An unexpected problem in the clinical assessment of cystinosis

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Sirs,
Barshop [1] recently described some key variables in the clinical assessment of nephropathic cystinosis. We wish to add to that list. Cystinosis is an autosomal recessive disease of altered intracellular organelle cystine transport that leads to kidney failure and other critical organ dysfunction when untreated. The current approved treatment requires frequent aminothiols (Cystagon®) administration. The efficacy of Cystagon® treatment is reflected in the leukocyte cystine level. It is essential to measure leukocyte cystine levels accurately, both to diagnose the disease and to monitor treatment, as well as for clinical trials testing new cystinosis therapies. In a recent phase 3 study of a novel, delayed-release form of cysteamine sponsored by Raptor Pharmaceutical, patients were enrolled worldwide, using a single contract laboratory, but at two sites for the leukocyte cystine level determinations. Each clinical center received training to use the same method of leukocyte preparation prior to off-site clinical assays. Unfortunately, an unexpected problem quickly arose. This letter describes the problem, how it was corrected, and warns the medical community of a potential problem in the care of cystinosis patients.

Blood was anticoagulated with heparin and leukocytes prepared promptly after the blood was obtained. The leukocytes were lysed and immediately acid-precipitated with

sulfosalicylic acid. The acid pH stabilized the cystine in the supernatant and the protein pellet was dissolved in base. Both the cystine and protein were stable until assayed under these conditions. Cystine concentrations were measured using a highly accurate mass spectrophotometric method.

Although the usual method of reporting the level of a substance is by its concentration, the volume of the leukocytes is much too small to measure and hence, relying on precedent set in 1967 by Schneider et al. [2], cystine levels in cell preparations have been arbitrarily reported as nmol half-cystine/mg total protein in the cell lysate. Based on the same precedent, the Lowry assay, with bovine serum albumin as the standard, has been used as the default quantitation method for the total protein content of leukocyte lysate.

To qualify for the delayed-release cysteamine study, patients had to have leukocyte cystine levels of less than 1 nmol half-cystine/mg total protein. As described, total protein was determined by the Lowry assay. Although all patients initially met the requirement, many were subsequently found to have cystine levels greater than 1.0 at the time they entered the study. This discrepancy was found to correspond to a change in the leukocyte lysate total protein assay used by the contract clinical laboratory that used the BCA protein assay rather than the Lowry assay. Comparison of BCA and Lowry total protein assay values, using a bovine serum albumin standard, from 106 clinical samples from patients with cystinosis, revealed a significant and consistent difference in values from identical samples. The mean and standard deviation of the ratio between BCA and Lowry results over all samples was 0.65 ± 0.07 .

Discovery of the discrepancy in total protein values allowed values to be normalized and the study to proceed. We suspect that the discrepancies observed are based on variable assay sensitivity to different protein types, as has been noted by others [3]. Obviously, the choice of protein

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assay is completely arbitrary. This is just one of the many things that can influence the results of leukocyte cystine assays. Laboratories that assay leukocyte cystine must establish an exact system of preparing cells and performing both the cystine and protein assays, and then establish their own standard values. Clinicians should understand which protein assay is being used in interpretation of the leukocyte cystine levels at diagnosis and in making therapeutic decisions.

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