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The Search for More Reliable Estimated GFR Biomarkers

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In this issue of *AJKD*, Inker et al[1] explore the role of the unconventional endogenous filtration markers, β-trace protein (BTP) and β_2-microglobulin (B2M), for estimating glomerular filtration rate (GFR). Because of challenges associated with precise measurements of kidney function in clinical practice and clinical research, more accurate estimation of GFR may have important implications in medicine.

Kidney function cannot be measured directly. Accordingly, GFR, which can be measured using exogenous filtration markers and can be estimated using endogenous filtration markers, is the most widely accepted surrogate marker of kidney function.[2] A GFR marker must have a stable concentration in plasma and must be physiologically inert, must be freely filtered by the glomerulus, and must not be secreted, reabsorbed, synthesized, or metabolized by the kidney, so that the amount filtered equals the amount excreted in urine.[3] The gold standard for GFR measurement is inulin clearance, a method introduced by Homer Smith in 1933.[4] Today, methods using iohexol, iothalamate, or EDTA have replaced inulin clearance, but all these are cumbersome, are invasive, have considerable imprecision, and involve exposure to exogenous substances, making them somewhat impractical.[2]

Reflecting these limitations, many endogenous biomarkers of GFR have been proposed over the centuries. The year 1773 saw the first attempts to quantify kidney function with the quantitation of serum urea.[5] A century and a half later, Moller introduced the concept of urea clearance as a measure of kidney function, defining clearance as "the volume of blood that a one minute's excretion of urine suffices to completely clear of urea."[6-9] Meanwhile, the history of creatinine dates back to 1847, when Liebig[10] heated creatine with mineral acids and named the resulting substance creatinine. In 1929, Rehberg[11] suggested that creatinine was filtered through glomeruli and concentrated in tubules, and 6 years later, Shannon[12] showed that creatinine clearance values approximate the clearance of inulin. The next year, Popper and Mandel[13] proposed the use of serum creatinine for GFR estimation. Two decades later, another marker of GFR was proposed, serum indican (indoxyl sulfate),[14] a protein-bound solute generated from the metabolism of tryptophan, phenylalanine, and tyrosine in the colon.[15]

Timed urine collection proved to be very unreliable, yielding a very high coefficient of variation (29%) even when using rigidly controlled conditions.[16] Because of intraindividual day-to-day fluctuations, creatinine excretion of an accurately collected 24-hour urine sample would have to be measured for several days, rendering this approach impractical.[16] As a result, 24-hour biomarker clearance was largely abandoned for single plasma concentrations, and serum creatinine has remained the most widely used marker for GFR estimation despite its many shortcomings.[2] Although more precise measurement of serum creatinine through widespread implementation of isotope-dilution mass spectrometry traceability in clinical chemistry laboratories has reduced some of the analytical problems,[17,18] considerable issues remain related to the properties of creatinine, including variability in muscle mass, dietary intake, creatinine production, and nonrenal elimination, especially in select populations such as individuals with atypically high or low muscle mass[19] and children.[2,20]

Additional options include other endogenous small-molecular-weight proteins. Jung and coworkers were the first to propose small-molecular-mass proteins as markers of GFR because typically they are essentially freely filtered through a healthy glomerular membrane.[21-23] Of these, cystatin C[24] appears to be the best marker to date, especially since Grubb et al[25] introduced a certified international standardized reference material for cystatin C.

However, although cystatin C has been demonstrated to be independent of muscle mass and clearly superior in its ability to detect a decrease in GFR in the so-called "creatinine-blind range,"[26] it subsequently became clear that combining 2 (or more) endogenous markers improves the accuracy of GFR estimation equations.[27-29] This is not surprising because cystatin C levels can be affected by patient characteristics, such as inflammation, hyperthyroidism, high-dose steroids, and even triglycerides.[24,30] In reality, there probably is no individual protein that has a totally constant production unaffected by changes in systemic metabolism and systemic inflammatory reactions. Today, the CKiD (Chronic Kidney Disease in Children)[31] and CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration)[29] equations are the most established formulas for the estimation of GFR in children and adults, respectively. Combining 2

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well-established markers of estimated GFR (eGFR) such as serum creatinine and cystatin C, with their different strengths and challenges, should be superior to the estimation of GFR using 1 biomarker alone. However, it should be noted that each formula works best in the population type and within the GFR range in which it was generated.[32]

