

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

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The authors sought to assess the repeatability of measurements of C-reactive protein, an independent predictor of coronary heart disease, in a large cohort of apparently healthy men and to correct earlier estimates of the association of C-reactive protein and coronary heart disease for the measurement error in this protein. They measured C-reactive protein by a high-sensitivity assay in 936 men aged 45–64 years in the MONICA (Monitoring of Trends and Determinants in Cardiovascular Disease) Augsburg cohort in 1984–1985 and remeasured it 3 years later. All men were subjected to an 8-year follow-up of their cardiovascular status. The analytical variation of the assay was small, with the analytical variance component at 1 percent of the within-subject variance component, a repeatability coefficient of 25 percent, and a reliability coefficient of 1.00. In contrast, the within-subject variation of C-reactive protein corresponded to a repeatability coefficient of 740 percent and a reliability coefficient of 0.54, indicating considerable within-subject variation. Based on the authors' estimates, three serial determinations of C-reactive protein should be done to achieve a reliability of 0.75, the value they found for total cholesterol. Correcting the hazard ratios in their original analysis of the association of coronary heart disease and high-sensitivity-assay C-reactive protein for the measurement error in C-reactive protein and covariables leads to a considerably larger estimate. The results suggest that the true association between C-reactive protein and cardiovascular risk is underestimated by a single C-reactive protein determination, and that several serial C-reactive protein measurements should be taken.

coronary disease; inflammation; predictive value of tests; proteins

Abbreviations: CI, confidence interval; MONICA, Monitoring of Trends and Determinants in Cardiovascular Disease; VC_a, analytical variance component; VC_b, between-subject variance component; VC_w, within-subject variance component.

It is now clear that atherosclerosis is an inflammatory, thrombotic disease, and there is powerful evidence of local inflammation and a systemic inflammatory response (1). Recent studies of C-reactive protein, the classic acute-phase protein, using new high-sensitivity assays, have revealed a consistent positive association with future cardiovascular events in both initially healthy subjects and patients with angina pectoris (2).

C-reactive protein represents an exquisitely sensitive objective marker of inflammation, tissue damage, and infection. Its plasma half-life (~19 hours) is rapid but identical under all conditions, in contrast to virtually all other major acute-phase reactants, so that the synthesis rate of C-reactive protein is the sole determinant of its plasma concentration (3). Excellent anti-C-reactive protein antibodies and a well-established World Health Organization international refer-

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ence standard for C-reactive protein (4) are available so that precise, sensitive, and robust clinical serum/plasma assays can be readily undertaken (5–7). The measurement of C-reactive protein thus has many advantages for detection and monitoring of the acute-phase response in general and particularly in relation to atheroma and its complications.

In long-term observational epidemiologic studies, risk variables are usually measured once at “baseline” and then related to outcome. Most physiologic variables, however, are not stable over time but show a more or less pronounced diurnal, seasonal, and long-term variation. The potential for long-term variation is of great importance, since such variation may have considerable impact on the accuracy of risk prediction by particular analytes. Relatively little information is available about this aspect of even the conventional risk factors in adequately sized samples and over longer periods of time (8), and only a few studies have investigated the hemostatic parameters commonly used in epidemiologic studies (9–12). Because the acute-phase response is nonspecific, highly sensitive, and induced by a wide range of different processes, including most forms of tissue injury and infection, long-term variation might be expected to be even more important for markers of inflammation than for other biovariables that are subject to such common and wide-ranging effects. In particular, for C-reactive protein, which is extremely sensitive and shows a dynamic range of up to 10,000-fold in response to a variety of stimuli (13), this information is needed to assess the risk prediction associated with elevated values reliably.

The objectives of the present study were to investigate the repeatability of C-reactive protein measurements in a large sample of middle-aged men from the general population and to correct earlier estimates of the association of C-reactive protein and coronary heart disease for the measurement error in C-reactive protein. Repeatability was assessed recognizing three potential sources of variation in C-reactive protein, that is, the error of the measurement process itself (analytical variation), the variability of values in individual subjects (within-subjects variation), and the variability of values between individuals (between-subjects variation).

