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Guest: Dr. Dina Greene serves as an Associate Laboratory Director for LetsGetChecked as well as a Clinical Associate Professor at the University of Washington.

Randye Kaye:

Hello and welcome to this edition of *JALM Talk* from *The Journal of Applied Laboratory Medicine*, a publication of the American Association for Clinical Chemistry. I'm your host, Randye Kaye.

Chronic kidney disease, or CKD, affects approximately 12% of the global population. The urine albumin-to-creatinine ratio or UACR is an important test, alongside serum creatinine and the estimated glomerular filtration rate, to diagnose and monitor CKD. According to guidelines from the Kidney Disease Improving Global Outcomes Program, a UACR result of at least 30 milligrams of urine albumin per gram of creatinine is considered abnormally elevated. Elevated results are considered diagnostic for CKD if they persist for at least three months. The extent of albuminuria provides prognostic value even as lower levels of urine albumen are associated with hypertension and cardiovascular mortality. However, some studies have demonstrated that UACR is an underutilized test.

Further, in people with normal kidney function and appropriate urine flow rates, the urine albumin concentration is typically low and maybe undetectable by conventional assays. When the urine albumin is below an assay's limit of quantitation, the UACR ratio cannot be calculated, which may require the test to be canceled.

The September 2022 issue of *JALM* includes a study in which the authors sought to challenge the performance characteristics of a commonly utilized, commercially available urine albumin assay. In the article, the authors illustrate the clinical screening implications of lowering the assay's lower limit of quantitation.

Today, we're joined by the article's first author, Dr. Dina Greene. Dr. Greene serves as an Associate Laboratory Director for LetsGetChecked, as well as a Clinical Associate Professor at the University of Washington. Welcome, Dr. Greene.

Dina Greene:

Hi, Randye. Thanks for inviting me to be here today to talk with you about this.

Randye Kaye: So, why is it important to maximize the tools we have for CKD screening?

Dina Greene: It's important because a lot of people are at risk for CKD and a lot of people have CKD. So, it's estimated that 37 million people have CKD, and about 90% of those people don't know it.

A recent study that was done, it was a collaboration between some folks at the NKF, which is the National Kidney Foundation and Labcorp, which is a large national reference lab, showed that as little as 20% of people with risk factors for CKD are appropriately screened. Meaning people with hypertension or diabetes or both. And these screening tests are pretty inexpensive for us to run, and they're easy for us to run in a high throughput fashion. And so, if we can maximize these screening tools, we can detect more people that are leading towards CKD or already have CKD, and we can slow their progression with new pharmaceuticals and lifestyle changes that are available for people that are entering these categories.

Randye Kaye: And that makes that really important. Thank you. How does urine albumin fit into CKD screening? What is complicated about its measurement and the result reporting?

Dina Greene: Yeah, so, when most people think, and particularly lab people when they think about kidney disease, and kidney function, and what we look at most importantly for the metric to evaluate that, we think of serum creatinine and the estimated glomerular filtration rate or EGFR. But a random urine sample actually provides a lot of information and that's where urine albumin fits in.

So healthy people will have little to no urine albumin. If they do, it's going to be negligible relative to the concentration of solutes in the urine, particularly the concentration of urine creatinine. But really, any amount of detectable urine albumin can be an indicator of early disease. So, what's complicated about its measurement? This sounds really straightforward. You take a urine sample. Do I have this kind of protein in it? And if I do, is that amount of protein kind of consistent with the solute concentration that's in this urine, or is it inconsistent?

And so, the lower limit of quantitation, the smallest amount that we can measure in urine albumin assays, has always been an issue and it's not necessarily been an analytical issue. I'm not sure that folks have realized that this is something that could possibly shift to a lower value, because in every lab I've worked in, this has been a problem.

The math is really simple. So you're going to get a ratio. You're going to calculate the urine albumin concentration by the urine creatinine. But if the urine albumin concentration is a 'less than' number, so say less than 12, because that's the assay that was used in this publication. The lower limit of quantitation or the LLOQ, as I may start referring to it as, the LLOQ was 12. If you get a value of like 10, what's classically thought is you would have less than 12, then you don't have a number for the numerator. So you have a no number for the numerator and you have a creatinine concentration as the denominator, and that doesn't let you have a result. And so, this creates a conundrum for the lab. We can either use that LLOQ as the numerator and that has been recommended by some groups, but it will grossly over-flag results.

