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Intra-individual variation in serum C-reactive protein over four years: An implication for epidemiologic studies

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Abstract

Background—Data on long-term intra-individual variability in high sensitivity C-reactive protein (hsCRP) are needed to determine whether one measurement adequately reflects usual levels in prospective studies of on the etiology of cancer and other chronic diseases; when not

reflective, the ability to statistically detect modest to moderate associations is reduced. The authors estimated the size of this source of variability and consequent attenuation of the relative risk (RR).

Methods—hsCRP concentration was measured using a high-sensitivity immunoturbidometric assay in sera collected at years 2, 4, and 6 from 50 men in the placebo arm of the Prostate Cancer Prevention Trial (PCPT). After natural logarithm-transformation of hsCRP, analysis of variance was used to estimate the within and between individual variances from which the intra-class correlation coefficient (ICC) was calculated.

Results—The observed RR due to an ICC <1 was calculated by $e^{(\ln \text{ true RR} \times \text{ICC})}$ for a range of true RRs. The four-year ICC was 0.66. Measuring hsCRP once and assuming no other error, if the true RRs were 1.50, 2.00, and 3.00 when comparing high with low concentration then the observed RRs would be 1.31, 1.58, and 2.06, respectively.

Conclusion—Investigators planning to measure hsCRP only once should design adequately sized studies to preserve inferences for hypothesized modest to moderate RRs.

Keywords

C-reactive protein; variability; relative risk

Introduction

The role of the innate immune response in the etiology of cancer and other chronic diseases is of current focus in the epidemiologic literature. C-reactive protein is an acute-phase reactant produced by the liver in response to stimulation by the pro-inflammatory cytokines interleukin-1 β , interleukin-6, and tumor necrosis factor- α . Thus, the production of C-reactive protein serves as an indicator of the activity of the innate immune system. Higher circulating hsCRP concentration has been found to be associated with future diagnosis of cardiovascular disease and diabetes [1] and with colorectal cancer in some, but not all studies [2]. C-reactive protein concentration can be validly and reliably measured in low plasma volume in apparently healthy individuals using high-sensitivity methods [3].

Typically in epidemiologic studies of chronic disease etiology, a blood specimen is collected only on one occasion. To be able to statistically detect modest to moderate associations of high sensitivity C-reactive protein (hsCRP) with chronic disease risk, the level measured at that single time point must adequately reflect the participants' usual levels. Thus, we assessed within- and between-individual variation in serum hsCRP concentration at three time points over a four-year period in healthy older men who participated in a large prostate cancer chemoprevention trial. To highlight an important implication of the choice using a single measurement of hsCRP concentration to reflect usual level in an epidemiologic study, we present the magnitude of attenuation of an association between hsCRP and an outcome due to the size of intra- relative inter-individual variability in hsCRP that we observed in this study.

Materials and methods

For 50 men randomized to the placebo arm of the Prostate Cancer Prevention Trial (PCPT) [4] and who were not diagnosed with prostate cancer, we selected serum samples collected at years 2, 4, and 6. At enrollment into the PCPT the men were ≥ 55 years old and had a normal digital-rectal examination and a serum prostate specific antigen concentration ≤ 3.0 ng/mL. Time of day and date were recorded for each blood collection. Height was measured at baseline and weight was measured at each annual visit; from these, body mass index (BMI; weight in kilograms divided by the square of height in meters) was calculated.

Current cigarette smoking status was collected at randomization. This study was approved by the Institutional Review Board at the Johns Hopkins Bloomberg School of Public Health.

hsCRP was measured using an automated latex-enhanced immunoturbidometric assay performed on a Behring BN II nephelometer (Dade Behring, Deerfield, IL). The three samples for each man were assayed adjacently, but in random order. The laboratory was blinded to quality control samples that we embedded; the coefficients of variation (CV%)s were 0.54% and 0.52% for six replicates each from two pools of serum. The laboratory also included quality control samples with known concentrations of 1.24 mg/L (reflecting a typical concentration) and 6.89 mg/L (reflecting an elevated concentration); the CV% for these samples were 0.60% and 1.20%, respectively. The lower limit of detection was 0.03 mg/L; all of the samples had concentrations above the limit of detection.

