# For CKD, work is on to refine and find biomarkers

## Karen Lusky

**July 2015—Getting the upper hand on chronic kidney disease requires taking maximum advantage**of existing CKD biomarker capabilities. It also means discovering new markers, though the trick is finding those that can expand treatment options. Some believe fibroblast growth factor-23 has the potential to fit that bill, with one researcher calling it "among the most exciting new targets in chronic kidney disease."

Urine albumin, creatinine, and newcomer cystatin C have their strong points. Each has its pitfalls, too, which stem from the nature of the biomarker itself, testing accuracy issues, or sometimes knowledge gaps about how to use or interpret a test result.

"Laboratory professionals should be aware that both clinicians and patients may look at these [test] results as absolute without understanding the variables that may affect measurements," says Andrew Narva, MD, director of the National Kidney Disease Education Program at the National Institutes of Health, who is often on the stump educating clinicians and sometimes laboratory professionals about laboratory assessment of chronic kidney disease.

"About half of the people identified as having CKD in the U.S. have that diagnosis only on the basis of increased urine albumin," Dr. Narva says. "You can have albuminuria or decreased glomerular filtration rate, or both."

Dr. Bachmann

Urine albumin is the best biomarker for the more common causes of kidney damage: diabetes, hypertension, and cardiovascular risk factors, says W. Greg Miller, PhD, professor of pathology at Virginia Commonwealth University Medical Center, chair of the NKDEP Laboratory Working Group, and member of the CAP's Accuracy-Based Testing Committee and of the International Standards Organization Technical Committee 212 Working Group 2 on Clinical Laboratory Reference Measurement Procedures and Materials. "A lot of different studies over time have shown very clearly that the albumin/creatinine ratio in the first morning void is very nearly identical to the 24-hour urine albumin excretion rate," Dr. Miller says. The albumin/creatinine ratio, or ACR, "compensates pretty nicely for hydration," he adds. The standard cutoff is 30 mg per gram of creatinine.

Misunderstandings about the ACR can sidetrack clinical care, however. In a talk at last year's AACC annual meeting, Dr. Narva reported that he frequently gets questions from clinicians such as, "'We did the microalbumin test. It came back 5,726. What do I do now? Do I get a 24-hour urine?' I say, 'No, that patient is in big trouble. That patient has about 6 grams of urinary albumin per day.'"

"The other issue," Dr. Narva said, "is the multiple names that are given to these tests, and many clinicians think that if you have a little bit of kidney disease, you have the little albumins, the microalbumins, and then if you have bad kidney disease, the big albumins come out. They don't understand that it's all albumin and that's a continuous risk factor."

Dr. Miller

Urine albumin isn't standardized, which can affect diagnosis and follow-up, especially if clinicians aren't aware of it. Dr. Miller and Lorin Bachmann, PhD, DABCC, associate professor of pathology, Virginia Commonwealth University Medical Center, are leading a standardization initiative. "The NKDEP and International Federation of Clinical Chemistry and Laboratory Medicine have formed a joint Laboratory Working Group to facilitate standardization efforts for urine albumin," says Dr. Bachmann, who chairs the IFCC Working Group for Standardization of Albumin in Urine. The aim is "a robust reference system so the manufacturers have a trusted accuracy base on which to calibrate their clinical assays," says John H. Eckfeldt, MD, PhD, former chair and current member of the NKDEP Laboratory Working Group and a professor of laboratory medicine and pathology, University of Minnesota.

How far off the mark are manufacturers' assays? A study that Dr. Miller, Dr. Bachmann, and colleagues conducted found as much as a 45 percent difference among the various commercial urine albumin procedures (Bachmann LM, et al. *Clin Chem.* 2014;60[3]:471–480).

"Most of the difference," Dr. Bachmann says, "could be accounted for by concentration-dependent bias, where the methods differed by a range of -35 to +35 percent at 15 mg/L and -15 to +18 percent at 30 mg/L."
That means patients who are near the ACR cutpoint of 30 mg/g could be classified as being above or below that threshold based on the laboratory method used rather than actual physiology, Dr. Miller says. "There's a lot of evidence that suggests that the threshold should actually be lower. There's an increased hazard ratio for progressing CKD at lower albumin/creatinine ratios, but the bias becomes even larger at lower values. So until the standardization is complete," he says, "it's not really practical from a clinical implementation point of view to consider lowering the thresholds."

