Yale researchers dig for new kidney biomarkers

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October 2016—An automated immunoassay has been created for symmetric dimethylarginine, or SDMA, a biomarker that can detect chronic kidney disease between 10 to 17 months earlier than creatinine, with 100 percent sensitivity and 91 percent specificity. And, unlike with creatinine, a patient's muscle mass does not influence the biomarker's reliability. SDMA has already been incorporated into the kidney-function testing advice that guides clinician ordering worldwide. Since the automated SDMA test was launched in July 2015, 5 million samples have been analyzed and 80 percent of clinicians are aware of the test.

There is a hitch in SDMA's forward march to a place of prominence in chronic kidney disease testing: It has gone to the dogs—and cats.

The automated SDMA assay is available only from Idexx Laboratories, a Westbrook, Me., company with a 40 percent share of the veterinary lab testing market. In veterinary medicine, the weaknesses of serum creatinine as a CKD biomarker are pronounced because there are no estimated glomerular filtration calculations for laboratories to use and report.

So veterinary clinicians have been faced with the task of how to accurately interpret the meaning of a creatinine level in a serum sample from a Chihuahua or a Saint Bernard. Creatinine levels are normalized by breed, but that solution is short of ideal given the scope of the problem: One-third of cats and 10 percent of dogs develop kidney disease during their lives. Idexx now includes SDMA in all of its routine chemistry panels.

SDMA's value should go beyond diagnosing CKD in dogs and cats, says Murthy Yerramilli, PhD, vice president of research and development at Idexx Laboratories.

"We want to take this test to human medicine," Dr. Yerramilli says. "We want to collaborate and show that similar clinical findings happen in human medicine. We do think that this is going to be extremely useful for human health as well."

That is more than just talk. The company has handed off its automated SDMA assay, which runs on Beckman and Roche instruments, to Yale University School of Medicine for validation in human samples.

Joe El-Khoury, PhD, is handling the validation task. He is an assistant professor of laboratory medicine at Yale and co-directs the clinical chemistry laboratory at Yale New Haven Health. His PhD research focused on developing a high-throughput, liquid chromatography-mass spectrometry assay to measure SDMA, along with asymmetric dimethylarginine and arginine (El-Khoury JM, et al. *Anal Bioanal Chem.* 2012;402[2]:771–779), so he has had a long-running interest in SDMA's potential utility. (Dr. El-Khoury has received honoraria and travel reimbursement for two speaking engagements at Idexx, but the company is not paying him for the validation studies on its SDMA assay.)

The need for an alternative CKD biomarker is great, Dr. El-Khoury argued in a presentation at this year's annual meeting of the American Association for Clinical Chemistry. The endogenous biomarkers used to estimate GFR based on creatinine and cystatin C have disadvantages. Creatinine, he said, has poor sensitivity and specificity and is affected by extra-renal factors such as age, gender, ethnicity, race, diet, muscle mass, and medication. While eGFRcystatinC is more accurate than creatinine-based eGFR, it is influenced by thyroid functioning and may be affected by obesity, inflammation, and smoking.

Using the more sophisticated Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI), there is a 30-point spread in eGFRcreatinine, Dr. El-Khoury said during the session, "Emerging Biomarkers of Acute Kidney Injury

and Chronic Kidney Disease."

"In somebody whose GFR value comes back as 60, your range could really be between all the way down to 30 or all the way up to 90," he said. "So you could be normal, or you could be at stage three or stage four chronic kidney disease. Even though the equations that have been developed have improved, there is still a lot of room for improvement, and there are still a lot of extra-renal effects and variability."

Cystatin C represents an improvement in accuracy, but in high-stakes clinical situations when diagnostic accuracy is critical—for example, whether to accept a donor kidney for transplantation—clinicians often double-check the organ's functioning using an exogenous marker such as iothalamate. But that silver standard for measuring GFR is time-consuming and involves radioactive exposure for patients and thus is not a good choice for routine CKD screening, Dr. El-Khoury said.

Dr. El-Khoury

The ideal biomarker for CKD "must have a constant production rate so that as you're rendering it into urine it is not affected by changes in production," he added. "And it must be freely filtered. Obviously, you want a concentrated marker and it can't be something that cannot get through the glomerulus. And it should neither be reabsorbed nor secreted by the renal tubules, so it's not affected by other factors related to the filtration process. Also, it should not be metabolized or eliminated in extra-renal pathways."

