Clinical Chemistry

Searching for New Biomarkers of Renal Diseases through Proteomics

Bob Barrett: This is the podcast from *'Clinical Chemistry'*. I'm Bob Barrett. The effectiveness of treatment for renal diseases is limited by the lack of diagnostic, prognostic and therapeutic markers. A tissue biopsy is often necessary to establish a diagnosis, particularly in the case of glomerular diseases.

Although, considered the gold standard diagnostic test, biopsy always carries risks including hemorrhage, pain and even death. The most widely used biochemical indicators are serum creatinine and urine protein, however, both have limitations.

Knowledge of more specific protein perturbations might better inform the management of renal diseases. The field of proteomics is expanding daily and in a review published in the February 2012 issue of *'Clinical Chemistry'*, Dr. Ana Konvalinka from the Division of Nephrology at the University of Toronto gave an overview of studies in which renal disease biomarkers were investigated by use of mass spectrometry and outlined a scheme for understanding the basics of proteomics.

Dr. Konvalinka is our guest in this podcast. Doctor, in this review, you have written about the use of kidney biopsy as the gold standard to make a diagnosis of kidney disease. Kidney biopsy has risks, which can be substantial. So the use of urine serum biomarkers for diagnosis of kidney disease would be a tremendous step forward.

As a nephrologist, do you see the urine serum biomarkers replacing a kidney biopsy for the purpose of diagnosing kidney disease?

Dr. Ana Konvalinka: Well, this is the million-dollar question. The discovery of reliable markers in either urine or serum that could differentiate kidney diseases without the need for a biopsy would be a monumental achievement.

There has been a lot of interest in this field for obvious reasons, but the progress has been slow. Why is that the case? Well, in part because disease-specific markers are truly difficult to uncover. Many of the markers are reflective of systemic perturbations as opposed to disease-specific mechanisms.

Also, the sensitive techniques used in proteomics usually uncover differences between the particular groups being studied, which can usually not be extrapolated to other groups of patients with the same disease.

There is at least one story of success, however, and that is the discovery of an antibody to M-type phospholipase A2

receptor, present in specialized kidney cells that are called podocytes.

Beck and his colleagues discovered that these anti-phospholipase A2 receptor antibodies were present only in the sera of patients with idiopathic membranous nephropathy, but not in patients with other glomerular diseases or in normal controls.

In fact, the evidence linking this antibody to idiopathic membranous nephropathy has since been substantiated in other groups of patients.

A polymorphism in the gene that's coding for this phospholipase A2 receptor predisposed patients to development of this condition. And Beck and his group then went on to show that depletion of anti-phospholipase A2 receptor antibody with a medication that's called Rituximab, resulted in these disease remission in a number of patients.

While additional studies are needed to determine the exact sensitivity and specificity of this antibody as well as the function of this phospholipase A2 receptor, this anti-phospholipase A2 receptor antibody starting to be used in the clinic to diagnose in the idiopathic membranous nephropathy.

Unfortunately, however, this example is an exception as for most other kidney diseases, there is still no reliable markers.

So I believe that these diagnostic markers are still out of reach, but with larger and well-designed studies and studies that are driven by disease mechanisms, such as Beck's study, this will be possible.

Bob Barrett: What about prognosis? Do you think then an adequate urine serum marker will be found that could be followed to indicate risk of disease progression?

Dr. Ana Konvalinka: Well, this is another area that is extremely clinically important. If we knew which patients were going to progress to end stage kidney disease, we could focus on aggressively treating this particular group of patients.

And related to this, if we had markers of responsiveness to therapy, we could administer toxic treatments only to those patients with favorable markers of response, thus bearing other patients of the potential side effects of treatment.

So we already have some markers of disease progression that are used in the clinic but that are imperfect. For

example, urine albumin excretion rate or urine total protein excretion rate are the best markers of chronic kidney disease progression that we have.

They are also problematic, because for example, in the case of patients with IgA nephropathy, urine protein excretion rate of one gram per day portends poor prognosis, while the same degree of proteinuria in membranous nephropathy or in focal segmental glomerulosclerosis is a predictor of good prognosis.

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It is thus hard to believe that looking at individual proteins will not offer more information than looking at the total protein content or albumin alone.

The advantage of examining disease progression is that one can focus on systemic disturbances as opposed to specific disease processes.

Also, we need not distinguish between all possible clinical cases but only the relevant ones.

For example, finding markers of disease progression from microalbuminuria to macroalbuminuria in diabetes mellitus requires looking only at two carefully selected cohorts; one that progressed from micro into macroalbuminuria, and one that never progressed from microalbuminuria or that regress to normal albumin excretion rate.

In this case, one require samples before and after progression or regression, and the cohorts should otherwise be very similar and have minimal other comorbidities and comparable medication intake.