MATERIALS AND METHODS

Study population

Our study population was part of the first cross-sectional survey of the MONICA (Monitoring of Trends and Determinants in Cardiovascular Disease) center in Augsburg, Germany, in 1984–1985. The objectives and design of the MONICA project have been described in detail earlier (14, 15). Briefly, 4,022 of the 5,069 eligible individuals 25–64 years of age, initially sampled at random from a study population of 282,279 inhabitants of a mixed urban/rural area, participated in the study (response rate, 79.3 percent). Of these, all men 45–64 years of age were subjected to an 8-year follow-up of their coronary heart disease status. All subjects of this cohort who had complete data on all the variables studied had been included in the analysis of the association of C-reactive protein and coronary heart disease reported earlier (16) ($n = 936$).

In 1987–1988, 852 subjects of this group participated in a reexamination (response rate, 91 percent) and submitted to the same protocol during the same time of the year (October through May). For 696 of these subjects, we were able to obtain a second set of complete C-reactive protein and covariable values. All C-reactive protein values were measured in triplicate on both measurement occasions. They were originally recorded as averages after elimination of the extreme value if the coefficient of variation was larger than 15 percent. For a systematic subsample of the initial study population (every other man, $n = 469$), however, we were able to recover the three individual C-reactive protein values of the first measurement occasion. The basic statistics of our variables in the various subgroups did not differ appreciably from those in the larger groups from which the subgroups were sampled.

Survey methods

Participants completed an identical standardized questionnaire in 1984–1985 and in 1987–1988, including medical history, lifestyle, and drug history. Blood pressure was measured after the interview (duration, approximately 30 minutes) by a random-zero sphygmomanometer (Hawksley & Sons, Ltd., West Sussex, United Kingdom) according to the recommendations of the American Heart Association. Body height (m), body weight (kg), body mass index (weight (kg)/height (m)²), smoking behavior, and alcohol consumption (g/day) were determined as described elsewhere (17). Leisure-time physical activity was assessed on a four-level graded scale for winter and summer (none, <1, 1–2, and >2 hours/week). The number of years of education was calculated from the highest level of formal education completed. The presence of diabetes was determined by history.

Laboratory procedures

Nonfasting blood samples were drawn from an antecubital vein of the seated participant according to the recommendations of the International Committee for Standardization in Haematology. Only short-term venous stasis and minimal suction were applied. Serum samples from all subjects at the baseline examination in 1984–1985 and during the survey in 1987–1988 were immediately put on ice and then stored at -70°C until analysis.

Serum concentrations of C-reactive protein were measured in triplicate in a high-sensitivity immunoradiometric assay using monospecific polyclonal and monoclonal antibodies produced by immunization with highly purified C-reactive protein (7). C-reactive protein was heavily skewed but appeared to be almost perfectly approximated by a lognormal distribution, such that the lognormal transformation of C-reactive protein (mg/liter) was used throughout. Serum total cholesterol and high density lipoprotein cholesterol were measured by routine enzymatic methods. High density lipoprotein cholesterol was measured on serum after precipitation with manganese chloride and phosphotungstate.

Statistical methods

All computations were performed in Windows NT 4 (Microsoft Corporation, Redmond, Washington), with the software products SAS (SAS Institute, Inc., Cary, North Carolina), S-PLUS (Mathsoft, Inc., Seattle, Washington), and WinBUGS (18); the graphics were made with SAS software.

