Clinical Chemistry

# Development of an Immunoassay for the Kidney-Specific Protein *myo*-Inositol Oxygenase, a Potential Biomarker of Acute Kidney Injury

**Article:**
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**Guest:**
Dr. Joseph Gaut is an Assistant Professor of Pathology and Immunology at Washington University School of Medicine.

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.

Acute kidney injury affects many critically ill patients and is a significant cause of morbidity and mortality. The diagnostic standard plasma creatinine is nonspecific and may not increase until days after injury. So there is a real need for renal specific biomarker detectable early enough that there would be a potential window for therapeutic intervention.

A paper in the May 2014 issue of *Clinical Chemistry* describes the development of an immunoassay for the kidney-specific protein myo-inositol oxygenase that may just be that biomarker. The lead author of that paper, Dr. Joseph Gaut, joins us in this podcast.

Dr. Gaut is currently an Assistant Professor of Pathology and Immunology at Washington University School of Medicine, and sub-specializes in Renal Pathology.

So Dr. Gaut, what exactly is acute kidney injury and how significant a problem are we talking about?

Dr. Joseph Gaut: Acute kidney injury is defined as the sudden loss of kidney function. This results in accumulation of waste products in the blood, things like urea and creatinine.

The spectrum of kidney injury is quite broad. May be it can range from just mild impairment to complete organ failure requiring dialysis. Acute kidney injury has a multitude of causes ranging from sepsis to major surgery, drug toxicity, trauma, and a variety of other things.

So the significance of this problem is actually becoming more-and-more recognized around the world. In fact, recent reports indicate that acute kidney injury accounts for about two million deaths per year worldwide.

Here in the United States about 5% of hospital, all hospitalized patients will develop acute kidney injury. Patients who are critically ill are at the highest risk of developing acute kidney injury with a frequency of about 40%, and this increases mortality dramatically up to about a 30%-60% increase from their baseline amongst the critically ill population. So it's quite a significant problem both here in the United States and globally with a variety of causes.

Bob Barrett: What are the issues with the current methods of diagnosing acute kidney injury?

Dr. Joseph Gaut: The current gold standard for diagnosing acute kidney injury is the measurement of creatinine in the blood and following urine output. These parameters have been around for well over 50 years and are established in the medical community. They are relatively inexpensive and easy to do. However, they suffer from a significant lack of both sensitivity and specificity.

For instance, creatinine might not change until over 50% of your kidney function has been lost. Take for instance, a patient who is a living-related kidney donor. These patients can have an entire kidney removed and placed into another person, yet their serum creatinine levels will barely change, and they go on and live relatively healthy lives, yet they have lost half of their kidney function.

Creatinine also depends on other factors that are completely unrelated to the kidney, things like muscle mass and liver function. Therefore, it is not necessarily entirely specific to things going on in the kidney.

Aside from creatinine, urine output is very problematic because it's difficult to measure this accurately unless you have a foley catheter or some mechanism in place to quantify the urine output accurately.

Furthermore, the urine output can change depending on the fluid status of the patient, and whether or not diuretics are being used.

Since these methods of diagnosing acute kidney injury are insensitive and not specific, there has been an increased push by a variety of entities including the American Society of Nephrology among others, to develop more effective and accurate ways to diagnose kidney damage.

The hope being that with earlier detection we can intervene more quickly and prevent the short and long-term effects, this significant morbidity, mortality associated with acute kidney injury.

In fact, the actual lack of early detection, our inability to diagnose this accurately and sensitively, is cited as the major road block to designing and testing new therapeutic strategies for acute kidney injury. So that's actually the goal of this study, to identify a new specific and sensitive biomarker of acute kidney injury.

Bob Barrett: Doctor, how did you identify the protein and I want to say this right, myo-inositol oxygenase as a potential biomarker of acute kidney injury.

Dr. Joseph Gaut: Yes, that is correct myo-inositol oxygenase. So we actually started by thinking of the ideal characteristics of a tissue injury biomarker. So an ideal kidney injury biomarker or a tissue injury biomarker in general should be specific to the tissue of interest, highly abundant within that tissue, and easily measurable.

So in order to identify such a marker in the kidney to see if it even existed we started by screening gene expression profiles from multiple organs isolated from mice.

We then identified mRNA transcripts that were either only expressed or very highly enriched in kidney tissue and also highly abundant within kidney tissue. We also specifically looked for genes that were expressed predominantly in the proximal renal tubule because the proximal tubule part of the kidney is highly susceptible to ischemic kidney damage.

The result of all of this gene expression profiling was identification of this gene that encodes the protein myo-inositol oxygenase. So the theory being that with acute kidney injury the proximal tubule cells will be damaged and their contents will be released either, released directly into the urine or released into the surrounding space where it can be reabsorbed into the blood and detected.