SDMA appears to fit this bill of particulars.

"It is a methylated product of arginine residues. It is produced by post-translational modification of arginine residues in proteins that are in histones," Dr. El-Khoury said. "So this is a very highly preserved process, and it is produced at a fairly stable rate. And you have constant turnover and production of the marker. Over 90 percent is renally cleared and filtered through the glomerulus."

Previous research has evaluated SDMA's performance in comparison with creatinine and cystatin C, where it frequently showed strong correlation in the 0.80 range. However, Dr. El-Khoury explained, it is not sufficient to compare SDMA with existing biomarkers that may be less accurate. What appears as a false-positive in comparison with creatinine, for example, could be a false-negative given by creatinine. Only a handful of studies have compared SDMA with more accurate silver-standard mGFR markers—finding correlations ranging from 0.78 to 0.90—and all of these were done only using samples from patients with CKD or diabetes (Schwedhelm E, et al. *Nat Rev Nephrol.* 2011;7[5]:275–285).

To prove its mettle as a biomarker useful in screening, Dr. El-Khoury said, SDMA must be evaluated in healthy patients too. And so that is what he and his colleagues did.

"The reason why you want to look at healthy and CKD patients is to try to separate normals from abnormals," Dr. El-Khoury tells CAP TODAY. "The marker has to perform equally well in both categories. It might only correlate in chronic kidney disease but have no correlation in healthy patients. Then we might not catch someone with declining function into the CKD stages."

Dr. El-Khoury and his colleagues tested a series of 40 consecutive patients who had some reason to get tested for mGFR using iothalamate.

“These are very tough patient populations to get,” said Dr. El-Khoury, co-director of Yale’s clinical chemistry fellowship program. “We don’t frequently get patients who need silver-standard GFRs or measured GFR. In this case, the reason these patients showed up is they were either being evaluated as kidney donors, so this was our healthy population, or they were CKD patients or drug-dosing patients who needed to have their drug concentrations adjusted and the clinicians wanted to make sure their kidney-function result was still accurate.”

In addition to the mGFR reference method, the patients were tested for creatinine and cystatin C using the Roche Cobas 8000 and SDMA using the LC-MS/MS method Dr. El-Khoury developed. Compared with the silver-standard reference method, creatinine had just a -0.70 correlation, while cystatin C showed a higher correlation of -0.86. SDMA, meanwhile, had a correlation of -0.84 (El-Khoury JM, et al. *Clin Biochem*. Epub ahead of print July 21, 2016. doi:10.1016/j.clinbiochem.2016.07.009).

“That is a similar performance to cystatin C and much better than creatinine,” Dr. El-Khoury said. He said further research on SDMA should be done among a larger pool of healthy patients, one that is more diverse as 88 percent of the participants in this study were white and all were adults. He added that SDMA is influenced by age and, to some degree, gender. Those factors must be better understood and equations developed to account for them in generating an SDMA-based eGFR.

However, that is unlikely to happen if the test available for SDMA in humans is done using an LC-MS or ELISA method. While the sample-to-result run time for the LC-MS SDMA method he used in the lab is about 90 minutes, it must be run in batches, Dr. El-Khoury says.
“Today, the biggest reason why SDMA hasn’t been picked up—other than a lack of data, though there is a lot of convincing data—is the lack of an automated assay,” he says. “What is available now is highly specialized techniques like LC-MS or very manual techniques like ELISAs. [Idexx] is the first one to offer an automated platform that could run just like creatinine or cystatin C. That’s really a game changer. Once you can do this in high-volume laboratories, a lot more research can be done with it [SDMA].”

An automated SDMA immunoassay such as the Idexx test already used in millions of cats and dogs is essential “to bring this test to general clinical practice,” Dr. El-Khoury adds. “When you’re looking at screening for kidney function, you want something that can be done relatively quickly. And you’re not going to switch from a test like creatinine or cystatin C to a test that would require hours and very slow throughput to give you a result. Typically, these results are needed right away. People order these things when patients are waiting for radioactive dye or drug-dosing information. You can’t do that for a test that’s super slow.”

