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Urinary Biomarkers: Alone Are They Enough?

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The field of "novel" biomarkers for AKI is now well over 10 years old. During this era, tremendous amounts of time, effort, and money have been invested to develop clinically useful biomarkers for determination of risk, diagnosis, and prognosis. The search for clinical surrogate end points has centered on the biochemical analysis of body fluids and has not enlisted the use of imaging or molecular analysis for determination of structural, molecular, and functional alterations. Numerous biomarkers have been tested in both plasma and urine, with urinary biomarkers potentially offering more specificity for events occurring within the kidney. Important information has been obtained with regard to pathophysiologic mechanisms, biomarkers that can be used to follow therapeutic approaches, and the potential use of certain biomarkers to provide early detection of nephrotoxin-induced injury in patients.[1,2] However, the initial enthusiasm and excitement over finding the "holy grail," and thus providing a point-of-care diagnosis for ischemic/septic AKI, has now been tempered as study after study has delineated less encouraging data with receiver operating characteristic curves ranging from 0.6 to 0.8.[3,4] The best studied clinical population has been the cardiovascular surgery population, allowing timed analysis of specific injury events to occur before, during, and after surgery.[5]

On this background, and with only one previous publication quantifying the utility of biomarkers to assess long-term outcomes,[6] the article by Koyner *et al.* in this issue of *JASN*[7] evaluating urinary cell cycle markers has added to the long list of knowledge that has been gained. However, one has to question whether the biomarker field has really evolved to a point of allowing for the clinical application of biomarkers to benefit the care of patients with AKI. In this editorial, I aim to provide a critical evaluation of the field in an attempt to stimulate development of synergistic approaches, beyond biochemical biomarkers, that will enhance the clinical value added to individual patient understanding and therapeutic approaches.

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CELL CYCLE MARKERS AND PROGNOSIS IN AKI

In the article by Koyner *et al.*,[7] there is little question that a stepwise evaluation of the two cell cycle markers indicated a progressive increased likelihood of adverse long-term outcomes in patients with AKI as urinary values increased. However, a close evaluation of the methods used and the analysis undertaken reveals certain insights. Appropriately, serum and urinary samples were drawn at the time of patient enrollment and within 24 hours of movement into the intensive care unit (ICU). Furthermore, the study by Koyner *et al.*[7] excluded patients who had documented moderate or severe AKI (Kidney Disease Improving Global Outcomes [KDIGO] stage 2 or 3) at the time of the study. This means that patients with mild and evolving AKI (KDIGO stage 1) were included in the study, but we are not told the number of these patients nor their effect on the outcomes monitored. Furthermore, Tables 2 and 3 in the article by Koyner *et al.*[7] indicate that serum creatinine values were highly significant for the unadjusted hazard ratios (HRs), and even more so in adjusted HRs for the development of death or dialysis within 9 months. Does this mean there is no added clinical value for the urinary cell cycle biomarkers? Furthermore, would stratification of serum creatinine, as was done for the cell cycle markers, lead to even an increased predictive value? We also know the maximum KDIGO stage occurring within 72 hours of enrollment was more severe in the patients that underwent dialysis or died (as shown in Table 1 by Koyner *et al.*[7]), again indicating the clinical utility of the existing classification scale to predict outcomes. Taken together, these data indicate that the two cell cycle markers studied have prognostic value when studied early in a critical care situation and will aid in identifying patients for therapeutic trials. However, do they add value to serum creatinine, which we know from numerous studies involving the RIFLE and Acute Kidney Injury Network classification schemes correlates retrospectively with overall longterm prognosis for dialysis and mortality?

ADDING CLINICAL VALUE IN AKI TO EXISTING PARAMETERS

When researchers in the biomarker field started to investigate the utility of urinary and serum biomarkers in the care of patients with AKI, optimism was rampant and the goal was to develop the "troponin equivalent" for acute myocardial infarction (MI). This panacea has not been achieved; on closer inspection, it likely will never be achieved, and perhaps should be abandoned as a goal for several reasons. First, troponin itself is not the holy grail of acute MIs because its interpretation without other evidence of acute MIs would often lead to false positives. Fortunately, and by design, cardiology does not depend on only one biomarker or one technique; rather, cardiology combines multiple complementary tools allowing for accurate diagnostic information as well as the ability to quantify severity of injury and response to therapy. This combination of objective technological evaluation of a particular clinical condition, primarily caused by one structural pathologic event, has led to dramatic clinical success in the diagnosis and treatment of patients with MI. The use of electrocardiography, echocardiography, nuclear medicine scans, cardiac catheterization, and thrombolytic therapy, as well as stent insertions, has been made possible by the careful and methodical development of synergistic technology. Furthermore, utilization of large-scale interpretable clinical trials, always asking what the added value would be for the clinician, has led to markedly improved outcomes for acute MI. This, in conjunction with determining, understanding, and modifying risk factors for acute MIs, has now resulted in a shift in focus of cardiovascular expertise away from acute MIs.

