOD MEASUREMENTS
Fasting (>12 h) venous blood samples were collected into EDTA-containing tubes between 0700 and 0900. Blood plasma was harvested by low-speed centrifugation at 4 °C, aliquoted into individual tubes, and quickly frozen to −70 °C. On a regular basis, plasma samples were packed in dry ice and shipped for analysis via courier service to the Centers for Disease Control and Prevention standardized laboratory for lipid testing at the University of Massachusetts at Lowell (24). Total cholesterol and triglycerides were measured in plasma by enzymatic methods using a Beckman System 700 automated analyzer (25, 26). HDL-cholesterol was measured in the resulting supernatant after heparin manganese precipitation of apolipoprotein-B-containing proteins (27). LDL-cholesterol values were calculated by the Friedewald equation using total cholesterol, triglycerides, and HDL-cholesterol in individuals with triglyceride values ≤4.52 mmol/L (400 mg/dL) (28).

hs-CRP METHODOLOGY
hs-CRP concentrations were determined at Children’s Hospital in Boston using latex-enhanced immunonephelometric assays on a BN II analyzer (Dade Behring) as described previously (29). The assay has a detection limit of 0.15 mg/L and day-to-day imprecision (CV) of ~5% for concentrations of 0.35 and 0.5 mg/L.
Variance components and intraclass correlations were estimated using a random effects analysis of variance model using the loneway procedure in Stata, Ver. 6.0. Categorical variables were compared by Fisher exact tests. Unweighted \( k \) statistics were computed for comparison of classification of the first and second measurements of cholesterol and hs-CRP.

Results

One-hundred thirteen subjects (20–70 years of age) in the SEASON blood substudy had measured hs-CRP values. Of the 113 subjects, 64 (56.6%) were male and 49 (43.4%) were female. Seventy-five (66.4%) had all five quarterly measurements. Twenty-six (23%) had four quarterly measurements and 12 (10.6%) had three quarterly measurements. The mean (SD) interval between blood measures was 96.4 (19.6) days. Table 1 lists the participant characteristics. The values in Table 1 are based on averaged values over all quarterly measurements.

The gender difference in hs-CRP was significant based on a rank-sum test \( (P = 0.04) \). This difference was entirely explained by differences in body mass index (BMI), and after controlling for BMI, there was no longer a significant difference between males and females in mean hs-CRP. Gender differences in hs-CRP have not been previously described \((14,32)\), but the relationship between BMI and hs-CRP has been noted by others \((32)\). Overall concentrations of hs-CRP were comparable to values noted previously in the literature, as obtained by similar methodologies \((3,33)\).

Further analyses were carried out using pooled gender data, both because of the lack of independent significance for this variable and to maintain comparability with total cholesterol, which despite important gender differences does not have gender-specific guidelines \((34)\).

Values of hs-CRP \( >10 \) mg/L were relatively rare in both men (4.8% of 292 hs-CRP measurements) and women (2.8% of 214 measurements). The gender difference was not statistically significant \( (P = 0.36) \). Eighty percent of the 20 values \( >10 \) mg/L were associated with a report of an episode of cold or flu within the prior 90 days, vs only 30.2% for those \(<10 \) mg/L. In subsequent analyses, values \( >10 \) mg/L were removed under the assumption that they represented acute illness or inflammation. After hs-CRP values \( >10 \) mg/L were excluded, the mean (SD) hs-CRP value was 2.05 (1.8) mg/L based on a mean (SD) of 4.4 (1.8) measurements per subject.

In characterizing the variation of hs-CRP, the scale used is an important factor. Fig. 1 illustrates the distribution of hs-CRP and, for comparison, that of total cholesterol (all subjects represented by their mean values for all measurements). The data for total cholesterol are relatively symmetric and can be modeled appropriately by a gaussian distribution. The distribution of hs-CRP was highly skewed even with the higher values removed. Fig. 1C shows the log distribution (hs-CRP), which is much more symmetric and less skewed. A Kolmogorov–Smirnov test indicated that there was no statistical evidence of a deviation from normality for total cholesterol \( (P = 0.78) \) or for log hs-CRP \( (P = 0.20) \), but the test rejected normality for hs-CRP \( (P < 0.001) \). Variation estimates were made in both scales for comparative purposes.