The other option is you report unable to calculate, but because the majority of people have these low concentrations of albumin that are being screened, right, if you're doing population screening, or even if you're doing at-risk screening, you're going to have this dubious result come back that isn't really a result. It's just not able to calculate or it's using an artificial number because it's the lowest number that you think you can confidently report.

Randye Kaye: Got it. So you may have already, in that answer, answered the next question. But I want to see if you have anything more to say about explaining any more potential advantages of lowering a urine albumin assay's limit of quantitation.

Dina Greene: Yeah, I can. And so, when we can lower this lower limit of quantitation, it allows for a much more supreme resolution of values that can allow for extended use of the lab result. So, for example, in the last several years, there have been the derivation of kidney failure risk equations, KFREs is what they're called. And these equations are really good at predicting someone's risk of kidney failure, which is what happens when people get CKD. They're on the road to that. Those equations require a numeric value to be input. And so, by doing this, by having a numeric value for the urine albumin to creatinine ratio, even if it's lower, you're allowing for that type of resolution.

In our study, first thing we had to do was analytically validate that this LLOQ was possible and then also, wanted to clinically look at what the effects would be. The analytical validation was fine. We showed that we could take this down to what we were hoping for was three, so from 12 to 3, and it was precise and linear at those concentrations, compared well between different lots of reagents between different labs, and then the clinical validation. So what we showed was that if we used an LLOQ of 11.9, so if the assays can only measure down to 12 and you get a value of 4, we would plug in 11.9

as the numerator and whatever the urine creatinine was as the denominator. So if you did that, you would flag 107 of 499 results, or 21.4% would be potentially abnormal because again, you're using the worst case scenario. We know this was less than 12. If I put an 11.9 that's the worst-case scenario. This may be greater than our cut off of 0.3, but what we showed is when we use the numeric value for these samples down to 3 milligrams per deciliter, this reduced alarm to 1%. So basically, you're reducing your flagging by twentyfold.

So instead of telling 21% of patients you may be at risk for disease, you're telling 1% of patients you may be at risk for disease. This is a much more manageable number and it allows clinicians to have a much better resolution.

Randye Kaye: It sounds like you would recommend that other laboratories consider this approach. Is that true?

Dina Greene: I would say absolutely to that, Randye! So, it's really an easy fix and it helps everyone. Like, I honestly feel like this is one of my most valuable contributions to the literature, and I find it embarrassingly basic. I would also like to give a shoutout to my dear colleague, Mark Marzinke, who had already implemented this at Johns Hopkins. I had called him. Every great project starts with a phone call. I had called him to ask if he thought this would be a reasonable solution. And he was like, "yeah, I already did this." I was like, "you did? You didn't publish it?" He was like, "no, I didn't publish it." Which anyone that knows Mark Marzinke is surprised by that because he's a very prolific scientist.

But his previous implementation gave me the confidence to continue with the analysis and to write this manuscript. Given that there's already some people that have been doing this in the field, and then given the additional validation boost that as publishing this led to, I really do hope that this will not only the other laboratories will adopt this, but that some of the guidelines that are out there may change to encourage laboratories to do this.

Randye Kaye: Fantastic. And as you say, sometimes the best solutions are really basic. Finally, do you think that assay manufacturers need to change their reporting limits for existing or new assays coming to the market?

Dina Greene: I really do, yes. And I don't blame the assay manufacturers. When these tests came out, we didn't know that we wanted the resolution down this low. And so, they picked a kind of realistic number and validated it. Getting things through the FDA is very complicated and sometimes picking something that is a little bit higher value, you're just going to get much more consistent results, particularly when you're not sure if

there's value of going lower. But what we've shown is that there is value of going lower, and we know that our instruments are capable of being quite precise with these measurements. And so, I highly encourage manufacturers, if they are revisiting their tests or if they have the bandwidth to revisit their tests, that they do reduce the LLOQ that they define in their package insert to be able to support more accurate and comprehensive results for urine albumin, and the urine albumin to creatinine ratio. Because ultimately, that gets at all of our goals, which is detect disease earlier and be able to use labs to support public health.

Randye Kaye: Wonderful. Thank you so much for that information in the article and for joining me today.

Dina Greene: Yeah. Thank you, Randye.

Randye Kaye: That was Dr. Dina Greene from LetsGetChecked, describing the *JALM* article, "Decreasing the Lower Limit of Quantitation for Urine Albumin Improves Clinical Utility." Thanks for tuning in to this episode of *JALM* Talk. See you next time and don't forget to submit something for us to talk about.