Inflammatory biomarkers foreshadow CKD, study finds

Anne Paxton

March 2018—The central idea of the film *Minority Report*—that a "precrime" police unit can predict and prevent crimes—still mostly inhabits the realm of science fiction. Luckily, in medicine, researchers studying "predisease" can make headway on prevention by analyzing the laboratory test results from samples collected years earlier, when patients showed no clinical symptoms, that might have been able to predict disorders such as chronic kidney disease (CKD) in those patients.

One of the latest such studies was possible thanks to the trove of data from the Diabetes Control and Complications Trial (DCCT) and Epidemiology of Diabetes Interventions and Complications (EDIC) study, which allowed investigation of biomarkers in two follow-up windows: up to three years (short term) and up to 10 years (long term). Through a prospective evaluation of markers of inflammation and endothelial dysfunction, a study team from the Medical University of South Carolina, the VA Medical Center of Charleston, SC, and Bristol-Myers Squibb found that these markers are associated with progression to kidney dysfunction in type 1 diabetes during both short- and long-term follow-up.

The study, "Association between inflammatory markers and progression to kidney dysfunction: examining different assessment windows in patients with type 1 diabetes" (Baker NL, et al. *Diabetes Care*. 2018;41[1]:128–135), confirmed that the biomarkers sE-selectin and sTNFR-1 and -2 are associated with the long-term development of macroalbuminuria. Higher levels indicated the possible development of macroalbuminuria in participants—all patients with type 1 diabetes—who had been free of clinically detectable kidney disease at baseline. Similarly, the biomarkers sTNFR-1/2, sE-selectin, PAI-1, and fibrinogen were found to be associated with progression to chronic kidney disease stage three or worse.

Baker

With this prospective evaluation of DCCT and EDIC test results, the researchers hoped to expand on observations from previous studies, says study coauthor Nathaniel L. Baker, MS, a statistician with MUSC. The study team had conducted two of those earlier studies. "We looked at some of these biomarkers of inflammatory, thrombosis, and endothelial dysfunction in a cross-sectional study of patients who progressed to kidney dysfunction in DCCT and early in EDIC," Baker says. In 2008, "We found that sE-selectin was strongly associated with concurrent abnormal albuminuria" (Lopes-Virella MF, et al. *Diabetes Care.* 2008;31[10]:2006–2012). In 2013, "We found that higher levels of sE-selectin and sTNF receptors 1 and 2 are strongly associated with long-term progression to macroalbuminuria" (Lopes-Virella MF, et al. *Diabetes Care.* 2013;36[8]:2317–2323).

While the pathological mechanisms of development and progression of kidney disease in patients with diabetes are not well understood, other studies such as the EURODIAB Prospective Complications Study had also provided strong evidence for the clinical significance of biomarkers of inflammation and endothelial dysfunction in predicting complications of diabetes, such as albuminuria, retinopathy, and cardiovascular disease, the coauthors of the new study note.

For this biomarkers study, "We looked at several samples that were collected during DCCT and EDIC," Baker says. "We wanted to explore the cross-sectional association to see if they could be measured earlier in the disease

progression." As the study notes, most of the biomarkers that have been shown to correlate with renal function deterioration in patients with diabetes seem to have long-term predictive value. But only a limited number can predict the onset of chronic disease closer to the development of macroalbuminuria and CKD. "We wanted to see how close we could get." For clinical trials of relatively short duration, the study found the most useful biomarkers for inclusion would be sTNFR-1 and sTNFR-2.

The DCCT, a large longitudinal study that ran from 1983 to 1993, collected data from two study cohorts with type 1 diabetes, a total of 1,441 patients ages 13–39. The first cohort had no retinopathy on the basis of fundus photography, diabetes for one to five years, and no microalbuminuria (

At their baseline visits, the DCCT participants underwent a physical examination, medical history, and routine laboratory analysis including serum creatinine, lipid profile, and HbA1c. Then they were assigned randomly to either intensive or conventional insulin therapy. The DCCT was halted in 1993, ahead of schedule, because of the consistent beneficial impact of intensive therapy on diabetes complications. But most of the DCCT participants went on to be enrolled in the EDIC observational follow-up study.

