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 intra-individual 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 Women’s 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, gentamicin, 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), gentamicin (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. T7183) 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-
bance/μg) was determined using the concentration of a sample that gave an absorbance value within the linear range of the assay and was expressed relative to the absorbance/μg of BSA (corresponding to the gradient of the calibration curve):

\[
\text{Interference} = \frac{\text{absorbance}/\mu g \text{ of aminoglycoside}}{\text{absorbance}/\mu g \text{ of BSA}} \times 100%
\]

Neomycin, gentamicin, tobramycin, and paromomycin gave higher responses (442%, 304%, 259%, and 135%, respectively) than BSA (100%), whereas geneticin, kanamycin, streptomycin, and dihydrostreptomycin gave lower responses (26%, 21%, 7%, and 3%, respectively; n = 5; CV < 5%). The aminoglycosides gave negligible interference in the CBB assay. The aminoglycosides gave negligible interference in the BEC assay.


turbidity in the BEC assay.

In conclusion, we have confirmed interference in the PRM assay by gentamicin, neomycin, and tobramycin (1–3); reported interference from additional aminoglycosides; and demonstrated a susceptibility of the Sigma PRM assay to interference from aminoglycosides in urine at 0.2 g/L. In contrast to the PRM assay, the CBB and BEC assays are resistant to aminoglycoside interference.

References

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New Enzyme Immunoassay for Salivary Cortisol

To the Editor:

The measurement of salivary cortisol is emerging as the simplest approach in the diagnosis of Cushing syndrome (1–3). One of the problems with previous methods is the lack of a Food and Drug Administration (FDA)-cleared method. We have evaluated an enzyme immunoassay (EIA) for salivary cortisol marketed by Salimetrics (State College, PA) and recently cleared by the FDA for in vitro diagnostic use.

We compared the new EIA with our modified RIA (2) in 147 samples. The first set of samples (n = 44) was collected at 2300 and 0700 from a group of apparently healthy adult individuals (n = 22; age range, 25–60 years; 11 females and 11 males). The second set of samples (n = 30) was collected at 2300 by patients (n = 30; age range, 12–84 years; 22 females and 8 males) to screen for Cushing syndrome. The third set of samples (n = 73) was collected between 0600 and 1000 from participants (n = 42; age range, 6–14 years; 21 females and 21 males) enrolled in a study of allergic rhinitis. The study was approved by the appropriate Institutional Review Boards, and consent was obtained. Saliva was sampled as described previously (2) with a collecting device (Salivettes with no preservative; Sarstedt).

Salivary cortisol was measured by two methods. The serum cortisol RIA [Coat-a-Count TKCO; Diagnostic Products (DPC)] was used as commonly modified for the measurement of salivary cortisol (2). The salivary cortisol EIA (product no. 1-1102; Salimetrics) was used as instructed without modification. The sample volumes were 200 and 25 μL.