Nonetheless, the biomarkers discovered do not need to differentiate these diabetics from all other patients, but need to be relevant only to this one single and specific question, and this is the realistic goal.

So, prognostic biomarkers will more likely consist of multiple markers that combine the prognostic value of established biomarkers such as proteinuria and clinical parameters as well.

And the stepping stone to any marker to inflation into the clinical practice is it's the validation.

Bob Barrett: Well, it appears that numerous studies searching for novel proteomic biomarkers of renal disease have been published, but only a few, if any, candidate markers have made it to

the clinic, why has it been so hard to find proteomic biomarkers, and how could this field move forward?

Dr. Ana Konvalinka: Yes, we have discussed some of the most important hurdles in the advancement of proteomics biomarkers in this review, and many of the issues were technical in nature and relate to standardization of urine collection, for example, and spectrometric methods to analyze urine.

However, I want to stress the importance of good study design and selection of patients with the consistent and accurately identified and defined clinical phenotype. Many studies have failed to include homogenous patient populations and mass spectrometry technique is exquisitely sensitive and so may reveal differences between selected groups that are not actually generalizable.

And ultimately, even the most solid discovery study has not been followed by appropriate validation studies.

So the scientific community will have to properly evaluate and validate the markers already discovered in a systematic fashion. A consortium of industries, nonprofit institutions and regulators such as, for example, the Predictive Safety Testing Consortium is a good example of how specific markers can be propelled into validation phases.

Also, future discovery studies should be carefully designed to address all of the mentioned issues. Biomarkers already discovered could also be employed in new studies that are designed to test novel hypotheses.

And finally, combining several biomarkers into clinical parameters, with clinical parameters, can increase the predictive power of potential prognostic biomarkers.

Bob Barrett: You talk about the potential of urine as a bio fluid for discovery of markers of kidney disease, would urine be promising to mine for other diseases and discover other diseases?

Dr. Ana Konvalinka: This is an interesting question and some research groups have turned from blood to urine for exploration of potential disease markers because of the ease of collection of urine and more importantly because of the relative simplicity of urine compared to blood.

The most yield in the field of urine proteomics will likely come from mining the proteomes of organs that have direct contact with urine such as kidney and lower urinary tract.

However, there have been efforts to discover urine biomarkers of distant cancers including hepatocellular carcinoma, ovarian cancer, non-small cell lung carcinoma, and upper gastrointestinal cancers.

And the idea here is as follows. Even though, these distant cancers do not have direct contacts with urine, they shed their proteins and peptides into the systematic circulation. Provided that these cancer-specific proteins or peptides are small and positively charged, they may be filtered across the glomerulus.

And if these proteins or peptides are able to reach high enough concentration to overwhelm the capacity of the tubules to reabsorb them or if the reabsorption capacity of the tubules here is decreased for another reason, for example, tubular disease, then they will end up in urine.

So the thought is that by mining the urine proteome of patients with a high cancer burden, these potentially unique proteins and peptides will be discovered.

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The classic example of this is production of light chains by monoclonal expanded population of plasma cells in multiple myeloma, which can be detected in urine and are called Bence Jones proteins.

The potential trap in these discovery experiments is that one is more likely to uncover peptides reflective of systemic perturbations or other local diseases such as kidney injury in patients with advanced cancer.

Bob Barrett: You have described studies that utilize blood, urine and tissue discovery of proteomics markers of kidney disease, are there any other sources of biomarkers to explore?

Dr. Ana Konvalinka: Yes. Besides the most obvious three sources of biomarkers that you have mentioned, there are other so-called proximal fluids that have been mined for biomarkers of disease, and for example, amniotic fluid, FIDs, cyst fluids collected during aspiration, saliva, vaginal fluid, etcetera.

I have focused on urine, blood and tissue as these are most relevant to kidney diseases.

Bob Barrett: But how do you envision the integration of knowledge between different bio fluids tissues and also between distinct platforms, such as proteomics and genomics?

Dr. Ana Konvalinka: I think that all these research platforms are complementary and should be used together whenever possible. Ideally, large multi-center studies addressing a clear question in as homogenous set of patients as possible should be conducted.

Ideally, also there should be biopsy tissue available for either targeted or discovery types of questions.

Also, in my opinion, bio banking of urine and blood should be included in any venture addressing kidney disease treatment or inquiring about natural history. And in this way, we could in a systematic way perform discovery and/or targeted proteomic and genomic studies to find markers of diagnosis, prognosis, responsiveness to therapy or markers indicative of the pathogenesis of disease.

This information should then be integrated with clinical information. There are some ongoing efforts by several groups to take similar approaches to bio-banking and to future studies, and one such example is the Nephrotic Syndrome Study Network or NEPTUNE Consortium, which is aimed at investigating primary glomerular diseases in North America in a systematic way.