Dr. Miller predicts standardization will be wrapped up in about six years. The best route for now may be for clinicians to use the same laboratory for urine albumin testing when monitoring a patient who, for example, has diabetes, hypertension, or increased cardiovascular risk, he says. "The advantage of using the same lab is then you see changes that probably reflect the patient rather than the lab method. If you are tracking a diabetic who doesn't yet have albumin in their urine, you could make a case that you should try two or three labs to see if one of them gives you a value above 30 mg/g as an early indicator, but it's not a practical recommendation."

Dr. Narva

Diurnal variation and many physiological parameters that affect urine albumin levels also can cause albumin/creatinine ratio results to vary. Dr. Narva points out that the Centers for Disease Control and Prevention's evaluation of NHANES (National Health and Nutrition Examination Survey) data found that only 43 percent of the participants with a positive random urine exceeding 30 mg/g of creatinine had a positive first void specimen. That information was useful, he says, because the timing of the two specimen collections reflects the usual clinical scenario in which a screening test is performed on a random basis and a confirmatory one on a first morning void.

"It would be nice to have a random followed up by two first voids in a study to confirm that's the way to do it," Dr. Narva says. "As part of the urine albumin standardization process, the Lab Working Group will provide data on the

best timing for reproducibility." The CDC data will be useful in that regard, he adds.

**In the use of glomerular filtration rate to diagnose CKD,** Dr. Narva finds there's confusion about the difference between measured and estimated GFR. He notes that the estimated GFR equations were developed in populations of people who had their GFRs measured. "They do provide a very good reflection of the population, but when you are looking at an individual across the desk or exam table from you, the uncertainty associated with the prediction equation result for that individual can be a significant matter," he cautions. "The performance characteristic that's used is P30, which is the likelihood of being within [plus or minus] 30 percent of the measured GFR. That's a pretty wide range, and it increases as eGFR increases. So you can be telling someone they have CKD when actually they do not."

The most useful aspect of the estimated GFR, he says, is identifying those with CKD who have a near normal creatinine. "People often don't understand that the creatinine doesn't have to be very high for the GFR to be much decreased. If someone sees a creatinine of three, they know that person has a problem. Its greatest purpose is as a warning flag that someone actually may have kidney disease."

The 2012 Kidney Disease: Improving Global Outcomes recommendations, released in January 2013, advise using cystatin C for patients who have an eGFR based on creatinine that falls between 45 and 59 without elevated urine albumin. "That's sort of an ambiguous area," Dr. Miller says, "and basing the eGFR on a cystatin C-based equation or ideally on a combined creatinine and cystatin C equation gives a more reliable estimate of GFR."

**Dr. Eckfeldt**

Dr. Eckfeldt says the group in Lund, Sweden, that discovered cystatin C recommends reporting estimated GFRs individually, with one based on creatinine and another on cystatin C to discover discordance and investigate why they are different. For example, if people are cachectic, they produce less creatinine than normal people, and their eGFRcreatinine will be artificially high, Dr. Eckfeldt says. Body builders will have an artificially low eGFRcreatinine.

An inaccurate cystatin C result could explain the difference between the two eGFRs. A study reported in Archives of Pathology & Laboratory Medicine says: "[D]espite the availability of an international reference material for more than 3 years, the variability in cystatin C measured values with several widely used clinical measurement procedures appears to be too large for the values to be very useful for diagnosing and managing patients with kidney disease" (Eckfeldt JH, et al. Arch Pathol Lab Med. 2015;139[7]:888–893).

Dr. Eckfeldt explains: "Very roughly, a given percentage error in cystatin C concentration translates into a similar percentage error in eGFRcystatin C in the opposite direction. Thus, if your measured cystatin C concentration is 25 percent low, then your eGFRcystatin C will be 25 percent high. This could put the patient in an entirely different category of chronic kidney disease." While the inverse relationship between serum creatinine and eGFRcreatinine is similar, the accuracy of serum creatinine measurements has improved dramatically over the past decade, says Dr. Eckfeldt, who is also a member of the CAP's Chemistry Resource and Accuracy-Based Testing committees and of the International Standards Organization Technical Committee 212 Working Group 2 on Clinical Laboratory Reference Measurement Procedures and Materials.
What's needed more broadly,

Dr. Eckfeldt says, are better biomarkers to diagnose kidney injury and predict development of end-stage renal disease. "Most of the people classified as at risk of developing end-stage renal disease or adverse symptoms from

the kidney disease itself never really develop end-stage renal disease. In fact, it's a relatively small percentage," he notes. The National Institute of Diabetes and Digestive and Kidney Diseases has a CKD Biomarkers Consortium of more than a dozen universities looking for biomarkers that can predict and improve outcomes.