Variation. Variation was analyzed from concepts described by Fleiss (19), Bland and Altman (20), and Fraser and Harris (21). Basically, we computed estimates of the three variance components implied by the data, a nested structure of subjects (936 "levels"), measurement occasions (two levels for 696 subjects, one level for the rest), and replicates (three levels for 469 subjects at occasion 1, one level for the rest), assuming the traditional nested normal random-effects model. For the computations, we used the SAS MIXED procedure (restricted maximum-likelihood method, RANDOM statement), weighting an observation by the number of values represented by that observation (single replicate value or average of replicates). The three variance components are designated VC_b (between subjects), VC_w (within subjects), and VC_a (analytical). For evaluating the within-subject variation, we used the 95 percent repeatability coefficient advocated by Bland and Altman (20) and computed as $1.96 \times \sqrt{2} \times (VC_w + VC_a)^{1/2}$. This coefficient (also called the critical difference (22) or the reference change value (23)) represents, when back-transformed by taking antilogs, for 95 percent of the subjects the upper limit of the ratio of the larger to the smaller of the measurements on two separate specimens of a single subject. A 95 percent confidence interval was obtained from the covariance matrix of the variance components. The intraclass correlation coefficient of reliability (reliability coefficient) was used to characterize the repeatability of measurements for comparing subjects or groups of subjects. It was computed as the $VC_b / (VC_b + VC_w + VC_a)$ ratio (18) with an approximate 95 percent confidence interval (24, 25).

For evaluation of the analytical variation (the error of the measurement process itself), analogous coefficients were computed as $1.96 \times \sqrt{2} \times VC_a^{1/2}$ (95 percent repeatability coefficient) and as $(VC_b + VC_w) / (VC_b + VC_w + VC_a)$ (reliability coefficient).

The number of measurements required to achieve a given reliability was computed with the Spearman-Brown prophecy formula (19). A variable was treated as being measurement error free if its reliability coefficient exceeded 0.75, following the recommendation of Fleiss (19). To assess the measurement error for the other variables, we estimated the reliability coefficient in an analogous way treating categorical variables as ordinal.

Association between C-reactive protein and coronary heart disease. The association of C-reactive protein and coronary heart disease had previously been analyzed with the Cox regression model (16). To correct the earlier results for the measurement error in C-reactive protein and the covariables, we repeated the analysis, utilizing the measurements at the second measurement occasion where available. The most used tool for measurement error correction is a structural approach with regression calibration (26). The indepen-

dent variables were C-reactive protein (lognormal mg/liter) and the following covariables: the continuous covariables age (years), body mass index (kg/m^2), total cholesterol (mmol/liter), high density lipoprotein cholesterol (mmol/liter), and systolic blood pressure and diastolic blood pressure (mmHg). The categorical covariables were smoking status (never smoked, former smoker, current smoker), alcohol consumption (men: 0, <40, ≥ 40 g/day; women: 0, <20, ≥ 20 g/day), years of schooling (<8, <11, >11 years), winter and summer leisure time physical activity (none, <1, 1–2, and >2 hours/week), and diabetes by history (no, yes). The continuous covariables were at least rather symmetrical such that they were used on their original scale. The dependent variable was again time to incidence of a first major coronary heart disease event. A subject was considered censored when he had died from another cause, when he had left the study area, or when the observation period had ended. The independent variables were C-reactive protein (lognormal mg/liter) and the covariables listed above. As a preliminary covariable check, the possibility of seasonal variation in C-reactive protein was investigated by fitting a sinusoidal curve with a period of 1 year to the data (details of the analysis method can be found elsewhere (27)). We reduced the number of covariables by the same forward-stepping procedure as before, at each step adding that variable that changed the absolute value of the C-reactive protein coefficient in the Cox regression the most and by at least 10 percent when added to the variables already in the model (28). Age was forced into the regression from the beginning. The categorical variables, recognizing their ordinal character, were treated as continuous variables. All variables were used with their linear terms. In this stepping process, the repeat measurements were utilized with the method of regression calibration by linear approximation of the calibration function (28). The final estimates of the regression models of interest were then obtained by Bayesian inference using Markov chain Monte Carlo methods (29), assuming the classic measurement model and "uninformative" priors.