Proteomics and genomics offer different but complementary information. Several studies correlating protein and gene expression in the same experiment discovered that only one-third of proteins have a concomitant change in gene expression.

This is perhaps not surprising when we take into account the different post-translational modifications of proteins, and the fact that multiple different protein isoforms can be created from the same gene.

Proteomics and genomics can thus be used to cross-validate the findings or to uncover different but complementary markers.

Bob Barrett: Well, in that case, do you think individualized medicine is on the horizon?

Dr. Ana Konvalinka: There is a clear and ever increasing recognition that each patient's disease and a response to a disease is a product of that patient's genetic background, environmental factors, medications, metabolic factors, other comorbidities, etcetera.

We also know that some patients respond extremely well to some therapies, while others do not respond to the same therapies. But how do we predict which patient will respond

to which therapy or which patients are more likely to have unwanted side effects?

And this is an area of active research, but the actual application of this in the clinical arena on a global scale is still science fiction.

I do, however, believe that a day will come that based on a patient's particular genome complement, proteomic perturbations, disease manifestations and susceptibility to known drug side effects, we will be able to prescribe individualized therapy.

Bob Barrett: You have shown in this review that numerous studies performed on different patient populations using different technology, in fact, identified overlapping proteins as being significant. For example, Hepcidin-25 is a marker of recovery from renal flares in lupus patients, and a marker of acute kidney injury in patients with cardiopulmonary bypass, you gave other examples too. Why would this be the case? In other words, do you think that these markers are meaningless since they appear in different settings and in different disease processes?

Dr. Ana Konvalinka: This phenomenon of finding the same protein biomarkers in very different patient populations, in different clinical settings or research questions, and in seemingly different processes appears to be relatively common. There are several explanations for this.

First, the proteins with high abundance are overrepresented in proteomic studies. If a protein is present in high concentration in a tissue or fluid, then its peptides will be more readily detected and will be sequenced by the mass spectrometer more often.

Second, many different disease processes converge on the same responses to injury or systemic perturbations.

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So when we see markers that are not disease or tissue specific, they will clearly be inferior candidates for diagnosis. However, they may still be excellent candidate biomarkers for disease progression or responsiveness to therapy.

And the key here is to further study them in the same clinical context in which they were discovered.

Bob Barrett: Well it's obvious, biomarkers of kidney disease is your area of study, what kind of approaches do you use in your own research to advance this field?

Dr. Ana Konvalinka: As a nephrologist, I am clearly interested in markers of kidney diseases, particularly kidney disease progression and response to therapy.

So as discovery methods perform directly in urine and blood for that matter have so far not been particular informative, I decided to adopt an approach that is used by the Cancer Biomarker Laboratory. Dr. Eleftherios Diamandis has led an extremely successful laboratory where the focus is on using cell cultures to derive markers of interests by means of proteomics studies.

Then these potential markers are confirmed using an alternative method and finally propelled into clinical studies, and in this way, Dr. Diamandis has discovered novel biomarkers for breast, ovarian and other cancers.

I am interested in finding markers on the renin-angiotensin system or RAS, which is believed to be active in almost all chronic kidney diseases as well as in transplanted grafts.

So blocking the renin-angiotensin system is also the mainstay of therapeutic chronic kidney disease.

Unfortunately, we don't have good markers to inform us how to dose this therapy, what to aim for besides reductions in blood pressure and proteinuria, whether to use dual therapy, etcetera.

Finding markers of activity of this system would allow us to use the renin-angiotensin system blockers in a more educated way. And this would be important for patient care and decreasing progression of kidney diseases.

So in order to explore this, I have used kidney cells grown in culture and these cells are then stimulated with one of the main effectors of the renin-angiotensin system, and then the proteome of simulated cells is compared to that of unstimulated kidney cells.

In this way, I have discovered close to 5,000 proteins and a small number of these proteins is increased or decreased in expression when this renin-angiotensin system effector is added.

These differentially regulated proteins are of interest because they may represent markers of renin-angiotensin system activity in patients with kidney diseases, and in fact, I am in the process of verifying these proteins in cell cultures and I will then go on to test their expression in urine of patients with kidney diseases in which the system is believed to be important in the chronic kidney disease progression.

So instead of looking for unique markers of each disease that are difficult to find, I have taken advantage of a systemic disturbance that could be applied to numerous kidney diseases.

Bob Barrett: Well, finally Doctor, are there any promising markers we should be paying attention to?

Dr. Ana Konvalinka: Well, it appears that markers of oxidative stress and response to oxidative stress are some of my top candidates for future biomarkers of renin-angiotensin system activity, but this still remains to be seen. So I can't tell you anything more at this point.

Bob Barrett: Dr. Ana Konvalinka is from the Division of Nephrology at the University of Toronto, and has been our guest in this podcast from *'Clinical Chemistry'*.

I'm Bob Barrett. Thanks for listening!

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