We transformed hsCRP using the natural logarithm because the distribution was right skewed. We calculated the crude geometric mean hsCRP and 95% confidence interval (CI) by year of blood collection. We also calculated the geometric mean for each time point adjusted for age, BMI, cigarette smoking status, time of day and season of blood draw, and the residuals of blood storage time regressed on trial year (storage time and trial year were highly correlated), and centered at the mean values for the covariates across the years for comparability. We calculated Pearson correlation coefficients between the hsCRP concentrations for years 2 and 4, years 4 and 6, and years 2 and 6. We performed analysis of variance (random effects) to estimate the within- and between-individual variances, from which the intra-class correlation coefficient (ICC) was calculated as the between-individual variance divided the sum of the within- and between-individual variances. To minimize the influence of acute infections on the results, we repeated the analysis after excluding the one sample for which the concentration was ≥ 10 mg/L. Analyses were performed using SAS v. 9.1 (Cary, NC) and STATA v. 8.2 (College Station, TX). Statistical tests are two sided.

The observed relative risk (RR) estimated in a prospective study if a single measurement of hsCRP were used was calculated for a range of hypothetical true RRs as $e^{(\ln \text{true RR} * \text{ICC})}$, and the extent of the attenuation was calculated as $[\text{true RR} - e^{(\ln \text{true RR} * \text{ICC})}] / \text{true RR}$ [5,6]. We used the ICC from this study and assumed that there were no other sources of error.

Results

The mean (standard deviation) age and BMI of the men at year 2 were 64.9 (6.6) years and 27.7 (5.2) kg/m², respectively (Table 1). Only 10% of the men currently smoked at randomization. Taking into account repeated measures, BMI was positively associated with hsCRP ($p=0.01$). Age (continuous), cigarette smoking status at randomization, season of the year (winter versus other season) and time of day of blood draw (noon or later versus before noon), and the residuals of blood storage time regressed on trial year (continuous) were not statistically significantly associated with hsCRP. Crude, age-adjusted, and multivariable-adjusted hsCRP at years 2, 4, and 6 were similar (Table 2).

Figure 1 shows that for most men there was little variability in hsCRP over time, although for a few the variability was large. The intra-individual correlations between each time point were as follows: years 2 and 4 (0.69, $p<0.0001$), 4 and 6 (0.70, $p<0.0001$), and 2 and 6 (0.64, $p<0.0001$). The between-individual variance was 0.66 and the within-individual variance was 0.34. The crude ICC was 0.66 both before and after excluding the one man who had an elevated hsCRP at one time point. The ICC was 0.68 after adjustment for age, BMI, cigarette smoking status, time of day and season of blood draw, and the residuals of blood storage time regressed on trial year.

The Table 3 presents the RRs that would be observed in an epidemiologic study when using a single measure of hsCRP to reflect usual levels for a range of hypothetical true RRs, assuming an ICC of 0.66 and no other sources of error. For RRs in the typical range for molecular epidemiology studies of 1.25 to 3.00, the percent attenuation might range from 7.3% to 31.2%, respectively.

Discussion

In healthy older men, we observed an ICC of 0.66 for hsCRP measured at three time points over a four-year period, which epidemiologists would consider to indicate good consistency over time. Nevertheless, with an ICC of this magnitude, attenuation of the association between hsCRP measured once and cancer and other chronic disease risk could occur in epidemiologic studies. For hypothesized moderate to large true associations between hsCRP and chronic disease, inferences might be preserved, that is the direction of the association, despite an attenuated RR. However, our work highlights the need for investigators to consider the extent of attenuation of the RR for hypothesized modest to moderate true associations, so that an adequate sample size may be selected to be able to statistically detect the attenuated association. When sample size is fixed, as in an existing prospective cohort study, investigators may consider collecting samples at future time points in a subset of participants so that methods to estimate a deattenuated association, such as regression calibration [5], may be used.

Several studies have investigated intra-individual variability in C-reactive protein concentration over the short [7–14] and the long (≥ 3 years) [15–20] term. Short-term studies have reported an ICC range of 0.59 over 20 weeks in 19 healthy men and women aged 20–46 years old [7] and over one year in 48 Chinese men with a mean age of 54.8 years [13] to 0.86 over six months in 20 healthy men and women aged 24–58 years old [8]. Long-term studies have reported an ICC range of 0.54 over three years in 946 healthy German men ages 45–64 [17] to 0.61 over five years in 65 Dutch men and women [19]. All of these ICCs would be considered to be in the good range by epidemiologists. In the JUPITER Study, which enrolled 8,901 men 50+ years old and women 60+ years old from 26 countries and who had had a hsCRP concentration of 2 mg/L or higher, the ICC was 0.50 over four years [20]. Other long-term studies reported intra-individual correlation coefficients of 0.43 over five years in 366 Japanese men and women aged 30–69 years old [16], 0.59 in 379 adult Icelanders with non-fatal myocardial infarction over 12 years [18], and 0.6 in 214 US men and women with a mean age of 59.3 years and with a prior history of myocardial infarction over five years [15]. Our ICC of 0.66 (and the correlation coefficient comparing years 2 and 4 concentrations of 0.64) over four years in healthy older US men unselected for baseline hsCRP is at the top of the range reported across long-term studies that included other demographic characteristics (women, nationality, race) and health states (prior myocardial infarction, elevated hsCRP).