In the past, other small-molecular-weight proteins have also been proposed as filtration markers, most notably BTP and B2M.[33,34] Some doubts were cast upon B2M when viewed in isolation,[33] reflecting the influence of acute-phase reactions on its levels, as was demonstrated in patients with lupus[35]; notably though, it is used at times as a dialysis adequacy marker. BTP appears to be similarly promising as cystatin C, with certain advantages in special populations such as pregnant women and neonates.[36] The advantages of BTP as a marker of GFR are discussed in detail in our recent review.[36]

In this context, Inker et al[1] evaluated the utility of BTP and B2M to estimate GFR. Using data from 3,551 participants in 3 large populations with CKD, they developed and validated a robust GFR estimating equation using these biomarkers and commonly available clinical characteristics. They carefully adjusted for covariates such as sex, ethnicity, and age. For BTP alone, sex and age remained significant influences. The impact of sex confirms our previous findings.[37,38] For B2M, there was no effect of sex, and the equation based on BTP and B2M was free of significant bias. Not unexpectedly, this study demonstrates that age, sex, and race are less influential for both these small-molecular-weight proteins in comparison to creatinine, but not cystatin C. Most importantly, the authors demonstrated that the coefficient of variation of B2M for sex was smaller than that of cystatin C, which is probably due to the increased fat mass of women because adipocytes are the only nucleated cells in the body that do not produce cystatin C.[24] The authors then combined B2M and BTP and the resultant formula had similar accuracy to the CKD-EPI creatinine–cystatin C equation. Inker et al conclude that BTP and B2M are less influenced by age, sex, and race than creatinine and less influenced by race than cystatin C. Their new equation appears to provide a methodological advantage, although they clearly point out the limitations of this post hoc analysis and mandate further prospective evaluations before clinical use is recommended.

This is good advice in view of the limited availability of BTP assays. Currently, BTP measurement is generally used for the detection of cerebrospinal fluid leakage,[36] and only a few laboratories routinely offer serum BTP for the assessment of eGFR.[36,37,39] In addition, it is likely that each biomarker will have some populations in which it is particularly well or poorly suited. Both BTM and B2M appear to have some advantages over serum creatinine for GFR estimation, but systemic inflammation and other factors clearly have some influence on the production of small-molecular-weight proteins, making the utility of these novel biomarkers less certain in people with acute and chronic systemic inflammatory states. The question is, how many markers should we combine? Clearly, a combination of all 4 markers may produce slightly higher agreement with measured GFR, but the clinical utility of equations containing too many components is limited.

It should not be forgotten that the GFR measurement method used in the 3 large patient cohorts in the Inker et al study under discussion has some plasma protein-binding tendencies that affect its accuracy in comparison to inulin.[2] Iohexol may currently be the second-best GFR method after inulin. In this context, Seegmiller et al[40] recently assessed the discordance between iothalamate and iohexol urinary clearances (the latter were used by Inker et al). Although they did not measure inulin clearance, they showed that that the mean proportional ratio of iohexol to iothalamate clearance was 0.85 (95% confidence interval, 0.83-0.88) across the range of GFRs, indicating that GFR measured using iohexol clearance is lower than GFR measured using iothalamate clearance. These relatively small but significant biases were opposite to each other, which may reduce the impact on the "gold-standard" method used by Inker et al.

Ultimately, to design the best possible estimating strategies, the community should unite to design collaborative worldwide prospective studies using inulin as the gold standard for clearance measured with tandem mass spectrometry to reduce the imprecision of the inulin measurement, using only biomarkers with certified international standardized reference materials (it is important to note that these are needed for B2M and BTP), and combining these studies with proteomics and metabolomics to identify additional internal biomarkers of GFR that perhaps are less influenced by acute-phase reactions. Combinations other than cystatin C and creatinine may be useful, especially in select populations. The cost difference of BTP and B2M over cystatin C and creatinine may render this new approach impractical, but with more widespread use, assay costs will come down. A more cost-efficient approach may be the determination of multiple serum proteins by mass spectrometry. Taking a high-level view, one should also question the emphasis on GFR when assessing kidney function. Tubular function is responsible for a large proportion of kidney function, especially with regard to drug clearance, and disturbance in GFR and tubular function do not necessarily change plasma

proteins in parallel. Nonetheless, the study by Inker et al forms a very important contribution to the search for the best biomarker of eGFR.

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