Nephrologist Paul L. Kimmel, MD, senior advisor at the institute and project scientist for the consortium for phases one and two, says there has been great interest at the NIDDK in developing CKD biomarkers that could be helpful in a number of ways. One would be to identify which patients will have a rapid course to end-stage renal disease, and another to predict which patients will respond to a particular medication, or which patients will have a complication seen in CKD.

"Also, for designing clinical trials, I think it's imperative that we have better markers, if they exist, to guide getting the patients who would be responsive to the therapy into the trial, and excluding patients who would not have a response to therapy or perhaps have an adverse response," Dr. Kimmel says.

In Dr. Kimmel's view, the most promising marker appears to be fibroblast growth factor-23 (FGF23), which he describes as "an early marker of mineral metabolism dysfunction in CKD."

The main finding of a recently published study (Rebholz CM, et al. *J Am Soc Nephrol.* 2015;26[1]:192–200) was that higher levels of intact FGF23 were strongly associated with development of end-stage renal disease. "We adjusted for a number of confounders, such as demographic characteristics, and risk factors for kidney disease as well as the baseline kidney function, eGFR, and then other mineral metabolism biomarkers," says Casey Rebholz, PhD, MS, MPH, assistant professor, Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health.

The researchers, the abstract says, assessed the relationship between baseline (1990–1992) serum levels of intact FGF23 and incident ESRD in 13,448 Atherosclerosis Risk in Communities (ARIC) study participants (56.1 percent women, 74.7 percent white) followed through 2010. At baseline, the mean age of participants was 56.9 years; the mean eGFR was 97 mL/min per 1.73m2. During a median follow-up of 19 years, 267 participants, or two percent, developed ESRD.

Dr. Coresh

Josef Coresh, MD, PhD, also of the Johns Hopkins Department of Epidemiology and a co-investigator for the study, says they focused on FGF23 instead of comparing it to other things. "When you look at CKD, the strongest risk factor is always estimated GFR by creatinine. That alone can have a 10,000-fold risk rate, but we know about that." The goal, he says, "is to get new biomarkers with information above and beyond that which is potentially actionable. The idea is the phosphate axis [which is affected by FGF23 levels] could be potentially actionable through dietary intake or other manipulation."

FGF23 researcher Myles Wolf, MD, MMSc, says FGF23 is one of the main regulators of serum phosphate. With dietary phosphate a contributor to FGF23 elevation, he too believes it could be actionable in CKD.

However, a number of small pilot studies have examined whether dietary phosphate restriction or dietary phosphate binders that prevent gastrointestinal absorption can lower FGF23, and the results have been mixed, says Dr. Wolf, Margaret Gray Morton professor of medicine and director of the Center for Translational Metabolism and Health at Northwestern University Feinberg School of Medicine.

The COMBINE study (CKD Optimal Management With Binders and Nicotinamide) is going to take a different tack, says Dr. Wolf, a member of the study's steering committee. Researchers will use a phosphate binder, lanthanum

carbonate, along with nicotinamide as a second method to reduce phosphate absorption, he says. The aim is to lower FGF23 and serum phosphate levels. Participants are being recruited now and the study is expected to be completed within 24 to 30 months.

Dr. Wolf

“The COMBINE study is a pilot study, the primary goal of which is to test whether these interventions can lower biomarkers of phosphate homeostasis, including serum phosphate and FGF23,” explains Dr. Wolf. If the study is successful, the next question will be whether the interventions can in turn improve clinical outcomes. “That would require a subsequent randomized, controlled trial that would need to be much larger and of substantially longer duration. So as far as changing management of chronic kidney disease, I believe that FGF23 testing and targeting is still a fair bit away.”

While the mechanisms of FGF23 elevation in kidney disease may be somewhat related to phosphate intake, it’s likely other mechanisms are involved too, Dr. Wolf says. “So exclusively targeting phosphate might be insufficient in many patients.”

He points out that several human studies have shown an association between higher FGF23 and left ventricular hypertrophy, and the latter is a well-known underlying mechanism that contributes to diastolic heart failure. “We published an [animal] study in which we showed that elevated FGF23 might play a direct causal role in the pathogenesis of LVH by stimulating hypertrophic growth of cardiac myocytes. And we showed that LVH was present in several experimental states of elevated FGF23” (Faul C, et al. *J Clin Invest.* 2011;121[11]:4393–4408).