The findings from studies of within- and between-individual variability in analyte concentrations are dependent on the characteristics of the study population, sample collection and handling, laboratory sensitivity and precision, and statistical analysis assumptions. We attempted to minimize extraneous variation through the use of well-characterized blood specimens that were collected systematically for a large clinical trial. We used a stringent protocol for specimen tracking, aliquotting, and shipping. We used a high sensitivity assay that we documented with quality control samples to have excellent reliability. However, because the specimens were collected in various settings around the country, we cannot preclude that extraneous variability was introduced.

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References

1. Tsimikas S, Willerson JT, Ridker PM. C-reactive protein and other emerging blood biomarkers to optimize risk stratification of vulnerable patients. *J Am Coll Cardiol* 2006;47:C19–31. [PubMed: 16631506]
2. Tsilidis KK, Branchini C, Guallar E, Helzlsouer KJ, Erlinger TP, Platz EA. C-reactive protein and colorectal cancer risk: a systematic review of prospective studies. *Int J Cancer* 2008;123:1133–1140. [PubMed: 18528865]
3. Rifai N, Ridker PM. High-sensitivity C-reactive protein: a novel and promising marker of coronary heart disease. *Clin Chem* 2001;47:403–433. [PubMed: 11238289]
4. Thompson I, Goodman P, Tangen C, Lucia M, Miller G, Ford L, et al. The influence of finasteride on the development of prostate cancer. *N Engl J Med* 2003;349:215–224. [PubMed: 12824459]
5. Rosner B, Spiegelman D, Willett WC. Correction of logistic regression relative risk estimates and confidence intervals for random within-person measurement error. *Am J Epidemiol* 1992;136:1400–13. [PubMed: 1488967]
6. Hankinson SE, Manson JE, Spiegelman D, Willett WC, Longcope C, Speizer FE. Reproducibility of plasma hormone levels in postmenopausal women over a 2–3-year period. *Cancer Epidemiol Biomarkers Prev* 1995;4:649–54. [PubMed: 8547832]
7. Clark GH, Fraser CG. Biological variation of acute phase proteins. *Ann Clin Biochem* 1993;30 (Pt 4):373–6. [PubMed: 7691039]
8. de Maat MPM, de Bart ACW, Hennis BC, Meijer P, Havelaar AD, Mulder PGH, et al. Interindividual and intraindividual variability in plasma fibrinogen, TPA antigen, PAI activity, and CRP in health, young volunteers and patients with angina pectoris. *Arterioscler Thromb Vasc Biol* 1996;16:1156–1162. [PubMed: 8792769]
9. Macy EM, Hayes TE, Tracy RP. Variability in the measurement of C-reactive protein in healthy subjects: implications for reference intervals and epidemiological applications. *Clin Chem* 1997;43:52–58. [PubMed: 8990222]
10. Sakkinen PA, Macy EM, Callas PW, Cornell ES, Hayes TE, Kuller LH, et al. Analytical and biologic variability in measures of hemostasis, fibrinolysis, and inflammation: assessment and implications for epidemiology. *Am J Epidemiol* 1999;149:261–7. [PubMed: 9927222]
11. Ockene IS, Matthews CE, Rifai N, Ridker PM, Reed G, Stanek E. Variability and classification accuracy of serial high-sensitivity C-reactive protein measurements in healthy adults. *Clin Chem* 2001;47:444–450. [PubMed: 11238295]
12. Nasermoaddeli A, Sekine M, Kagamimori S. Intra-individual variability of high-sensitivity C-reactive protein: age-related variations over time in Japanese subjects. *Circ J* 2006;70:559–63. [PubMed: 16636490]
13. Lee SA, Kallianpur A, Xiang YB, Wen W, Cai Q, Liu D, et al. Intra-individual variation of plasma adipokine levels and utility of single measurement of these biomarkers in population-based studies. *Cancer Epidemiol Biomarkers Prev* 2007;16:2464–2470. [PubMed: 18006938]
14. Shemesh T, Rowley KG, Jenkins AJ, Best JD, O'Dea K. C-reactive protein concentrations are very high and more stable over time than the traditional vascular risk factors total cholesterol and systolic blood pressure in an Australian Aboriginal cohort. *Clin Chem* 2009;55:336–341. [PubMed: 19074519]
15. Ridker PM, Rifai N, Pfeffer MA, Sacks F, Braunwald E. Long-term effects of pravastatin on plasma concentration of C-reactive protein. The Cholesterol and Recurrent Events (CARE) Investigators. *Circulation* 1999;100:230–5. [PubMed: 10411845]

