Effect of Addition of Hemolysate on Urine and Cerebrospinal Fluid Assays for Protein

To the Editor:

The presence of hemolyzed erythrocytes is common in urine and spinal fluid samples, and the released cellular contents, primarily hemoglobin (Hb), may affect protein measurements. Hematuria may be seen with or without the presence of other proteins in urine. Similarly, in hemorrhagic stroke, erythrocytes in the cerebrospinal fluid (CSF) begin to lyse within 2–3 h (1). Hb can be measured by protein assays, but may produce spectral and chemical interferences.

We investigated the effect of hemolysis on Pyrogallol Red (PYR) (2), benzethonium chloride (BTC) (3), and benzalkonium chloride (BC) (4) methods for measurement of protein. We prepared 3 urine and 3 CSF pools with different protein concentrations and added hemolysates at Hb concentrations of 48–3840 mg/L for urine and 186-5580 mg/L for CSF samples as described previously (5). We measured Hb hemolysate concentrations with a Coulter® LH 750 hematology analyzer.

For the PYR method (2), we included sodium dodecyl sulfate at 25 mg/L (6) and measured absorbances with a Shimadzu UV-1208 spectrophotometer. The BC and BTC methods were performed on a Roche Modular P analyzer with bichromatic analysis at 450 and 700 nm and a sample blank (4). In-house reagents were used for all of the methods. The protein concentrations were determined in duplicate by each method on the same day.

Hemolysis affected the PYR and BTC methods at an Hb concentration of 48 mg/L, the lowest concentration tested (see Fig. 1 and Table 1 in the Data Supplement that accompanies the online version of this letter at http://www.clinchem.org/content/vol52/issue1/), consistent with the report that the PYR method gives increased protein results at Hb concentrations ≥16 mg/L (7). These concentrations were considerably lower than the Hb concentrations (~200 mg/L) required for hemolysis to be detected visually (8). PYR was the method most affected by hemolysis in our study, and PYR results were increased 30% even at 48 mg/L Hb. For reasons that remain unclear, BC was not affected by hemolysis at low Hb concentrations (<192 mg/L for urine or <372 mg/L for CSF samples; Fig. 1), even in the pool with the lowest protein concentration.

Freedom from an effect of Hb on CSF and urine protein assays may be useful for clinical assays of urine and CSF because the presence or absence of proteins other than Hb provides an indication of the localization and type of the defect in the urinary tract. For example, cystitis, urinary tract neoplasia, and urinary tract stones are associated with hematuria without concomitant proteinuria. Similarly, for CSF protein assays, intracerebral hemorrhage is associated with high erythrocyte count and Hb concentration and a mild increase in CSF protein concentration. In contrast, subdural hemorrhage is associated with parallel increases in protein concentration and erythrocyte count or Hb concentration.

In conclusion, hemolysis interference in urine and CSF is method dependent, and microhemolysis may contribute to unexpected urine and CSF protein results in laboratories using BTC and PYR, whereas Hb does not seem to interfere in the BC method.

References
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Fig. 1. Effect of hemolysate on protein concentrations in urine (A) and CSF (B) as measured by the BC method. Relative error = [100(A0 - A1)/A0], where A0 is the apparent protein concentration in the presence of Hb, and A1 is the protein concentration in the absence of Hb. •, pool 1; ■, pool 2; ▲, pool 3. The dashed line in A is the 10% error limit.
Errors in the Assessment of Estimated Glomerular Filtration Rate

To the Editor:

The article by Grubb et al. (1) is an important contribution to the developing field of clinical chemistry and medicine. However, we believe that several factors possibly detract from it. First, the application of the Modification of Diet in Renal Disease (MDRD) formulae (2,3) in children is inappropriate, as those equations are not applicable to this age group. As a result, the scales of the y axes in Figs. 1 and 3 in the article (1) are inappropriate from the adult viewpoint. This makes graphical interpretation of the adult data more difficult—the data for children and adults should have been separated graphically and statistically.

Second, Grubb et al. (1) used a population different from the population studied by Levey and coworkers (2,3): i.e., relatively normal vs abnormal, respectively. The intent of the MDRD formulae is as a screen for patients at risk for end-stage renal disease and not as a screen for the general population.

Third, the cystatin C–based equation involving the factor 83.93 does not appear to be linear, as none of the ~27 data points above an iothalamate clearance of ~110 mL/min in Fig. 3A (1) seems to have a positive percentage error. Although errors in this range of glomerular filtration rate (GFR) may not be critical, it does beg the question concerning the derivation, and thus the validity, of the cystatin C–based equation throughout the whole GFR range. The authors state that they built “linear regression models based on log-transformed plasma clearance of iothalamate [in mL·min⁻¹·(1.73 m²)⁻¹] and cystatin C concentrations (in mg/L) because these transformations produced roughly symmetrically distributed regression residuals homogeneous variance” (1). As a result, we wonder whether the model put to use is actually the optimal model and whether it is mathematically justifiable. The formulae developed by both groups (1–3) were developed by empirical means with no attempt to apply an appropriate mathematical model. Empirical models are acceptable as rough sketches of relationships, but one should not depend on them for making precise clinical decisions. Empirical models, especially those built with log-transformed data, are weak because of their capacity to propagate errors.

The reports by Grubb et al. (1) and Levey and coworkers (2,3) represent the beginnings of the process to develop a simple laboratory method to identify patients at greater risk for renal failure, but we must caution clinicians not to believe that the estimates are infallible or to apply great clinical weight to their results. For example, 20% of patients had predicted cystatin C–based GFR values that differed more than 30% from the measured GFR values (1). A significant number of patients will have a measured GFR of 60 mL·min⁻¹·(1.73 m²)⁻¹, but one-fifth of them will have an estimated GFR >78 or <42 mL·min⁻¹·(1.73 m²)⁻¹. This is very important from both a wider population and an individual point of view, as it will have a major influence on the selection of diagnostic tests as well as on the selection of therapeutic agents for the treatment of patients. Before the laboratory community adopts estimated GFR as an approach to identifying patients at risk, we should ensure that a modern approach to model building is applied.

Finally, there are issues concerning the comparison of the various equations. The population used in the derivation of the cystatin C–based equation appears to be the same population used in the comparison study (1). If this is the case, then the cystatin C comparison data are not independent and will lead to overestimation of the accuracy of the cystatin C–based equation. Even if this is not the case, because the cystatin C–based equation was calibrated with iothalamate clearance (1) and the MDRD formulae were calibrated with iothalamate (2,3), any study comparing the equations with iothalamate clearance should favor the cystatin C equation. The alternative reference methods to the gold standard inulin clearance are similar but not the same.

Despite the above, the differences in the data above the age of 50 years in Fig. 1 in the article by Grubb et al. (1) raise some interesting questions: is the MDRD equation more likely to have a significant positive bias in patients >50 years of age than the cystatin C–based equation, and if so, why?

References


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