The within-subject data for total cholesterol and hs-CRP, rank-ordered by mean values, are illustrated in Fig. 2. The first hs-CRP plot (Fig. 2, middle panel) is in the

**STATISTICAL METHODOLOGY**

Variance components and intraclass correlations were estimated using a random effects analysis of variance model using the loneway procedure in Stata, Ver. 6.0 \((30)\). Categorical variables were compared by Fisher exact tests. Unweighted \( k \) statistics were computed for comparison of classification of the first and second measurements of cholesterol and hs-CRP \((31)\).
Variation is usually separated into analytic, within-individual (biologic), and between-individual variation. Analytic variation is the variation attributable to measurement error (a sample is analyzed multiple times and variation is estimated). Individual, or biologic, variation is the variation within an individual accounting for or adjusting for analytic variation. Multiple measures within a subject are used to estimate variation, and analytic variation is subtracted out. Between-individual variation is the variation in average response between individuals. With these data, we can estimate within- and between-individual variation where within-individual variation is a combination of analytic and biologic variation.

Variation can be expressed by dividing overall variation into between- and within-subject variation and describing the percentage of overall variation attributable to each component. If we let \( \sigma_b^2 \) be the between-subject variance and \( \sigma_w^2 \) be the within-subject variance, then \( \frac{\sigma_b^2}{(\sigma_b^2 + \sigma_w^2)} \) is the intraclass correlation and 100 × intraclass correlation is the percentage of variation explained by between-subject variation. The remaining variation is the within-subject variation, which is the combined biologic and analytic variation. This is an appropriate comparison if we have a representative sample of individuals.

A random-effects analysis of variance estimated the between-subject standard deviation for total cholesterol to be 0.946 mmol/L (36.6 mg/dL) and the within-subject standard deviation to be 0.447 mmol/L (17.3 mg/dL); for hs-CRP, the between-subject standard deviation was 1.66 mg/L and the within-subject standard deviation was 1.19 mg/L. The estimated intraclass correlation for total cholesterol was 0.82 (82% of variation explained by between-subject variation and 18% by within-subject variation); for hs-CRP, the estimated intraclass correlation was 0.66. Table 2 lists the variance components for each of the measures.

### Table 2. Variance components for total cholesterol and hs-CRP.

<table>
<thead>
<tr>
<th>Value</th>
<th>Mean (Range)</th>
<th>( \sigma_b ) (% total variance)</th>
<th>( \sigma_w ) (% total variance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mmol/L (mg/dL)</td>
<td>5.78 (223.7)</td>
<td>0.95 (36.6)</td>
<td>0.45 (17.3)</td>
</tr>
<tr>
<td>LDL, mmol/L (mg/dL)</td>
<td>2.46–8.94 (95–346)</td>
<td>0.85 (32.7)</td>
<td>0.41 (15.8)</td>
</tr>
<tr>
<td>HDL, mmol/L (mg/dL)</td>
<td>3.85 (148.8)</td>
<td>0.91–6.83 (35–264)</td>
<td>0.85 (32.7)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L (mg/dL)</td>
<td>1.18 (45.8)</td>
<td>0.57–2.43 (22–94)</td>
<td>0.28 (10.9)</td>
</tr>
<tr>
<td>hs-CRP, mg/L</td>
<td>1.71 (151.5)</td>
<td>0.28–13.08 (25–1159)</td>
<td>1.05 (39.3)</td>
</tr>
<tr>
<td>Log hs-CRP, log mg/L</td>
<td>1.96</td>
<td>0.1–9.9</td>
<td>1.66</td>
</tr>
</tbody>
</table>

### Classification Accuracy

To determine classification accuracy, values of hs-CRP were divided into four groups: <0.50, 0.50–0.99, 1.00–1.99, and ≥2.0 mg/L (2).