RESULTS

Distribution of C-reactive protein

Figure 1 shows the distribution of the baseline triplicate C-reactive protein values, plotted against a standard normal distribution (Q-Q plot), using a logarithmic scale for C-reactive protein. The data points closely approximate a straight line with no apparent outliers, such that a normal distribution may be assumed for log-transformed C-reactive protein. The same conclusion was reached when adjusting C-reactive protein for the covariables and on the other two levels of measurement (within and between subjects). One can see that the minimum of C-reactive protein was 0.05 mg/liter, which was the lower detection limit of the assay used. The maximum was 70 mg/liter, and the 99th percentile was 25 mg/liter. A value of more than 3 mg/liter was reached by 32.2 percent of the values, and a value of more than 10 mg/liter was reached by 3.6 percent.

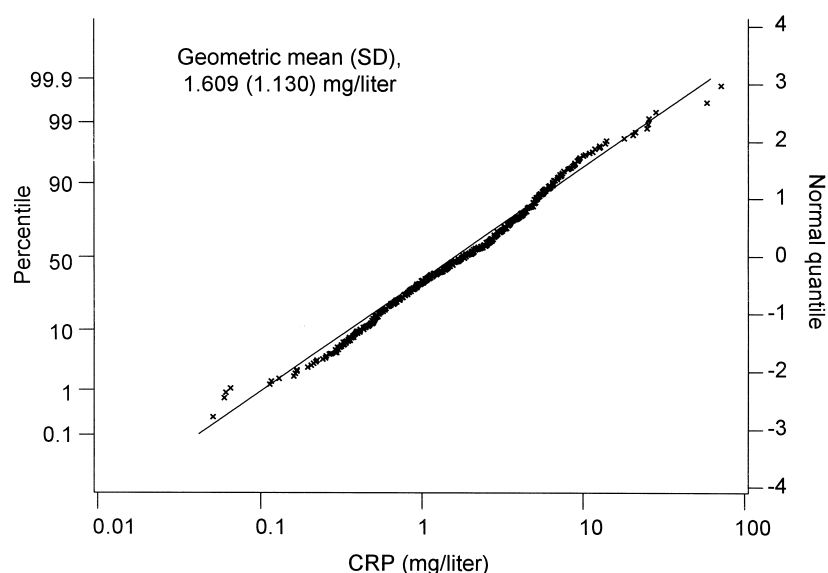


FIGURE 1. Normal quantile-quantile (Q-Q) plot of C-reactive protein (CRP) replicate measurements at occasion 1 ($n = 469$), MONICA Augsburg studies, 1984–1987. SD, standard deviation; MONICA, Monitoring of Trends and Determinants in Cardiovascular Disease.

Variation

Table 1 presents statistics relating to the variation of C-reactive protein. The first block of rows presents the total mean, back-transformed by taking antilogs to yield the geometric mean, and the ratio of the geometric means at the two measurement occasions. The 1.04 ratio is very close to 1 with a 95 percent confidence interval of 0.96, 1.13 that

includes the value 1. Therefore, negligible overall bias may be inferred.

The next block of rows presents the estimates of the three variance components VC_b , VC_w , and VC_a . For assessment of the measurement process, it can be seen that VC_a was very small in relation to VC_w (about 1 percent). For the analytical variation, the 95 percent repeatability coefficient was 1.25,

TABLE 1. Variation statistics for C-reactive protein with 95% confidence intervals ($n = 936$), MONICA* Augsburg studies, 1984–1987

Statistic	Value	95% CI*
Geometric means		
Total (mg/liter)	1.59	
Ratio of measurement occasion 2 to measurement occasion 1	1.04	0.96, 1.13
Variance components ($(\ln^* \text{ mg/liter})^2$)		
VC_b , between subjects	0.6847	0.5932, 0.7991
VC_w , within subjects	0.5831	0.5259, 0.6502
VC_a , analytical	0.0063	0.0057, 0.0069
Analytical repeatability		
Repeatability coefficient	1.25	1.23, 1.26
Reliability coefficient	1.00	0.99, 1.00
Within subject repeatability		
Repeatability coefficient	8.40	7.55, 9.46
Reliability coefficient	0.54	0.48, 0.59
No. of specimens required for a reliability coefficient of 0.75	3	3, 4

* MONICA, Monitoring of Trends and Determinants in Cardiovascular Disease; CI, confidence interval; ln, lognormal.