The new biomarkers study looked at markers of inflammation, endothelial dysfunction, and fibrinolysis from samples taken at four study time points: DCCT baseline, DCCT closeout, EDIC years four through six, and EDIC years eight through 11. "The samples from DCCT baseline and closeout were collected by the centers at the time of the DCCT trial and we received a frozen aliquot when we requested it from the NIDDK [National Institute of Diabetes and Digestive and Kidney Diseases]," Baker explains. "The first time the sample was unfrozen it was aliquoted, and from there on each new test was performed on aliquots from the original samples. The blood glucose samples were received frozen from the centers where they were collected and the same process was followed. The first time they were used they were aliquoted."

The aim was to see if those test results would be associated with development of kidney dysfunction within follow-up windows of up to three years and up to 10 years. The kidney dysfunction of interest included incident macroalbuminuria and CKD stage three or worse. Using levels of eGFR to define progression to CKD, and albumin excretion rate values to define progression to macroalbuminuria, the researchers analyzed 4,378 paired biomarker and disease outcome measurements taken in 1,396 participants.

Of those participants, 94, or 6.7 percent, progressed to CKD stage three or worse during DCCT or during the first 18 years of EDIC follow-up (21–28 years of follow-up time since enrollment in the DCCT study). The median progression time from the DCCT baseline was 19.3 years. Progression to CKD stage three or worse occurred, on average, within 5.3 years after the last available biomarker measurement. Within the 10-year window, increased levels of some inflammation markers (cytokines) and endothelial dysfunction were associated with higher risk of progression to CKD.

A higher percentage of the DCCT participants (161, or 11.5 percent) progressed to macroalbuminuria during that trial or during the first 18 years of the EDIC follow-up. Forty-four of those progressed within a three-year window and 143 progressed to macroalbuminuria within a 10-year window since the last available measurement. The biomarkers sTNFR-1/2, sE-selectin, and PAI-1 were associated with progression to macroalbuminuria during the 10-year follow-up window, but more weakly associated within the three-year window.

Within the population studied, levels of C-reactive protein, fibrinogen, sTNFR-1, and active PAI-1 increased over time, exhibiting a sustained trend with age, while levels of sE-selectin decreased over time. By contrast, for the participants who developed impaired eGFR and macroalbuminuria compared with those who maintained normal kidney function, the overall levels of all the biomarkers increased, except IL-6, which did not show an appreciable difference, and VCAM-1, which was lower in participants who developed nephropathy.

Two main goals drove the study, says coauthor Maria F. Lopes-Virella, MD, PhD, an endocrinologist and clinical pathologist at MUSC. "One was to find biomarkers that would identify patients at high risk of developing CKD and MA [macroalbuminuria]. And that, of course, could be applied clinically." The other goal was to help in recruiting

the best patients for clinical trials of promising drugs. “We cannot do trials that extend 15 years,” she says. “We have a shorter period of time to test proposed treatments, so we need to be aware of biomarkers that can help us recruit patients who will develop a disease during the follow-up period and therefore allow us to determine whether or not a drug works.”

The study concluded that biomarkers associated with diabetes complications in a close temporal relationship with CKD or macroalbuminuria “could be useful in clinical trials for the recruitment of patients more likely to have [a kidney] event during the trial, thus increasing the number of events and, therefore, enhancing the power of the study and validity of the conclusions.”

Dr. Lopes-Virella

Says Dr. Lopes-Virella: “Our idea was to see if some biomarkers could help us choose patients who start with more of a chance of having the event. Then, if you are looking at a drug and the effects of a drug, you can see more easily if some do not develop the disease when they are treated.” The study team also wants to help other trials expose fewer patients to the drugs being studied, Baker says. “You can do that if you can choose patients for your study pool who are more likely to acquire the disease.”

