How Should We Measure the Albumin in Urine?

Laboratories almost universally use immunochemical methods to detect microalbuminuria, defined variously as an albumin excretion of 30–300 mg/day, an albumin/creatinine ratio of 30–300 mg/g, or an albumin excretion rate of 20–200 μg/min. (The term microalbuminuria is a misnomer, of course. No small form of albumin exists that is comparable, for example, to β₂-microglobulin, but the term now seems too deeply imbedded in the diabetic literature to be replaced.)

During the last 3 years, size-exclusion HPLC has been promoted to detect forms of albumin that are not reactive immunochemically (1, 2). The authors of these studies claimed that this approach is more sensitive in detecting increases in urinary albumin and detects them earlier than do the immunochemical methods (3). They isolated this nonimmunoactive albumin form after adsorption on immobilized anti-albumin and found that it contained <1% of the potential contaminants transferrin, α₁-acid glycoprotein, and α₁-antitrypsin by immunochemical tests and little, if any, by mass spectrometry.

A challenge to these findings appeared in the March issue of Clinical Chemistry (4). A group at NIH studied the performance of size-exclusion HPLC at the bench and found that 4 purified plasma proteins of comparable size are not resolved from albumin and that some larger ones can be included through tailing of the peaks. They tested 2 actual urines and showed the presence of transferrin, α₁-acid glycoprotein, and α₁-antitrypsin in the “albumin” peak by mass spectrometry. They estimate that 20%–30% of the albumin peak is attributable to serum globulins. Thus, the findings of the 2 groups are at odds, and the user is left in the lurch as to which to believe.

There were differences in the techniques used in the 2 reports, however, that may have a bearing on the dissimilarity of their findings.

Preconcentration of urines. Sviridov et al. (4) concentrated their urine specimens an unstated amount by use of a filter with a molecular mass cutoff of 10 000, whereas Comper’s group (1–3) apparently used unconcentrated urine to the columns. This could cause differences in the resolution of peaks on the columns, which were of the same dimensions.

Number of samples. Sviridov et al. (4) reported results on only 2 urine specimens, which contained 33 and 5 mg/L albumin by immunoassay. Comper et al., in their 2004 article (1), analyzed 10 urines with various concentrations of albumin. Other studies also found appreciably higher amounts of total albumin by HPLC than by immunochemical techniques with much greater numbers of specimens (2, 5, 6).

Contaminating plasma proteins. Both groups reported some contamination by plasma proteins, detected with mass spectrometry by Sviridov et al. (4) and seen in small amounts on native polyacrylamide gel electrophoresis by Comper’s group (1). Their report (1) that there was <1% contamination by 4 plasma proteins on testing with ELISA raises the question, however, whether these plasma proteins, like albumin, may also be present in nonimmunochemically reactive forms, which were not measured.

Application of other techniques. By scanning native polyacrylamide gel electrophoretic patterns of diabetic urine samples, Comper et al. (1) showed that the amounts present in the albumin band corresponded much better with HPLC values than they did with RIA values.

What is the clinical chemist to draw from these reports? First, that the size-exclusion technique should be applied with caution and with considerable judgment. Sviridov et al. (4) showed that selection of the chart baseline for delineation of the albumin peak can make a large difference in the “purity” of its value on integration. Second, based on reported values of an average of 67% more albumin measured by HPLC than by immunonephelometry for 30 cases (2) in the 20–200 μg/min range and 35% for 283 cases (5) in the 30–300 mg/L concentration range, interpreting the HPLC albumin value as containing ~25% globulins in these ranges may still yield a useful measurement.

Another finding that deserves further explanation is the detection in diabetic urines of “nicked” but intact albumin, i.e., albumin containing 1 or more cleavages of the peptide chain but that remains intact by virtue of its numerous S–S bonds (1, 7). Sviridov et al. (4) did not rule out the presence of this component. When analyzed by reducing polyacrylamide gel electrophoresis, the albumin-sized band disappeared and a large number of smaller peptides appeared. This form was nonimmuno-reactive. Its isolation and a search for the mechanism and site of its origin are of interest. It should be possible to isolate the peptides seen on reducing polyacrylamide gel electrophoresis and identify them by mass spectrometry as originating from human albumin.

The current situation implies that there is more albumin in early diabetic urine than immunochemical methods detect and that the incipient renal damage of early diabetes might be detected earlier if total albumin were reliably measured. The challenges to chemists are to devise improvements on methodologies to measure total urinary albumin and to establish reference intervals for the findings in pertinent groups of individuals.

References


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DOI: 10.1373/clinchem.2005.065284