As diabetic CKD takes toll, work on tests continues

Kevin B. O'Reilly

September 2016—When nephrologist Katherine Tuttle, MD, first saw the photo of two women holding young children, she thought it captured the mother of the boy and girl sitting on a couch with the children's grandmother.

The younger-looking woman, 33 at the time the photo was taken, works in the clinical research group at Providence Health Care, Spokane, Wash., where Dr. Tuttle is executive director for research. Flashing a smile in the photo, Dr. Tuttle's colleague held in her arms a baby girl who munched on her toy. Seated next to her was a woman whom diabetologists would recognize as having lost sight in one eye, with a two-year-old boy on her lap. Her face, deeply lined with wrinkles, bore a glum expression.

Contrary to Dr. Tuttle's first impression, that second woman was no grandmother. She, too, was 33 years old, a cousin of Dr. Tuttle's colleague and the mother of the two-year-old boy. Diagnosed with type 1 diabetes at 12, she had been on hemodialysis for two years by the point the photo was taken and had lost vascular access. Due to her son's birth, the woman was highly sensitized and no kidney donor could be found.

"She was dialyzing via a hemodialysis catheter, and if you are a nephrologist you'd say she was probably not receiving very good dialysis based on the way she looked," Dr. Tuttle said during a talk at the American Association for Clinical Chemistry's annual meeting in August. "She had been thinking about stopping dialysis, but she didn't have to make that choice because she was found dead about six weeks after this picture was taken."

"This is what youth-onset diabetes looks like by the 30s, when people are supposed to be enjoying the prime of life," she added. "It doesn't matter if you're type 1 or 2. It doesn't matter what color you are. It's really tragic, and it should be preventable."

But preventing kidney failure requires new therapies and better biomarkers to help develop, test, and monitor the effect of those treatments. Slashing the rates at which patients with diabetes develop and die of kidney disease also depends on improved clinical use of existing tests and standardization of urine albumin and the cystatin C-based estimate of glomerular filtration rate, said Dr. Tuttle and her co-panelists during the AACC session, "Diabetic Nephropathy: Where Are We Now?"

About 30 percent of patients with type 1 diabetes eventually develop chronic kidney disease, and CKD is diagnosed in about 40 percent of those with type 2 diabetes. Between 66 percent and 86 percent of American patients with end-stage renal disease have diabetes (the rate varies by race).

Dr. Tuttle

"Those of us in nephrology, we proportionally take care of more people with diabetes than most endocrinologists," said Dr. Tuttle, who also trained as an endocrinologist-diabetologist. "There are proportionally more diabetic patients in the nephrology clinic and dialysis centers than there are in the endocrine clinics. That's a sobering fact."

Looking at the big picture, the problem is only getting worse, Dr. Tuttle argues. The prevalence of diabetic kidney disease, or DKD—defined as persistent albuminuria and reduced eGFR—has not changed significantly despite decades of attempts to improve clinical management of diabetes. Examining cross-sectional studies of adults from

the National Health and Nutrition Examination Surveys, Dr. Tuttle and her colleagues found a DKD rate of 28.4 percent from 1988 to 1994. In the most recent period, 2009–2014, that rate fell slightly, to 26.2 percent (Afkarian M, et al. *JAMA*. 2016;316[6]:602–610).

"I don't think it's really good news," Dr. Tuttle tells CAP TODAY. "During this time period, the rate of the use of effective treatments of diabetes has improved, but it has not translated to a clinically impactful reduction of this disease. When you look at overall diabetes complications, they're going down, and down remarkably. Cardiovascular event rates are down across the board more than 50 percent, and myocardial infarction in particular is down 67 percent. That's what better diabetes care did. However, this care did not reduce kidney disease in the same way."

An interesting wrinkle in the NHANES data is that the prevalence of albuminuria fell from 20.8 percent in the 1988–1994 period to 15.9 percent during 2009–2014. Meanwhile, the prevalence of decreased eGFR went in the opposite direction, rising from 9.2 percent to 14.1 percent.