Dr. El-Khoury believes that a combination of cystatin C and SDMA may someday overtake creatinine in testing for chronic kidney disease.

“Hopefully, in the future SDMA will overtake creatinine, because creatinine is good but it does have known limitations,” he told the AACC crowd. “Let’s use the best biomarkers available and not have them impacted by one that has poorer performance.”

“Creatinine,” Dr. El-Khoury said, “is so 1950s.”

The defects of creatinine also was a recurring theme of the other portion of the AACC session, which focused on emerging biomarkers in acute kidney injury.

Nephrologist Chirag Parikh, MD, PhD, compared the slow progress made in understanding and treating acute kidney injury since the 1950s and the great strides made in treating acute myocardial infarction. In the ’50s, the principal way to diagnose a heart attack was white blood cell count. That moved to LDH in the ’60s, CPK in the ’70s, CK-MB in the ’80s, troponin T in the ’90s, and troponin I in the new millennium.

Dr. Parikh

“In acute kidney injury, creatinine was the first test and we quickly figured out you can find AKI by noting the change in serum creatinine,” said Dr. Parikh, director of the Yale Program of Applied Translational Research. “That started being used in the 1950s, and here in the 2000s we’re still talking about the same test. So no wonder the therapeutics [in AKI] are lagging behind.”

“In acute kidney injury, we need to reduce the dependence on serum creatinine,” Dr. Parikh said. “It doesn’t increase very quickly. It’s very nonspecific. If we had specific biomarkers of structural injury from when the first nephron got injured, we’d pick up on these episodes of subtle injury and try to make therapeutic progress.”

Responding to a call from the American Society of Nephrology for better AKI biomarkers, Dr. Parikh and colleagues formed Translational Research Investigating Biomarker Endpoints-Acute Kidney Injury in 2005. The TRIBE-AKI consortium of nine medical centers has enrolled more than 3,000 patients who are being actively followed for outcomes related to AKI and major cardiac surgery.

To test various biomarkers of interest, Dr. Parikh and his co-investigators selected patients undergoing coronary artery bypass graft or valve surgery deemed to be at high risk for AKI (serum creatinine greater than 2 mg/dL or other clinical or surgical criteria). One 10-mL preoperative blood sample was taken, as was a 10-mL preop urine sample. After surgery, 10-mL samples of blood were collected each of the first five days following the procedure. On the day of surgery, 10-mL samples of urine were collected four times in the first 24 hours. On each of the following four days, one 10-mL sample of urine was collected.

The researchers took care to ensure specimens were handled the same in all steps of the testing process to avoid introducing bias in comparing the biomarkers’ performance, Dr. Parikh said. Elements that, if done differently, could skew results included: the time from blood draw to spin/freeze; the number of thaw-freeze cycles; the duration of storage; the type of blood-collection tube; time from thawing to assay; and the addition of protease inhibitors.

In a slide showing how the urinary biomarkers interleukin-18 (IL-18) and kidney injury molecule-1 (KIM-1) performed, Dr. Parikh highlighted that they demonstrated signs of kidney injury well before serum creatinine. IL-18 became elevated on day one while KIM-1 went up on day two, but it was not until day three that serum creatinine rose in the patients who developed AKI (Parikh CR, et al. *J Am Soc Nephrol.* 2011;22[9]:1748–1757).

“You can see that in the first 24 to 48 hours, it [serum creatinine] is not elevated. That’s almost two days’ lead time, which can be very helpful if you wanted to design a therapeutic study,” said Dr. Parikh, a professor of medicine (nephrology) at Yale University School of Medicine and a professor in the Clinical Epidemiology Research Center at the VA Connecticut Healthcare System.

Patients with elevated IL-18 had nearly a seven-fold higher odds of developing AKI than patients who did not, after adjusting for age, gender, race, type of surgery, preoperative eGFR, diabetes, hypertension, and cardiopulmonary bypass duration. Patients with elevated KIM-1 had nearly five times higher risk of AKI, as did those whose plasma neutrophil gelatinase-associated lipocalin (NGAL) rose. The liver-type fatty acid-binding protein (L-FABP) found in urine was less predictive of AKI, showing a 1.8 adjusted odds ratio.