“FGF23 is extremely strongly predictive of death and heart failure,” he adds.
Dr. Wolf says his group’s theory is that kidney disease results in increased FGF23 levels. “This helps patients maintain a normal serum level of phosphate, but chronically elevated FGF23 levels, especially in the setting of chronic kidney disease, promote LVH which puts patients at risk for heart failure and perhaps death.”

Are there other ways to lower FGF23 levels? To answer that question, Dr. Wolf notes a paper published online last month in Circulation on a secondary analysis of the EVOLVE trial, which had 4,000 dialysis patients. The new analysis showed that a medication called cinacalcet (Sensipar) decreased FGF23 and that participants with the largest reduction had a significant survival benefit and significantly fewer cardiac events, particularly heart failure (Moe SM, et al. Circulation. Epub ahead of print June 9, 2015. doi:10.1161/circulation aha.114.013876).

“So it all fits with the hypothesis that elevated FGF23 increases risk of heart failure and death, and suggests that cinacalcet is a non-dietary approach to lowering FGF23,” Dr. Wolf says. There’s a catch: Cinacalcet is not FDA-approved for use by CKD patients who aren’t on dialysis. Part of the reason is that the drug causes excessive hypocalcemia, he says.

“Our hope,” Dr. Wolf says, “is that the cardiac receptor that mediates the toxic effects of FGF23 is distinct from the receptor that mediates the beneficial effects of FGF23 to regulate phosphate excretion in the kidney. If so, that could create the opportunity to block FGF23 effects on the heart selectively.”

“I think FGF23 is a reasonably good biomarker, but I think its promise may prove to be ultimately greater as a target or mediator than as a biomarker,” Dr. Wolf concludes. He says it could lead to important clinical advances in the future and considers it “among the most exciting new targets” in CKD.

Dr. Eckfeldt, a co-investigator with Dr. Rebholz of the study reported in the Journal of the American Society of

Nephrology linking FGF23 levels to future ESRD risk, thinks that FGF23 could be clinically useful as a solo biomarker or in conjunction with others but that the jury is still out. At this point, more clarity about how to measure the marker is needed.

“There are several different IVD manufacturers’ assays for FGF23 that give somewhat discordant answers. So the findings are somewhat assay-dependent, which is partly due to different forms of FGF23 being present in serum of certain patient groups. The problem,” Dr. Eckfeldt says, “is somewhat similar to early parathyroid hormone assays where C-terminal assays got very different values from N-terminal or ‘intact’ assays, particularly in patients with renal disease where non-biologically active PTH accumulates.”

**As for other potential biomarkers, Dr. Kimmel says** there’s been a finding for about five years that variants in apolipoprotein L1 (APOL1) alleles confer a very high risk of susceptibility to developing common renal diseases, such as focal glomerular sclerosis, hypertensive nephrosclerosis, and HIV-associated nephropathy. While everyone has the APOL1 gene, says Johns Hopkins’ Dr. Coresh, it’s only present as a susceptibility variant in populations of African origin. In people who have the two variant alleles, “early on the risk [of CKD] isn’t that high, but later on in kidney disease the risk probably accelerates,” he says. “So it will likely be an important marker, but right now it’s not ready for widespread screening because we aren’t completely clear about the risk or appropriate action.”

That’s the issue for the CKD Biomarkers Consortium, Dr. Kimmel says: “Whether we can come up with something meaningful that a doctor can use during a patient visit that will be better than proteinuria so the physician can say to a patient: ‘You are 62 years old and have a long history of hypertension. Now your kidney function is slightly diminished, you have a certain level of proteinuria, and there’s this great test that says, We don’t have to worry, because if you take a certain drug, your renal function isn’t going to change for 30 years.’”

“We prescribe ACE inhibitors or ARBs to control blood pressure, carefully watch the medication list for [nephrotoxic drugs], and make sure no obstructive uropathy develops, but we don’t now have a test or a therapy where we are going to radically change things in most cases of hypertension-attributed or diabetic kidney disease,” Dr. Kimmel says. “We need that kind of information.”

He holds in high regard the clinicians who back in the day developed the tests still in use—serum creatinine and urinary protein. “It’s very, very hard to do better than those in predicting outcomes,” Dr. Kimmel says, “which is the challenge for the kidney biomarker community.”

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