"The presentation is changing, to older adults presenting with low eGFR rather than with high albuminuria," says Dr. Tuttle, also regional principal investigator and clinical professor of medicine at the University of Washington's Institute of Translational Health Sciences. "Albuminuria screening alone is insufficient. I think that message has been out there, but these data make a clear argument for why it is insufficient . . . especially among older adults."

Something more than GFR is needed too.

Kidney disease develops in patients with diabetes in a slightly different fashion than in other patients with CKD and may not initially be reflected in a reduced glomerular filtration rate, said Andrew Narva, MD. He is director of the National Kidney Disease Education Program, which is part of the NIH's National Institute of Diabetes and Digestive and Kidney Diseases.

"Initially, patients become hyperglycemic and their GFR actually goes up," Dr. Narva told the AACC crowd. "The physiologic response to higher filtered loads in glucose is to reabsorb water and salt and that results in expanded volume and increased GFR. Over time, however, damage to the kidney from diabetes becomes apparent. The GFR decreases and at approximately the point where it reaches the preexisting GFR, urine albumin increases. That's why increased urine albumin, classically and especially in type 1, is the very first clinical sign of diabetic kidney disease—because, at this point, urine albumin has increased but the GFR appears normal. It is normal, but it's decreased from its higher level. From then on, there's a steady decrease in GFR and an increase in urine albumin until the GFR's so low that there's so little of anything being filtered."

Dr. Narva noted this classical course of DKD is not universal.

"There's more than one kind of diabetic injury," he said. "We can see this clinically. We know that albuminuria can decrease, and that decreased GFR can develop without albuminuria. Again, that heterogeneity we see in this disease is often not reflected in GFR. So you need something beyond that."

When it comes to kidney-function tests already available for use by clinicians and laboratories, good progress has been made in some areas in understanding how to test, which tests to use, and in standardization among test manufacturers. But in other areas, much work remains to be done, said Greg Miller, PhD, professor of pathology, director of clinical chemistry, and director of pathology information systems at Virginia Commonwealth University Health System in Richmond.

The National Kidney Disease Education Program's Laboratory Working Group, which Dr. Miller now chairs, began in 2003 to standardize and reduce the variability in eGFRcreatinine reporting. In the developed world, that was achieved by 2011, Dr. Miller said, pointing to a CAP Survey demonstrating as much (Killeen AA, et al. *Arch Pathol Lab Med.* 2013;137[4]:496–502).

"Congratulations to the laboratory community for solving that problem," he said. But, he noted, that standardization does not resolve the problem of interfering substances in creatinine testing (and, subsequently,

creatinine-based eGFR). He cited research on seven commercially available procedures using enzymatic and Jaffe methods. About four percent of the samples from diabetes patients run using enzymatic methods had biased results caused by interfering substances, while half of the Jaffe method results were biased (Greenberg N, et al. *Clin Chem.*2012;58[2]:391–401).

Dr. Miller

“Both Jaffe and enzymatic methods are affected by some drugs,” Dr. Miller said. “It’s not correct to say that enzymatic procedures are definitely free of interferences. However, as you can see, so far the Jaffe methods do appear to be somewhat worse, particularly in that diabetic group, which is a major clinical subgroup.”

In the question-and-answer session, Dr. Miller expanded on his view of this controversial matter of whether Jaffe methods for measuring creatinine ought to be avoided.

“In my opinion, I think clearly the enzymatic-reaction procedures are superior and preferred,” he said. “The problem is that while there is sufficient evidence that says in the diabetic population it’s pretty clear that enzymatic should be recommended, when you look outside the diabetic population you can’t make as strong of a case. And because of the difference in cost, laboratories are reluctant to want to make the change, so there hasn’t been a recommendation from our lab community to make that switch. Personally, our laboratory has used enzymatic creatinine for 20 years.”