Dr. Parikh also published data showing how tightly correlated these biomarkers were with serum creatinine, but noted the same objection as Dr. El-Khoury did in his talk.

“If you compare the new test to the existing test, the new test cannot look better because the existing test is

treated as the gold standard," he said. "So, with most of these biomarkers there's a 0.75 to 0.8 correlation, but there is no way to get better because the false-positives could actually represent an improvement in the clinical test."

To overcome that hurdle, Dr. Parikh and his colleagues followed a subset of patients they classified as having subclinical acute kidney injury—ones who were negative for AKI by creatinine but positive by one or more of the newer biomarkers. How well did these patients do in the years following their cardiac surgery and apparent kidney injuries? Researchers followed them through a combination of phone calls and search of vital records, medical records, and government databases.

The TRIBE-AKI researchers found a consistent pattern. Patients with no AKI—who were negative by the newer biomarkers as well as creatinine—had a mortality rate of 40 per 1,000 patient years. But the patients who were positive by any one of the biomarkers consistently had a death rate twice as high, 80 per 1,000 patient years.

"So we know that any AKI puts a patient at a long-term risk of higher mortality," Dr. Parikh said.

The researchers further stratified the newer biomarker results by tertile depending on how elevated they were. The death rates among the highest tertile group were greater than 100 per 1,000 patient years for each of the new biomarkers, except urine L-FABP, illustrating how pharmaceutical investigators, clinicians, and health systems might focus their efforts to aid this particular group of patients in better surviving their kidney injuries.
Dr. Parikh drew the AACC audience's attention to another pattern in the results. For each of the biomarkers examined, the mortality rate—ranging from about 45 to 60 deaths per 1,000 patient years—for the lowest tertile of patients positive for AKI by creatinine was strikingly similar to that for the patients in the subclinical AKI category (negative by creatinine but positive by the new biomarker).

"Both of these have a very similar risk as far as long-term mortality is concerned," he said. "Clearly, the new biomarkers would add a lot of additional information if they were available."

This pattern of catching subclinical AKI that increases patients' odds of death also held true for another biomarker Dr. Parikh and his team studied, not a novel biomarker but an old standby—urine albumin.

A questioner from the AACC audience made note of the fact, asking, "The urine albumin results looked pretty good. . . . Why not just use albumin?"

Dr. Parikh responded: "Absolutely . . . we don't need to get fancy. And if albumin does the job, then people can offer it and clinicians can make use of that. It is very available, so that is great."

The urine albumin option is especially important because, Dr. Parikh says, the chances seem low that one of the other biomarkers will be commercialized anytime soon despite the promise unveiled in the research.

"I've come to realize that the diagnostic companies are small companies, not like the pharmaceutical companies," he tells CAP TODAY. "And the regulatory burden is so high that if they really like a biomarker, they have to pour a lot into it before they'll see the profits. I think it's a gap in our translational development. In order for more biomarkers to become available, they need to anticipate that financial return. At the same time, you can't charge $100 for each new test because you're going to break the bank of the country's health care budget. I go back and forth between my optimism that comes out of our studies and then the cynicism arises when I think about how to get it [a novel AKI biomarker test] to each and every patient."

The biggest gap in AKI biomarker development—and, consequently, therapeutic development—has been the lack of long-term patient follow-up, Dr. Parikh says. That is a space the TRIBE-AKI consortium was made to plug, to help explain why patients who undergo AKI have a higher death rate in the years that follow.

Because serum creatinine is relatively insensitive, it provides a "false sense of security" about patients who experience acute kidney injuries, he adds. Dr. Parikh cautions, however, against the view that creatinine, despite

its flaws in AKI and CKD diagnosis, will or should disappear entirely from clinical use.

“Replacing serum creatinine is a tall order because we have over 100 years’ worth of knowledge around creatinine,” he says. “Even if that happens, I believe it will be several decades away. I think the simplest thing which can happen, in small increments, is to add other biomarkers that provide supplementary information that is not easily available from serum creatinine. And once we start using those and get comfortable with those, I think we’ll have a lot more information to help manage patients with kidney disease.”
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