Thirty years ago, a physician whose patient had diabetes and was urine protein dipstick–positive could only say, “You will most likely progress to end-stage renal disease in 10 years and need dialysis or a kidney transplant, and there is not much we can do about it.” Following the landmark microalbuminuria study of Mogensen and Christensen in 1984 (1), clinicians could say to patients with diabetes and microalbuminuria, “The good news is, we can now tell you 20 years ahead of time that you will likely progress to end-stage renal disease, but the bad news is that we still cannot do anything about it.” However, by the early 1990s, it was shown that treatment of microalbuminuric diabetic patients with ACE inhibitors to control blood pressure, diet modification, and intensive glucose control could slow or prevent progression to end-stage renal disease (ESRD) (2). Microalbumin quickly became a textbook example of a new laboratory test with profound impact on treatment and long-term outcomes of patients with a chronic disease.

Since then, the term microalbumin has been abandoned in favor of urine albumin because there is nothing “small” or “low molecular weight” about the albumin measured in urine. Urine albumin assays are simply immunoassays capable of measuring albumin concentrations that are more than 3 orders of magnitude lower than those encountered in serum, which are measured by dye-binding methods. Since its first use in patients with diabetes, urine albumin concentration has shown to predict progression to all-cause chronic kidney disease (CKD) and be an independent risk factor for cardiovascular disease (3). Today, numerous clinical practice guidelines exist for the use of urine albumin concentration to predict progression to CKD and cardiovascular risk (4). Two of the most cited are the Kidney Disease Improving Global Outcomes (KDIGO) and the American Diabetes Association (ADA) Standards of Medical Care in Diabetes (5, 6). Both of these define increased albumin excretion as an albumin:creatinine ratio (ACR) of ≥30 μg/mg creatinine (mg/g creatinine) and severely increased albumin excretion as >300 μg/mg creatinine (formerly called macroalbuminuria). The KDIGO guidelines define normal albumin excretion in “young” adults as <10 mg/g creatinine, thus leaving a gray-zone albumin excretion of 10–30 mg/g creatinine. It is interesting that these values, which correspond to approximately 30 mg/24 h, are remarkably close to the original cut point of 15 μg/min (20 mg/24 h) determined by Mogensen and Christensen in a study of only 43 patients!

In this issue of Clinical Chemistry, Bachmann et al. point out the rather dismal current state of the art for urine albumin measurement (7). In a well-controlled and -performed study, 332 patient urine samples were analyzed by 17 different commercial methods and a reference isotope dilution mass spectrometry (IDMS) method. The careful control of sample handling, strict statistical treatment of the causes of bias, and the broad concentration range examined make the conclusions from this study indisputable. Namely, differences in median bias between methods can be as much as 37%–45% across the concentration interval of 15–1000 mg/L. It is hoped that such a definitive study as this one will convince manufacturers to harmonize their methods to remove this variable from urine albumin measurement. Working together, the laboratory and clinical communities have done a remarkable job of standardizing and/or harmonizing tests used in clinical guidelines such as cholesterol (http://www.cdc.gov/labstandards/lsp.html), glycated hemoglobin (Hb A1c) (http://www.ngsp.org), and serum/plasma creatinine (8). It is likely that because of studies such as that of Bachmann et al. the community can do the same for urine albumin.

However, fixing the biases among urine albumin methods will not address the numerous other issues that confound the measurement of urine albumin excretion rates. These issues are addressed in a great detail in a recent review written by several of the same authors as the current study, but the points warrant repeating (4). First, there is a very large intr individual variability, with estimates ranging as high as 103% but centering around 28%–47%. There may be less intradi-
idual variability for first morning voids, but in 1 study of healthy individuals even such variability was considerable [31% coefficient of intra-individual variability (CVi)] (9). It is also clear that first morning void samples have lower ACR than random afternoon urine samples, since upright posture may enhance protein excretion. Although assay standardization cannot impact this biologic variability, clinical guidelines should be consistent and recommend first morning voids. For instance, the ADA guidelines recommend calculating the ACR using a random urine sample, whereas KDIGO recommends a first morning void sample. Both recommend confirming an increased value by obtaining and measuring another sample because of the high CVi. Second, there is the issue of whether immuno-unreactive albumin is present in urine and may contribute to the lack of harmonization among methods. It is known that fragments of albumin are present in urine, but whether they are of significance and contribute to the lack of harmonization between methods is controversial and unclear (10).

A third issue has nothing to do with the measurement of albumin but rather with urine creatinine. Equations for estimating glomerular filtration rate (GFR) (Cockcroft–Gault, Modification of Diet in Renal Disease, Chronic Kidney Disease Epidemiology Collaboration) account for sex and body mass (or race) because creatinine production is related to muscle mass. Daily production can vary by a factor of 2 or more between patients, but this is not considered when determining a normal ACR. Thus, African Americans will have a lower ACR based on increased creatinine filtration, and females will have a higher ACR for the opposite reason. None of the guidelines take this fact into account.

Yet another issue is that urine creatinine measurement is performed using a method originally designed to measure serum creatinine, thus requiring a large (100× is typical) dilution of the urine sample before analysis. This is an important fact since the 2013 College of American Pathologists survey shows that 75% of laboratories in the US use a compensated Jaffe reaction to measure creatinine. The “compensation” in these Jaffe methods essentially subtracts 0.2 mg/dL from the creatinine determination to account for the non-creatinine-reducing substances that react with picric acid (mainly serum proteins). However, these non-creatinine-reducing substances are not present in urine, and thus when a compensated Jaffe method (designed to measure serum creatinine) is used to measure urine creatinine in a highly diluted urine sample, urine creatinine will be underestimated by 10%–20% and the ACR overestimated. If an enzymatic creatinine assay that is traceable to an IDMS method is used, the “compensation” is not present and the urine creatinine will be measured accurately. The guidelines for ACR should indicate whether separate sex- and race-specific cut point values are needed and that urine creatinine should always be measured with enzymatic methods that are traceable to an IDMS method.

So, with all this biologic and analytic variability, why does urine albumin continue to be a useful and predictive marker for CKD progression, cardiac risk, and long-term treatment decisions? The answer is that an abnormal ACR detects early injury, and progression of CKD is a gradual process. With annual or biannual screening, affected individuals will be identified in time to implement treatment regardless of the issues discussed above.

The same argument cannot be made for other common laboratory tests used in similar KDIGO guidelines. There is a tremendous variation and a lack of harmonization in the measurement of parathyroid hormone (PTH), ferritin, and vitamin D assays (11–13). Various KDIGO guidelines recommend that specific targets or cut point values for these tests be used to guide treatment decisions that can have major sequelae and complications. For example, clinicians are advised to consider treatment with intravenous iron if the ferritin concentration is ≤500 ng/mL; but for such a concentration, different assays yield ferritin results with a range of 439–632 ng/mL (12). Similar situations exist in guidelines related to PTH, serum albumin, and vitamin D. Bias in a PTH assay led to one of the largest settlements ever for Centers for Medicare & Medicaid Services (CMS) against a medical device manufacturer (14). Immunoassays are difficult to harmonize when various manufacturers use different antibodies or when the source of the antibodies ceases to exist (goat dies!) and when multiple fragments of the analyte are present in the specimen (15). However, we believe that similar efforts to those shown by Bachmann et al. are needed for these problematic tests. So, should we sweat the “micro” things? Absolutely! But the laboratory and the renal communities should focus more efforts on bigger things, and hopefully the laboratory community will continue to take a lead in these efforts (16).

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