Problems with High-Sensitivity C-Reactive Protein

To the Editor:

Last year, Ockene et al. (1) published a report in this journal that made the claim that high-sensitivity C-reactive protein (hs-CRP) has a degree of measurement stability that is similar to that of total cholesterol and that this provides further evidence of the potential clinical utility of hs-CRP screening as a novel tool for vascular risk prediction.

The key evidence that Ockene et al. (1) present to justify their claim is a histogram showing an almost identical agreement in terms of group classification between first and second measurements for hs-CRP and total cholesterol. This apparent agreement is spurious and is attributable to the way in which Ockene et al. partitioned the hs-CRP data. Although the total cholesterol data in the histogram are divided into quartiles, the hs-CRP data are partitioned into arbitrary intervals that contain ~15%, 20%, 30%, and 35%, respectively, of the sample.

Ockene et al. (1) provide two graphs showing the data for all 113 patients for serial cholesterol and CRP values ranked by mean concentration. These values are different for the two analytes. For cholesterol, the average intraindividual variation is 18.2%, and the intraindividual variation is roughly constant across all the range of data. For CRP, the average intraindividual variation is higher, at 44.2%. It is lowest at low CRP concentrations and then increases as the mean CRP concentration increases.

Ockene et al. (1) also provide graphs showing the distributions of the total cholesterol and hs-CRP results. As expected, the total cholesterol distribution is approximately gaussian and the hs-CRP distribution is skewed. A result of the skewed distribution of CRP concentrations is that even if true quartiles had been used, the interquartile spacing would increase markedly as the mean CRP concentration increased. The arbitrary intergroup spacings used by Ockene et al. amplify this effect. The third group concentration range is twice as wide, and the fourth group concentration range is 16 times as wide as each of the first two groups. The group cutoffs used by Ockene et al. also allow the upper 65% of the study participants, who among them encompass the majority of the intraindividual variation in hs-CRP, to fall within these two wider intervals. It is readily apparent that the probability that a second or subsequent CRP value will fall outside the original group is lower if the original value was in the wide third or fourth group. The net result is a bias toward reducing misclassification in the hs-CRP data.

It might be argued that it is reasonable to use these particular CRP intergroup cutoffs because they are based on risk. However, inspection of the reference provided by the authors shows that the cutoffs used are apparently based on cutoffs that divided the all-male control group of a previous case-control study into quartiles (2). Because the selection criteria for the control group in that study (2) and the experimental group in the study by Ockene et al. (1) are clearly different, use of these cutoff values cannot be justified.

Even if the hs-CRP data and the total cholesterol data had been divided into true quartiles before comparison, it could be argued that such a comparison is inappropriate because of the differences in both the sample distributions of values and the distributions of the intraindividual variation between the two analytes. Basing the comparison on arbitrary division of the hs-CRP data into unequal groups further invalidates the conclusions drawn from the study.

Total cholesterol and hs-CRP are not equivalent in terms of clinical utility for predicting cardiovascular risk in individual patients. A recent study (3) has demonstrated that hs-CRP assays have limited clinical utility in this situation because of the unacceptably large intraindividual variation in values.

References


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The authors of the article discussed in the above letter respond:

To the Editor:

Campbell et al. argue that our report (1) describing a degree of measure-ment stability for high-sensitivity C-reactive protein (hs-CRP) that is similar to that of total cholesterol draws an unjustifiable conclusion. They argue that our histogram showing agreements of group classification between first and second measurements for hs-CRP and total cholesterol is spurious because the cholesterol data were divided into quartiles whereas the hs-CRP data were divided into arbitrary intervals based on clinical utility. We did this because it seemed appropriate to us to use clinical utility as the driving factor for a clinically useful test, but we appreciate the opportunity to describe additional analyses that space constraints prevented us from presenting in the original report. We repeated the analysis, dividing CRP into four equal quartiles. This produced an overall agreement of 59.3% compared with 62.8% in our original analysis, with a k statistic of 0.452 (95% confidence interval, 0.34–0.56) compared with 0.479 (0.39–0.60) for the original.