16. Kayaba K, Ishikawa S, Gotoh T, Nago N, Kajii E, Nakamura Y, et al. Five-year intra-individual variability in C-reactive protein levels in a Japanese population-based study: the Jichi Medical School Cohort Study at Yamato, 1993–1998. *Jpn Circ J* 2000;64:303–308. [PubMed: 10783054]
17. Koenig W, Sund M, Frohlich M, Lowel H, Hutchinson WL, Pepys MB. Refinement of the association of serum C-reactive protein concentration and coronary heart disease risk by correction for within-subject variation over time: the MONICA Augsburg studies, 1984 and 1987. *Am J Epidemiol* 2003;158:357–64. [PubMed: 12915501]
18. Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *N Engl J Med* 2004;350:1387–97. [PubMed: 15070788]
19. Al-Delaimy WK, Jansen EH, Peeters PH, van der Laan JD, van Noord PA, Boshuizen HC, et al. Reliability of biomarkers of iron status, blood lipids, oxidative stress, vitamin D, C-reactive protein and fructosamine in two Dutch cohorts. *Biomarkers* 2006;11:370–382. [PubMed: 16908443]
20. Glynn RJ, Macfadyen JG, Ridker PM. Tracking of high-sensitivity C-reactive protein after observation of an initially increased concentration: The JUPITER Study. *Clin Chem* 2009;55:305–312. [PubMed: 19095726]

Figure 1.

Intra-individual variability in hsCRP concentration at three time points each two years apart in 50 men in the placebo arm of the Prostate Cancer Prevention Trial. Each vertical set of three filled circles shows the difference between hsCRP concentration at Years 2, 4 and 6 and the mean hsCRP across Years 2, 4, and 6 for the same man. The men are plotted on the x-axis in ascending order from the least to greatest variability in hsCRP among the three time points.

Table 1

Characteristics of 50 Men Selected from the Placebo Arm of the Prostate Cancer Prevention Trial by Year of the Trial

	Year 2	Year 4	Year 6
Mean (SD) age, years	64.9 (6.6)	66.9 (6.6)	68.9 (6.7)
Mean (SD) BMI, kg/m ²	27.7 (5.2)	27.6 (5.6)	27.6 (5.1)
Season of blood draw, %			
Winter	20	20	22
Spring	26	24	20
Summer	32	32	32
Fall	22	24	26
Time of day of blood draw, %			
6 am – 11:59 am	44	54	52
12 noon – 6:59 pm	50	44	48
6 pm – 11:59 pm	6	2	0
Mean (SD) blood storage time, years	11.5 (1.2)	9.5 (1.2)	7.4 (1.2)

Table 2

Median, Range, and Geometric Mean (95% Confidence Interval) Serum hsCRP Concentration (mg/L) by Trial Year, 50 Men in the Placebo Arm of the Prostate Cancer Prevention Trial

	Year 2	Year 4	Year 6	<i>p</i> -trend
Median	0.93	0.92	0.77	
Range	0.08–4.73	0.07–15.5	0.04–6.08	
Geometric mean (95% CI)				
Crude	0.84 0.65, 1.09	0.95 0.72, 1.26	0.77 0.57, 1.04	0.50
Age-adjusted [*]	0.81 0.62, 1.05	0.95 0.73, 1.25	0.80 0.59, 1.08	0.76
Multivariable-adjusted [†]	0.80 0.62, 1.03	0.96 0.74, 1.26 [‡]	0.77 0.58, 1.04	0.73

^{*} Centered at the mean value for age across all years.

[†] Adjusted for age, BMI, current smoking, time of day and winter season of blood draw, and residuals of blood storage time regressed on trial year. Centered to mean values for these covariates across all years.

[‡] After excluding 1 observation at Year 4 with hsCRP concentration ≥ 10 mg/L: 0.90 (95% confidence interval: 0.70, 1.15), *p*-trend=0.74.

Table 3

Observed Relative Risk (RR) for a Given True RR When Using a Measure of hsCRP Only at One Point in Time, Assuming an ICC=0.66 and No Other Sources of Error

	True RR						
	1.25	1.5	1.75	2	5	10	20
Observed RR	1.16	1.31	1.45	1.58	2.89	4.57	7.22
Extent of attenuation	7.3%	12.8%	17.3%	21.0%	42.1%	54.3%	63.9%