The researchers chose to study short-term and long-term time frames because they have different purposes, Dr. Lopes-Virella says. “The short term was mostly to help in recruitment of patients if you are doing clinical studies. Those patients are in more immediate need of treatment because they are not doing well. But the long term, in my opinion, is more important because they are the ones who tell you which patients need help and need to be treated more intensively to prevent the development of complications.”

With most clinical trials funded by the National Institutes of Health, Baker notes, “you don’t get 10 or 20 years of follow-up data. You get three. So being able to use biomarkers to identify at-risk patients is very important.”

But he is interested not only in how the biomarker works to help but also in how the biomarker represents one of the mechanisms of the disease. “I personally don’t like biomarkers that I don’t think are somehow related to the pathophysiology of disease,” he says. As the study notes, identifying biomarkers associated with long-term disease progression helps illuminate the underlying mechanism of disease development.

“There are a lot of publications and a lot of data to suggest that the pathogenesis of diabetes complications has a strong link to the complement system, and also indicators of signaling operations in membrane structures that lead to different signal pathways. All of that seems to be very much involved in the complications of diabetes. So we were looking partly at what we know was involved in the complications and trying to figure out the biomarkers,” Baker says.

Baker and Dr. Lopes-Virella note that this study does not provide added clinical utility for disease prediction beyond the traditional risk factors, and that further classification analysis, including prediction models, should be conducted.

“In a sense, many of the clinical implications depend a lot on which pathway is implicated in the disease process,” Dr. Lopes-Virella says. Clinicians do not want, when trying to prevent one problem, to create another, she points out. For example, with inflammation, “you need to be very careful with your conclusions because inflammation is a

necessary process in the body." Since inflammation is part of a protective immune response, "if you attack inflammation, you may have unwanted side effects." Sometimes researchers can find very specific pathways that do not have general effects. "Targeting these pathways, I think, may have clinical implications and help with the discovery of drugs to treat the disease."

"I think as we work toward a more discriminative model, a panel-type model" for predicting kidney dysfunction, "we'll get closer to clinical utility," Baker says. "But with individual biomarkers, we are still kind of exploring the relationship not just to CKD but to the heart as well. Once we get a panel together, we will have a lot more clinical utility from our findings."

That milestone is still a step or two away. "We are talking about building a full panel and possibly developing a risk score that will help assess patients with additional biomarkers. But we haven't yet concluded which ones," Baker says. "In this study, we were looking at sE-selectin and sTNFR-1/2 because they seem to have a tremendous impact in the kidney."

The biomarkers study had a well-defined population that was followed for a significant time, but limitations of the study do temper the results, the authors note. The number of events in the cohort studied was relatively small in the three-year follow-up window, detracting from its statistical significance. There were variations in testing, including participant albumin excretion rate values that were measured annually during DCCT but only every other year during EDIC, making it difficult to measure persistent macroalbuminuria in the presence of treatment with ACE/ARB medications.

The NIH and pharmaceutical companies are paying attention to different biomarkers and how they could help in treating and preventing complications in diabetes and other diseases, Dr. Lopes-Virella says. "They want to use, as much as possible, plasma/serum/urine biomarkers and avoid invasive procedures to identify patients at high risk. That way you can more easily identify patients who are at high risk and really need treatment. You cannot treat everyone, but it helps if you considerably reduce the number of patients who will progress if left untreated."

A population of only type 1 diabetes patients was the subject of the study. But could the study's findings be helpful in predicting type 2 diabetes? Maybe, says Baker. "Diabetes is a very interesting disease. With type 2, I don't think we really know when the disease starts. It's a continuum from metabolic syndrome to prediabetes to diabetes. So I think these biomarkers are definitely something that could be helpful." But, he says, since the population of patients in this study all had diabetes and various stages of kidney disease, the biomarkers were not predicting progression to diabetes.

Dr. Lopes-Virella believes the biomarkers shown to be correlated with renal function deterioration in patients with diabetes could potentially help with prediction of prediabetes. "These biomarkers will probably help identify patients at risk. Could they help more than the traditional markers we already know? I don't know. But there are studies looking at this question."
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