Campbell et al. also note that the skewed distribution of CRP concentrations leads to the higher quartiles being much wider than the lower quartiles, and from this they conclude that a second or subsequent CRP value is less likely to fall outside of the original group if its first value
was in the third or fourth quartile, as these quartiles encompass a greater range. This argument, however, is not logical, for as Campbell et al. themselves note, the average intra-individual variation for cholesterol is 18.2% and is relatively constant over the range of values, whereas intra-individual variation is 44.2% for CRP with variation much higher at higher concentrations. Thus the wider range of values in the higher quartiles is proportional to the greater variation at these concentrations and thus would not produce a lower probability that a subsequent CRP value would fall outside of the original group. As noted in Table 2 of our original report, the use of log hs-CRP reduces variance to 21.7%, a value very comparable to the 18.2% for total cholesterol, but the quartile ranking is the same for the log-transformed value as for the untransformed variable because the log is a monotonic transformation.

CRP may reflect and respond strongly to acute inflammatory insult, including common viral infections, whereas cholesterol will not; to the extent that CRP concentrations are measured after such insults, repeatability will be reduced, and the clinician needs to take care to avoid such sources of variation.

Highly skewed analytes are not uncommon in clinical practice. Plasma concentrations of triglycerides are metabolically and pathophysiologically important, and their measurement is of value, despite a nongaussian distribution and considerable intraindividual biological variability. We believe that the use of cutpoints related to clinical utility is justifiable and produces levels of classification agreement that are little different from other possible choices.

Paul Ridker is listed as an inventor on patents, filed by Brigham and Womens Hospital, that relate to inflammatory biomarkers and cardiovascular disease.

Reference


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Total Protein Determination in Urine: Aminoglycoside Interference

To the Editor:

The aminoglycosides gentamicin and tobramycin interfere in the Dade Behring pyrogallol red-molybdate (PRM) assay used for determination of urinary protein (1). This is an important finding because aminoglycosides are nephrotoxic and accurate determination of urinary protein is necessary to detect renal damage in patients receiving aminoglycosides. We have investigated interference in the PRM assay (2, 3) from other aminoglycosides (dihydrostreptomycin, geneticin, kanamycin, neomycin, paromomycin, and streptomycin) and extended the study to the Coomassie Brilliant Blue (CBB) and the benzethonium chloride (BEC) protein assays, which are also used routinely for urinary protein determination (4–6).

Dihydrostreptomycin (cat. no. D7253), geneticin (cat. no. G5013), gentamicin (cat. no. G3632), kanamycin (cat. no. K4000), neomycin (cat. no. N6386), paromomycin (cat. no. P9297), streptomycin (cat. no. S6501), and tobramycin (cat. no. T1783) were purchased from Sigma-Aldrich Co. Ltd., and solubilized in 0.1 mol/L phosphate buffer (pH 7) at concentrations of 10, 5, 1, 0.5, and 0.1 g/L. Bovine serum albumin (BSA; cat. no. A7906; Sigma) was solubilized at 10 g/L, calibrated with the Sigma biuret assay, and diluted to 2 g/L for calibration of the PRM assay and 1 g/L for the CBB and BEC assays. Sigma urine control (cat. no. U9631) was reconstituted in water or aqueous aminoglycoside (final concentration, 0.2 g/L).

We mixed 20 μL of aminoglycoside, 20 μL of urine control (± aminoglycoside), or 5–20 μL of protein calibrator (adjusted to 20 μL with phosphate buffer) with 1 mL of either Sigma Microprotein-PR Reagent (PRM assay) or Sigma Protein Assay Solution (CBB assay)). The absorbance at 600 nm (PRM assay) or 595 nm (CBB assay) was measured with a Jenway 6100 spectrophotometer zeroed against a reagent blank. For the BEC assay, we mixed the samples (as above) with 0.8 mL of 0.5 mol/L sodium hydroxide containing 33 mmol/L EDTA. We then added 0.2 mL of 2 g/L BEC and measured the turbidity (A660 nm) after 50 min.

Aminoglycoside interference in the PRM assay (calculated as absor-