Cystatin C-based eGFR holds the promise of overcoming the limitations of eGFRcreatinine related to differing patient muscle mass. Patients with muscle-wasting diseases, very young or very old patients, cachectic patients—they all have eGFRcreatinine results that are less reliable in assessing kidney function.

“Cystatin C is a protein molecule that is larger than creatinine but still freely filtered,” Dr. Miller said. “It’s present in all tissue and there’s a minimal influence of muscle mass. That’s really the primary reason that cystatin C has become an important biomarker for kidney function, which makes it independent of age after about year one, whereas creatinine changes dramatically as children grow and turn into adults, and men and women are different.”

The Kidney Disease: Improving Global Outcomes guideline on CKD prognosis says that patients with normal to mildly increased albuminuria and eGFRcreatinine of 45–59 ought to be measured again with eGFRcystatinC. The catch, however, is that while a reference material for cystatin C became available in 2010 and the commutability of the material has been validated, standardization of cystatin C is not yet universal in the U.S. (Eckfeldt JH, et al. *Arch Pathol Lab Med.* 2015;139[7]: 888–893).

“There’s a pretty broad distribution of cystatin C values among all the various methods represented in the Survey,” Dr. Miller said of the CAP’s findings. “So cystatin C is not yet completely standardized, which is its fundamental limitation.”

For laboratories today, use of eGFR-cystatinC “is kind of like the cart going before the horse,” Dr. Miller said. “It’s not ordered very much, because it’s not standardized . . . so we’re waiting for that to be solved, yet the manufacturers are reluctant to pour money into standardizing cystatin C because nobody’s ordering it. So I think that at some point in time the laboratory community simply starts to make cystatin C available. When you do, make sure to use a standardized procedure, and I think we’ll proceed down the course of using cystatin C where appropriate.”

Urine albumin is another analyte, used in the urine albumin-to-creatinine ratio (ACR), that is critical in assessing

kidney function and yet is not standardized, Dr. Miller said, citing an examination of results from commercially available urine albumin measurement procedures (Bachmann LM, et al. *Clin Chem*. 2014;60[3]:471–480).

"There is roughly about a 40 percent difference in the median values for the different commercially available urine albumin assays. And at the extremes, if you go from the lowest to the highest values, there was well over a 100 percent difference between methodologies," he said. "Dilution introduced bias for four out of the 16 methods, and there was nonlinear calibration for six of the 16 methods. Clearly, in the laboratory community we have some work to do."

However, the analytical precision was good for more than 80 percent of the urine albumin assays. "This is important," Dr. Miller said, "because it means we can standardize these assays once we get the right tools in place."

That is what Dr. Miller and the NKDEP Laboratory Working Group are working on now.

"The focus is to collaborate with NIST [National Institute of Standards and Technology] and the renal reference laboratory at the Mayo Clinic to develop a reference measurement procedure and reference materials that can be used with a standardization scheme with the manufacturers to improve agreement among different measurement procedures," he tells CAP TODAY. "That is still two to three years away."

Following that is the implementation challenge among manufacturers, "which itself can take several years."

Standardizing urine albumin is critical because "the cutpoint that is used to discriminate between low-normal and moderately elevated is fixed at a value of 30 mg/g of creatinine," Dr. Miller says. "There is a lot of evidence that a lower cutpoint is suitable and that there should be different cutpoints for men and women. But right now, the variability among methods is too great to allow that distinction to be made. Once we standardize, we can get a more uniform assessment of patient status. Then we will be able to consider lower thresholds for identifying patients at increased risk, and identify them a little sooner and undertake treatment earlier."

Despite the lack of standardization with urine albumin, Dr. Miller said a urine-collection controversy with regard to testing that analyte can be put to rest. Another study based on NHANES data found that just 44 percent of those showing elevated urine albumins based on urine samples collected at any time of the day were confirmed by a second sample collected at the first morning void (Saydah SH, et al. *Clin Chem*. 2013;59[4]:675–683). Other research has shown the first morning void sample is equivalent in accuracy to a 24-hour collection.