Ideally then, identifying a kidney specific protein such as MIOX would then specifically serve as a specific indicator that the kidney has been damaged.

Bob Barrett: So how did myo-inositol oxygenase perform as an acute kidney injury biomarker?

Dr. Joseph Gaut: So yes, once we identified myo-inositol oxygenase which I'm just going to refer as MIOX, is a potential biomarker, we generated both polyclonal and monoclonal antibodies that were common on human form of this protein. We discovered that two of our monoclonal antibodies worked very well as a pair of antibodies to detect recombinant MIOX in a sandwich immunoassay format, with a limited detection of about a 115 picograms per ml and with very acceptable inter and

intra-assay coefficient of variation with less than 20% and less than 8% respectively.

Using this immunoassay that we developed, we were able to detect endogenous MIOX from both human and mouse kidney homogenate, and we found that MIOX was detectable in the blood of mice subjected to acute kidney injury, just in an ischemic model of acute kidney injury where the renal artery is clamped for 30 minutes and released. We found that after 24 hours, the protein was detected in the blood. We have not yet done a full-time course experiment in the animals.

Importantly, MIOX was completely undetectable in animals who were not subjected to injury or subjected to sham types of surgeries. In humans our first attempt in what's described in our paper is a retrospective study of human patients who were all critically ill and some of whom developed kidney injury to varying degrees. So some had very severe acute kidney injury, they required dialysis; and some had minor acute kidney injury without even a change in urine output; and some who had acute kidney injury would they drop in urine output where they were actually clinically defined as oliguric.

And what we found was, surprisingly to us, that the plasma MIOX concentrations were much higher in the patients who had acute kidney injury, but they were actually -- that wasn't surprising -- they were actually also higher about 54 hours before serum creatinine increased in these patients, which was quite striking.

So this particular result indicates to us that MIOX certainly has potential to be an early indicator of kidney damage. I should also note that MIOX increased the most in patients who had the most severe injury, namely those who ended up on dialysis and those who had acute kidney injury defined by a serum creatinine change, as well as a drop in urine output.

So there certainly are some unanswered questions and much work left to be done. But we believe based on our initial data that this biomarker definitely show some promise and that we need further studies to explore its utility as a clinically relevant diagnostic biomarker of kidney injury.

Bob Barrett: Well, looking ahead, doctor, what are your plans for the future with this biomarker?

Dr. Joseph Gaut: Well, there are many avenues to explore, and first and foremost, we are planning to evaluate MIOX in a prospective study of human patients who are highly susceptible to developing kidney injuries. So this includes patients that are

undergoing major surgery, particularly cardiopulmonary bypass surgery, and patients who are critically ill.

It's also important to further clarify whether or not this protein whether or not MIOX is effective in identifying different types of injury. For instance, could this also work as a marker of drug toxicity; animal models are very well suited to this and that's certainly an active area of investigation.

As I mentioned briefly before, we had not yet done the time course of analysis of how MIOX is released into the blood in an animal model and that is certainly something that needs to be characterized along with whether or not MIOX is up-regulated, or changed in some way, in other organs, following acute kidney injury.

We know based on all of our studies that it is very specific to the kidney under normal circumstances but what happens to it and other tissues after kidney damage, that's an unanswered question that certainly needs to be investigated. And of course, it would be interesting to compare MIOX with other more established acute kidney injury biomarker.

So there is a variety of these that have come on and been widely studied over the last decade or so. Things like Kidney Injury Molecule-1 or Neutrophil Gelatinase-Associated Lipocalin among many others, and it would be very interesting to look at MIOX which is a structural protein native to the kidney in contrast to things like NGAL is up-regulated following kidney damage.

So it would be interesting to just look at the time course of release and whether or not it can tell us different things in a panel format, you can measure both of these markers at the same time. Could that tell us different things then just analyzing one molecule at a time?

There are many opportunities to explore this very interesting molecule and its role in identifying kidney injury, but of course, the ultimate question is whether or not we will be able to improve patient care by diagnosing acute kidney injury early, and that goes for whether or not it's MIOX or one of the other kidney injury biomarkers, and that's a much more difficult question to answer and it's going to take a significantly greater amount of time and energy to figure that one out.

But the field is definitely promising and it's certainly moving forward at a rapid pace and it's quite an exciting time to be involved in this.

Bob Barrett: Dr. Joseph Gaut is an Assistant Professor of Pathology and Immunology at Washington University School of Medicine. He has been our guest in this podcast from *Clinical Chemistry* on acute kidney injury biomarkers.

I am Bob Barrett. Thanks for listening!