The agreement between the first and second measurements in all 113 individuals is shown in Fig. 3, which for comparison provides equivalent data for total cholesterol divided into quartiles. Overall, for hs-CRP, 71 of 113 (62.8%) of the results were in agreement. The \( \kappa \) statistic was estimated to be 0.479 (95% confidence interval, 0.39–0.60). When we removed measurement pairs where a value was >10 mg/L, there was a small increase in overall agreement (63.5%), with a \( \kappa \) statistic of 0.511 (95% confidence interval, 0.40–0.62). When we examined the classification accuracy across the 12-month follow-up period (excluding pairs with values >10 mg/L), correct classification was observed for 237 of 374 pairs (63.4%). For total cholesterol, agreement between the first and second measurements was 59.3% with a \( \kappa \) value of 0.456 (95% confidence interval, 0.35–0.56). Thus, variability of repeat
hs-CRP measurements was comparable to that of total cholesterol. In those individuals having at least four hs-CRP measurements, a similar analysis was carried out comparing the average of two randomly selected measures with the average of two other randomly selected measures. The use of four measurements in this manner increased overall agreement to 68%.

We also carried out a similar analysis classifying cholesterol into three groups using the classification methodology of the National Cholesterol Education Program (<200, 200–240, and >240 mg/dL) (34). Use of this scheme improved overall agreement between the first and second measurements to 71.7%, but the improvement was related to the smaller number of groups (three vs four).

**Discussion**

In epidemiologic studies, hs-CRP has been shown to be a useful predictor of future cardiac events (35). In making the transition from large-scale epidemiologic studies to clinical utility in individual patients, several requirements must be met, including a sensitive, accurate, and affordable assay and demonstration of relative stability of classification into risk categories (10). Although hs-CRP is an acute phase reactant, and as such is subject to marked and rapid shifts secondary to intercurrent illness, we have demonstrated that there is considerable stability in the measurement of hs-CRP and that correction for outlying high values yielded only minor changes in these findings. In particular, the stability of hs-CRP measurements compares favorably with that of total serum cholesterol, a standard measurement widely accepted in CHD risk stratification methodology. The analytic component of hs-CRP variability is relatively small, with between-subject variation accounting for most of the observed variability.

Although the stability of hs-CRP measurements is similar to that of total cholesterol, considerable within-subject variability does exist in both, and a single test will have a wide confidence interval (36). Because an analysis using four measurements per individual yielded only a modest increase in classification agreement (68% vs 63% with two measurements), the use of two sequential measures is appropriate for clinical use.

We are not recommending the use of log-transformed data for the clinical use of hs-CRP. A log-transformed number has a fixed relationship to the original number from which it is derived and so is not intrinsically of greater value. The use of log-transformed data does produce interquartile distances that are equal on a measurement scale, but in a clinical setting this is not important because the guideline cutpoints are defined for the clinician, and log-transformed data are not used for other measurements that are equally skewed (e.g., serum triglycerides).

Because hs-CRP is an acute phase reactant, it could be argued that the lowest of several measurements should be used as the predictive value, as opposed to the mean. Although this approach has biologic as well as clinical appeal, insufficient data exist at the present time to make this recommendation.

In conclusion, we believe the current data have several important clinical implications. (a) In a recent study directly comparing the magnitude of predictive value of 12 putative risk factors, including lipoprotein(a), homocysteine, and a full lipid panel, hs-CRP was the single strongest marker of risk for future myocardial infarction and stroke (3), data that underscore the critical role of inflammation in atherothrombosis. (b) Several studies have now shown that hs-CRP concentrations predict coronary risk even in the absence of hyperlipidemia (2, 3, 8), an important issue because one-half of all cardiovascular events occur among individuals with cholesterol concentrations not defined as increased by National Cholesterol Education Program criteria (37). (c) Because statin therapy appears to lower hs-CRP in a cholesterol-independent fashion (19), monitoring the inflammatory response has been suggested as a method to improve utilization of statin therapy, particularly in the primary prevention of vascular events. Thus, the observation in the current data that hs-CRP has a measurement stability similar to that of total cholesterol provides further evidence of the potential clinical utility of hs-CRP screening as a novel tool for vascular risk prediction (38).

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**References**