"This suggests that when patients go to the outpatient physician to get evaluated for their urine albumin-to-creatinine ratio, they should always come back and confirm with a first morning void sample," Dr. Miller said.

Several speakers at the session, and a questioner from the audience, said the kidney-function tests now available are not being put to their best use by clinicians and health systems. While guidelines call for patients with diabetes to have their kidney function tested annually, or sooner if their medication is changed, Dr. Tuttle said research has found that even well-functioning health systems fail to meet that goal roughly half the time. It is unclear how well her clinical colleagues at Providence Health Care are doing in ordering this recommended testing, she said.

"The way you know how you're doing is by measuring," she said in an interview. Providence recently created a CKD registry that identifies those at high risk of progression, such as patients with diabetes and hypertension.

Dr. Narva

“When we are able to pull out the group with diabetes, we can ask what the rates of recommended testing are,” Dr. Tuttle adds. “We tried to align that by creating a diabetes order set, and after that point we did see an uptick in people being properly tested.” A final analysis of before-and-after improvement has not yet been conducted.

For his part, Dr. Narva drew on his experience as director of the kidney disease program for the Indian Health Service (IHS). He still serves as chief clinical consultant for nephrology for IHS and conducts a telemedicine clinic at the Zuni IHS hospital in New Mexico from his office at the NIH.

“When I started with IHS, the Am-erican-Indian population had four times the incidence rate of Caucasians for end-stage renal disease. Implementing care in a systematic way in the Indian Health Service has been associated with a decrease in the incidence rate of end-stage renal disease among diabetics of about 35 percent,” Dr. Narva said. “The incidence rate of end-stage renal disease has gone from four times the white rate to almost identical to the white rate. And that’s with a rate of spending that’s 40 percent lower than the rest of the U.S. population and with no novel therapy.”

He said that about 60 percent of patients with diabetes have been screened with a creatinine/eGFR and a urine ACR within the past 12 months. Use of ACE inhibitors to control blood pressure and avoiding nephrotoxic drugs are other elements of the evidence-based interdisciplinary approach.

Improving implementation of current testing and therapies also is at the heart of a pragmatic clinical trial launched in April and funded by the National Institutes of Health. The Improving Chronic Disease Management With Pieces, or ICD-Pieces, trial involves using pharmacist and nurse care managers to help coordinate care for patients with type 2 diabetes, CKD, and hypertension. Proper testing and use of laboratory data are key elements of the trial, Dr. Narva tells CAP TODAY. More information about the trial is available at https://bit.ly/icd-pieces.

While work proceeds to improve the use of available kidney-function tests, the limitations of those biomarkers make it harder for researchers to make progress on the therapeutic front, Dr. Tuttle said.

Aside from issues with imprecision, both eGFRcreatinine and the urine ACR are prone to “a lot of intraindividual variability day to day,” she said. “That’s particularly true in the setting where the urine albumin-to-creatinine ratio goes up with intermittent illnesses such as fever, influenza, and exacerbation of heart failure that occurs so commonly in people with diabetic kidney disease.” She estimates there is intraindividual variation in results of about 40 percent from such factors.

With regard to eGFR what is most important to clinicians is not a single reported number but how that number varies with time, Dr. Tuttle said. As an estimate, after all, there is only an 80 to 90 percent chance that a given eGFR will be within 30 percent of the patient’s measured GFR.

“We just need to know these are ballpark estimates,” she said. “What’s still important is the ballpark they’re in and whether they’re progressing.”

As mentioned earlier, diabetic patients will see their GFR spike before falling. However, eGFRs above 60 are not reliable.

“There’s a stage of hyperfiltration before decline and we can’t detect that,” Dr. Tuttle said. “There’s a lot of debate about what that means, but I think most experts would agree that patients with higher GFRs are the highest-risk patients, paradoxically, for long-term GFR loss, and we are not able to detect them by clinical methods at this time.”