TABLE 2. Reliability coefficients with 95% confidence intervals of the covariables (*n* = 936), MONICA* Augsburg studies, 1984–1987

Variable	Reliability coefficient	95% CI*
Body mass index	0.93	0.92, 0.94
Total cholesterol	0.75	0.70, 0.78
High density lipoprotein cholesterol	0.70	0.65, 0.75
Systolic blood pressure	0.65	0.59, 0.70
Diastolic blood pressure	0.57	0.51, 0.63
Smoking status	0.87	0.85, 0.89
Alcohol consumption	0.54	0.47, 0.60
Years of schooling	0.71	0.66, 0.75
Winter leisure time physical activity	0.61	0.55, 0.67
Summer leisure time physical activity	0.54	0.48, 0.61
Work activity	0.61	0.55, 0.66
Diabetes	0.71	0.67, 0.76

* MONICA, Monitoring of Trends and Determinants in Cardiovascular Disease; CI, confidence interval.

meaning that for 95 percent of the subjects the ratio of the larger to the smaller of two C-reactive protein determinations on the same blood sample of a single subject is smaller than 1.25. The reliability coefficient of 1.00 is extremely large, attaining its maximum. These results suggest that there is very little measurement error in the C-reactive protein assay.

The next block with three rows of data reports the analogous indices for the within-subject variation quantifying the repeatability of a subject's expected C-reactive protein value over time (usual value, homeostatic set point). The 95 percent repeatability coefficient (critical difference) was 8.40 (95 percent confidence interval (CI): 7.55, 9.46), meaning that for 95 percent of the subjects, the ratio of the larger to the smaller of two C-reactive protein determinations on different blood samples of a single subject is likely to be smaller than anywhere between 7.55 and 9.46. The reliability coefficient was 0.54, representing only moderate reliability. Therefore, for the assessment of the association between C-reactive protein and coronary heart disease, a correction for the fluctuation of C-reactive protein should be considered. As seen in the last row of table 1, a value of 3 (95 percent CI: 3, 4) determinations on separate specimens of a single subject is required to achieve at least the reliability of total cholesterol of 0.75 in our data estimated by the Spearman-Brown prophecy formula.

For the covariables, only the average of the triplicate measurements was available for analysis, such that the within-subjects variation was confounded with the analytical variation. Table 2 reports reliability coefficients for both the continuous and the categorical variables, the latter all being ordinal variables allowing continuous analyses. The coefficients ranged from 0.54 for alcohol consumption and summer leisure time physical activity to 0.93 for body mass

index. The sizes of the reliability coefficients suggest that in regression analyses at least body mass index and smoking status may be treated as measurement error free.

Association between C-reactive protein and coronary heart disease

Of the 936 subjects of the study group, 53 (5.7 percent) developed a first major coronary heart disease event during the follow-up (maximum, 8.2 years). The average annual incidence rate was 7.64 (95 percent CI: 5.72, 9.99) per 1,000 person-years. Revision of our earlier estimates of the association of C-reactive protein and coronary heart disease, utilizing the repeat measurements, started again with the stepwise variable selection procedure indicated in Materials and Methods. The preliminary covariable analysis did not suggest that subjects be differentiated by the amount or direction of change of C-reactive protein, nor did it produce substantial evidence of seasonal change in C-reactive protein. Because of the extremely low analytical measurement error, we used only the averages of the triplicate measurements. The only covariable that changed the age-adjusted C-reactive protein regression coefficient by more than 10 percent when added to the variables already in the regression was body mass index (10.8 percent). Table 3 presents results of Cox regressions of coronary heart disease on C-reactive protein, using baseline measurements of C-reactive protein only, and results from Bayesian Cox regressions, using also the repeat measurements of C-reactive protein (age and body mass index treated as measurement error free). The results are presented as hazard ratios for an increase in C-reactive protein by 1 lognormal mg/liter, unadjusted, adjusted for age, and adjusted for age and body mass index. The table also contains 95 percent confidence intervals (uncorrected hazard ratios) and 95 percent credibility intervals (corrected hazard ratios) that may be interpreted in a way similar to 95 percent confidence intervals. It can be seen that the corrected hazard ratios were considerably higher than the uncorrected ones. The other rows of the table present hazard ratios and their confidence intervals derived from the most-adjusted model, for an increase of C-reactive protein by 1 standard deviation on the lognormal scale (computed as the square root of the sum of the three variance components) and for the comparison of the C-reactive protein medians of the fifth and the first quintiles.

