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Urinary Amino-Terminal Pro–C-Type Natriuretic Peptide: A Novel Marker of Chronic Kidney Disease in Diabetes

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Guest: Dr. Timothy Prickett of the University of Otago, New Zealand. Dr. Prickett currently holds a New Zealand Heart Foundation Senior Fellowship and is a member of the Christchurch Heart Institute.

Bob Barrett: This is a podcast from *Clinical Chemistry,* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I am Bob Barrett.

The last two decades have seen an explosive global increase in diabetes. There is now a greater than 400 million people estimated to have diabetes in the 20-80 year old age range, with a worldwide prevalence of 9%. A significant proportion of these people are likely to develop chronic kidney disease, which markedly increases morbidity and mortality of cardiovascular disorders and is a leading cause of end stage renal failure.

However, early detection of these disorders is challenging; albuminuria when used for detection of kidney disease is both insensitive and nonspecific, whereas a subnormal estimated glomerular filtration rate – or eGFR – is a relatively late sign and usually indicates irreversible renal disease. Because of these deficiencies, many groups are pursuing novel approaches to improving early detection of renal injury. Upregulation of C-type natriuretic peptide (or CNP) gene expression occurs in response to renal inflammation in experimental animals, and nothing is known of the molecular forms of CNP products in urine of human subjects with diabetes mellitus or links with renal function.

A paper appearing in the October 2019 issue of *Clinical Chemistry* found that fragments of proCNP could be measured in urine from subjects with diabetes mellitus. The urinary NT-proCNP to creatinine ratio was also more reproduceable than the now commonly used albumin to creatinine ratio and strongly associated with the presence of chronic kidney disease. The lead author of that paper is Dr. Timothy Prickett, who is senior research fellow at the University of Otago, New Zealand. He currently holds a New Zealand Heart Foundation Senior Fellowship and is a member of the Christchurch Heart Institute.

Dr. Prickett is our guest in this podcast. So doctor, what is the significance of your findings of fragments of pro C-type natriuretic peptide and your new test?

Dr. Timothy Prickett: Okay. For the first time, it provides a markup of renal C-type natriuretic peptide production in diabetes and how urine measurements of C-type natriuretic peptide, which I'll abbreviate to CNP for short, relates to normal renal function. For many years, it's been known that CNP is anti-inflammatory and increases in renal tissues of animals exposed to renal injury. For example, Hu et al, in 2012, using a rodent model of kidney injury showed that CNP urine excretion is greatly increased following unilateral urethral obstruction, and that this increase occurred before albumin was affected. The urine CNP concentration is related well to the degree of renal tubulointerstitial fibrosis.

Bob Barrett: Has C-type natriuretic peptide or its products been measured previously in urine? And if so, what is new about your technique?

Dr. Timothy Prickett: Well, previous work has shown that the material to be present in human urine using CNP radioimmunoassays. However, these measurements were not fully validated and the effects of interfering urine metrics components were not assessed. Also, CNP is susceptible to rapid clearance by enzymes such as neprilysin, and also clearance by the natriuretic peptide clearance receptor. And both of these routes are highly expressed in renal tissues. So this new test measures a cleavage product of the CNP-pro hormone, which is not subject to those same clearance pathways. And we took special pains to separate out the urine matrix effects and characterize exactly what molecular forms of proCNP were present in human urine using size-exclusion HPLC. Once this was completed, we developed an in-house assay to specifically measure the major NT-proCNP form that was predominant in DKD. And this essay was shown to give closely similar results to the area under the HPLC peak for a given individual's chromatogram.

Bob Barrett: Most diabetic patients have the albumin to creatinine ratio test in spot urine to assess renal status. So are you saying, your test should replace this if larger studies reproduce your results?

Dr. Timothy Prickett: No, no, certainly not at this stage. Larger studies will be required to determine the relative predictive paths of these two tests. But we do find that the new test is more reproduceable and variability of serial ACR results are well known, and indeed, a repeat ACR test is often requested. In our dataset we found that the coefficient of variation for repeat urine albumin creatinine ratio measurements were some 50% versus the coefficient of variation for a repeat NT-CNP creatinine measurement in those same urine samples, the variability was some 14 percentage points lower.

Bob Barrett: What additional information is this test providing over and above the now commonly performed albumin to creatinine ratio test?

Dr. Timothy Prickett: The urine albumin to creatinine ratio measurement reflects glomerular capillary damage. Whereas, NT-CNP creatinine ratio does not. The NT-CNP creatinine ratio may be detecting tubulointerstitial damage, not assessed by the albumin creatinine test. Indeed, within our dataset, if one combines the ACR and the NT-CNP creatinine ratio results using defined cutoff values, we find that specificity of detecting DKD has increased from 88% to 97%.

Bob Barrett: Is this relevant only to diabetes? What about non-diabetic chronic kidney disease?

Dr. Timothy Prickett: Yes, this needs to be tested; however, we expect similar results, especially as renal tubulointerstitial disease is a final common path. We have now initiated the studies looking at urine NT-CNP creatinine measurements in cardiac patient's development CKD.

Bob Barrett: There are, of course, other questions. What about the cost, availability, and turn around time of measuring fragments of proC-type natriuretic peptide relative to albumin?

Dr. Timothy Prickett: Yes, the currently available commercial essays are sold as research-only essays. And we found an ELISA test manufactured in Austria by Biomedica to be reliable and provide similar results to our in-house essay. This assay is marketed around the world by a number of vendors, and in the U.S., it costs around $630. Each kit can analyze 37 samples making the first sample kit cost some $17. And this assay takes approximately five hours from sample addition to read out. So should this test prove to be useful, with the development of faster high throughput clinical analyzers can be (00:06:57) with turnaround times that would not be too dissimilar to a urine albumin creatinine measurement.

Bob Barrett: Well, finally, Dr. Prickett, let's look ahead. Where will you and this field go from here?

Dr. Timothy Prickett: Well, it's important now to look at sequential changes in NT-CNP creatinine in individuals who are nearly diagnosed type two diabetes to determine the utility of this test as an early biomarker of kidney damage, also longer longitudinal studies are needed to document changes in NT-CNP creatinine ratios to determine its predictive value for those with rapidly declining renal function. Also, with new medications being available with it, it now delays

progression to end stage renal disease. For example, SGLT2 inhibitors or selective endothelial AMP antagonists. This new test may have the potential to predict, or be of utility to monitor the improvements and potentially titrate medication.

Bob Barrett: Dr. Timothy Prickett is Senior Research fellow at the University of Otago, New Zealand and a member of the Christchurch Heart Institute. He has been our guest in this podcast on the measurement of urinary amino terminal proCNP as a novel biomarker of chronic kidney disease and diabetes. That paper appears in the October 2019 issue of *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.