On the therapeutic front, Dr. Tuttle painted a resolute portrait.

“We have not had a new, approved therapy for diabetic kidney disease in 15 years,” she said. “The last approval was for ARBs [angiotensin II receptor blockers] in type 2 diabetes characterized by macroalbuminuria. There is tremendous preclinical and experimental science that’s pointed us in many mechanistic directions. But for various reasons, there has been a failure in clinical translation.”

She showed a slide listing more than three dozen novel therapies that have been investigated ("that's all I could fit on here," Dr. Tuttle said). Many are still under study, while others were terminated for safety, regulatory, or business reasons.

"To me, what that list looks like is a Jackson Pollock painting," she said. "It's kind of pretty in its own way, but I'm not sure where we're going."

The lack of biomarkers for diagnosis, prognosis, and action have proved to be a major barrier in translation research, she said. That having been said, Dr. Tuttle presented research that she and her colleagues have done on the JAK/STAT signal transduction pathway where inflammation is overexpressed in humans with diabetic kidney disease. And if it is overexpressed in mice, they "develop a very human-looking form of diabetic kidney disease," she said.

Partnering with Eli Lilly, which already markets the JAK1/2-inhibitor baricitinib for treatment of rheumatoid arthritis, the researchers did a phase two clinical trial with 129 patients with type 2 diabetes, median macroalbuminuria of 1,800 mg/g, and eGFR of 57. Four-mg doses of the drug appeared to be effective over three months, reducing the urine ACR, at the median, by about 0.3-fold from baseline while the ACR among patients taking placebo rose. The results could lead to a phase three trial, Dr. Tuttle said.

Another inflammatory pathway in diabetes that Dr. Tuttle and her colleagues are investigating is serum amyloid A. Plasma levels of SAA are higher in patients with DKD, and the kidney tissue of those with type 1 and type 2 diabetes shows obvious immunohistochemical staining due to the kidney disease (Anderberg RJ, et al. *Lab Invest.* 2015;95[3]:250–262). A paper recently published in the *Journal of Diabetes and Its Complications* (Dieter BP, et al. Published online ahead of print July 27, 2016. doi:10.1016/j.jdiacomp.2016.07.018) shows that patients in the highest third of SAA levels—more than 1 mg/mL—have three times the risk of death and end-stage renal disease when compared with patients in the lowest third of SAA levels of 0.55 mg/mL or lower.

Those findings suggest that SAA could have prognostic value, while correlation of higher SAA blood levels and lower eGFR independent of other DKD risk factors could offer predictive value. Last, Dr. Tuttle said, SAA could be actionable because JAK2 regulates SAA expression in the kidney.

Dr. Sacks

"That's just a preview," Dr. Tuttle said. "Other people have their favorite biomarkers. But I think that it gives us hope that maybe some day we'll go beyond albuminuria and eGFR not only to risk-stratify people but to identify patients who are at high risk where we can predict a certain therapeutic response and we can use those actionable biomarkers to measure whether or not the patient is improving and whether we're hitting the target as a result of our therapy."

David Sacks, MB, ChB, FRCPath, senior investigator at the National Institutes of Health and chief of clinical chemistry at NIH Clinical Center, convened and moderated the AACC panel discussion. He described Dr. Tuttle's presentation as "very interesting," while noting that "some of her stuff is in a very preliminary stage and is not going to be translated into patient care in the next few years."

"What she's doing is what's necessary, often, to understand the pathophysiology of disease, in order to comprehend where the breakdown is and what's causing the problem," Dr. Sacks added. "Once you understand what is disrupted and what goes awry in disease, then you can try to fix the problem. . . . I look at it as a piece of the jigsaw puzzle. Other people are identifying other pieces. Then someone will presumably put everything

together, but it’s going to take a long time.”

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