DISCUSSION

Our results demonstrate several important findings related to the use of C-reactive protein as a risk marker in coronary heart disease. First, the precision of the immunoradiometric assay we used is excellent. Second, the within-subject variation of C-reactive protein is considerable. Third, without taking the within-subject variation of C-reactive protein into account, we found that the true association between C-reactive protein and coronary heart disease is considerably underestimated.

TABLE 3. Hazard ratios with 95% confidence intervals from Cox regressions of coronary heart disease on C-reactive protein ($n = 936$), using baseline measurements only (uncorrected hazard ratios), and hazard ratios from Bayesian Cox regressions corrected for within-subjects variation in C-reactive protein, using repeat measurements (corrected hazard ratios), MONICA* Augsburg studies, 1984–1987

Adjustment	Uncorrected HR*,†	95% CI*	Corrected HR†	95% CI
None‡	1.57	1.25, 1.97	2.62	1.69, 4.05
Age‡	1.51	1.20, 1.90	2.41	1.54, 3.77
Age, body mass index‡	1.55	1.23, 1.95	2.59	1.61, 4.16
Increase by 1 SD* (1.133 ln* mg/liter)§	1.64	1.26, 2.12	2.93	1.72, 5.00
Top- vs. bottom-quintile median (6.5, 0.38 mg/liter)	3.38	1.77, 6.45	14.29	3.80, 53.70

* MONICA, Monitoring of Trends and Determinants in Cardiovascular Disease; HR, hazard ratio; CI, confidence interval; SD, standard deviation; ln, lognormal.

† For arbitrary C-reactive protein values a and b (mg/liter), $b > a$, the hazard ratio is $HR^{\ln(b/a)}$.

‡ For an increase in C-reactive protein of 1 ln mg/liter.

§ For a multiple of 1 SD, raise the hazard ratio to that multiple.

Problems of comparison of our results with those of the literature

Comparison of our variation results with published results turned out to be difficult for a number of reasons. One reason is the apparent lack of agreement on what statistics to report; for example, whereas Bland and Altman (20) strongly recommend the repeatability coefficient, Fleiss (19) makes extensive use of the classic reliability coefficient to which Bland and Altman are somewhat opposed (30). However, it seems that both coefficients are justified in their own right, the repeatability coefficient relating to measurements taken on a single individual and the reliability coefficient relating to the comparison of measurements on different individuals. Still others recommend using the repeatability coefficient, not basing it on the variance components obtained from analysis of variance computations, as do Bland and Altman, but on the variance components divided by the total mean (31), this way producing a “relative critical difference.” Comparability is also impaired by many authors doing analyses for the untransformed values when the assumptions implied by the methods of analysis are not even approximately met. Lognormal C-reactive protein can almost perfectly be approximated by a normal distribution, such that most results obtained from the untransformed values are not comparable to those obtained from the transformed values.

Analytical variation

The measurement error of the assay was extremely small compared with within-subjects’ variation, the variability of C-reactive protein values free of assay measurement error within subjects. As an “acceptable goal,” the analytical variation, expressed as the standard deviation ($\sqrt{VC_a}$), should be less than 50 percent of the biologic within-subjects standard deviation (21), 25 percent being considered “optimal” (23). Our analysis yielded 10 percent, which is of the same magnitude as reported by Clark and Fraser (32) using a less sensitive assay (7 percent, computed from their “components of variation” for the untransformed C-reactive protein

values). Our reliability coefficient of 1.00 was at its possible maximum. These results suggest that there is very little measurement error in the measurement device. Thus, the method used seems suitable for both clinical and scientific applications, even with a single determination.

Within-subject variation

The 95 percent repeatability coefficient for C-reactive protein was 8.40 (95 percent CI: 7.55, 9.46), which indicates considerable variation. The reliability coefficient of 0.54 was only of moderate size.

A number of studies have evaluated the biologic within-subject variance, mostly in smaller samples using various assays over either short periods of time (5, 9, 32) or medium time (12). Of these, only one presented analyses comparable to ours, at least with respect to the fact that they used lognormal-transformed C-reactive protein values (9). However, the within-subject variability was a little higher than ours, and the reliability coefficient of 0.86 for the healthy volunteers was considerably so because of the very large between-subjects variance component.

Two studies have reported on the long-term variability of high-sensitivity C-reactive protein. Ridker et al. (33), using data from 214 patients of the placebo group in the Cholesterol and Recurrent Events trial, found an acceptable correlation for lognormal-transformed C-reactive protein levels over 5 years with an age-adjusted correlation coefficient of 0.60 ($p < 0.001$), which is comparable to our age-adjusted reliability coefficient of 0.52. In a population-based study from Japan (34), 388 apparently healthy subjects had repeat measurements of C-reactive protein after a 5-year interval. For the 104 men, again for the lognormal-transformed C-reactive protein values, the correlation coefficient was 0.37 (95 percent CI: 0.19, 0.53), indicating moderate reliability. Thus, the variation of C-reactive protein found in our study, as measured by the reliability coefficient, compares with that reported in two other long-term studies.

If C-reactive protein measurements are used for cardiovascular risk assessment, based on our reliability estimates,

three serial determinations (with a single assay measurement in each) should be done to achieve a reliability of 0.75, the reliability we found for total cholesterol, which still represents the standard in the clinical situation to which a new risk marker should be compared. Similar results had been obtained by Ockene et al. (35) when they applied the Spearman-Brown prophecy formula to their data. The reliability coefficient computed by the intraclass coefficient was 0.66 compared with our repeatability coefficient of 0.54. It would increase to 0.79 for two specimens and to 0.85 for three specimens. Since the reliability coefficient of total cholesterol in their data reached a value of 0.82, only a number of three or more specimens for the measurement of C-reactive protein would achieve a higher reliability for C-reactive protein than for total cholesterol.

Correction of the association of C-reactive protein with coronary heart disease for measurement error

Eleven prospective studies in apparently healthy men and women reported on the risk of increased C-reactive protein on future coronary heart disease. These subjects were followed for up to 12 years, and studies comprised a total of 2,557 cases. Results were remarkably consistent and showed an almost twofold increased risk if the top third of the C-reactive protein distribution was compared with the bottom third (2). In all of these studies, risk prediction was based on a single ("baseline") measurement. This increased risk associated with elevated C-reactive protein values was similar to that of classic risk factors.

We corrected the hazard ratios for C-reactive protein in the prediction of coronary heart disease obtained in our earlier analysis (16) for the variability of C-reactive protein over time. The corrected hazard ratios were considerably larger than the uncorrected ones. Although this may not be true universally (36), in the case of C-reactive protein, where there are only little variation over time in stable subjects free of disease and various unspecific, mainly environmental stimuli (37, 38), the true association between C-reactive protein and coronary heart disease is subject to considerable underestimation if no correction for the sources of such variability of C-reactive protein is made. In case there is a true change in the individuals' habitual C-reactive protein over time, this certainly would correspond to a real change in coronary heart disease risk, and the correction would not be valid. Another issue relates to the additional adjustment of other biologic factors for measurement error (table 2). Better classification of traditional risk factors might have improved their predictive ability and thus may have resulted in significant associations with C-reactive protein, with the need to adjust for them and the potential consequence of attenuation of the initial association between C-reactive protein and coronary heart disease.

In summary, C-reactive protein measured by a high-sensitivity immunoradiometric assay showed extremely low analytical variation but a considerable within-subject variation over time. Cardiovascular risk prediction with C-reactive protein can be improved by taking this into account.

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