Now one can say that an acute MI has the advantage of clinical symptomatology that leads to a more accurate diagnosis and timing of the event. Although this is undoubtedly true, nephrologists have, in their search for the Holy Grail biomarker, tended to minimize useful information that can be gained by clinical histories as well as previously utilized techniques such as serum creatinine, urine analysis, and fractional excretion of sodium (FENa). For example, specific evaluation of FENa has been made in patients with AKI and the results of a receiver operating characteristic curve were <0.6, indicating little if any value. This was used as justification to totally disregard the measurement. Why so? Because the search is for one universal biomarker that is more equivalent to a pregnancy test, either positive or negative. Does this really make sense given the complexity in etiology, timing, and systemic influences on AKI and FENa?[8] Do cardiologists totally disregard an electrocardiogram as a screening tool given the number of false negatives that occur when a troponin is positive? Studies on the value of urine analysis have been both positive and negative, with recent evidence suggesting that a urine analysis done by a careful investigator provides both insight into the diagnosis and severity of injury.[9] Yet the urine analysis is negative in many cases often being useful in differentiating the "prerenal" state from tissue injury. Combining a panel of urinary and serum biomarkers to improve the sensitivity and specificity or timing in the diagnosis of AKI over one urinary biomarker has also yielded very little or no additional benefit. Why not do like the cardiologists and combine different synergistic techniques to improve the diagnostic accuracy of our approach?

Finally, and most importantly, the use of serum creatinine, once described as the "gold standard," has now been called into question because there are clearly patients that are serum creatinine negative and biomarker positive that have worse clinical outcomes in the hospital.[10–12] Although it seems this latter point is difficult to understand, one only has to return to freshman physiology to shine light on interpretations of AKI positive but negative serum creatinines. Landmark articles by Chertow *et al.* and Lassnigg *et al.* delineated the importance of small changes in serum creatinine of 0.3 mg/dl as having important clinical implications in a cross-section of patients in the hospital setting.[13,14] To many nephrologists, a change of 0.3 in serum creatinine having important clinical ramifications was difficult to believe, regardless of ideology. However,

this has held up and is clearly useful in diagnosing clinically meaningful AKI in the hospital setting. What does a change of 0.3 really mean? One first has to realize that a change of 0.3 mg/dl is dependent upon the patient's underlying kidney function. For a patient population, the serum creatinine (*y* axis) versus GFR curve does not start bending upward until 40%–50% of normal baseline GFR has been lost.[15] This is exactly why transplant donors have very little change in their serum creatinine after donation of half of their baseline kidney function. A second component to this is the renal reserve, which is a little thought-about component of overall kidney function that is utilized daily after meals to increase GFR approximately 50% above baseline in patients with normal kidney function. We do not understand the role of renal reserve in minimizing serum creatinine change in patients with AKI or CKD, but we do know that a percentage of patients in the ICU can activate their renal reserve to produce ongoing continuous GFRs in the 150–180 range. The importance of antibiotic underdosing in these patients was recently pointed out.[16] Now, in a patient with CKD, a change of 0.3 mg/dl is much more easily detected as one is on the upward portion of the creatinine versus GFR curve. In fact, a change of 0.3 in the idealized 70-kg man with a CKD baseline serum creatinine of 4.0 is a loss of only 1.0 ml of GFR and if the starting serum creatinine was 2.0, it would only require a loss of <5 ml GFR. Patients with CKD are thus more likely to have AKI diagnosed because the injury-induced GFR loss necessary to result in a measurable change in serum creatinine is small compared with a patient with normal kidney function. Thus, a change in serum creatinine with regard to the severity of the injury to one's kidney depends on the individual patient's underlying baseline function. This fact must always be remembered when thinking about the pathophysiology and consequences of injury to the kidney. Stated in another way, it is highly likely that a loss of 25% of kidney function or 25–30 ml/min per 1.73 m^2 of GFR in a patient with normal baseline function will be undetectable by serum creatinine and thus will be AKI negative even though there has been substantial injury to the kidney. These are likely the very patients that are biomarker positive, are serum creatinine negative, and do have renal injury and potentially could benefit from present-day biomarkers and future therapies.[11,12] This is exactly why we need screening biomarkers that are sensitive in the detection of this loss of kidney function. However, we need additional physiologic and imaging techniques that quantify the degree of injury, lead to an understanding of the pathologic mechanism, and thus allow for more individualization with regard to treatment.

INNOVATION AND INDIVIDUALIZATION OF CARE?

AKI has a broad spectrum of causes and pathophysiologies resulting in a complex clinical puzzle. Nephrologists act as detectives in obtaining the necessary information from the patient history and physical examination, blood and urine chemistries, urine analysis, ultrasonography, and, rarely, kidney biopsies. They must then integrate and individualize the data balancing the value of each information source to arrive at the diagnosis. On the other hand, acute MI results from a structural mechanism in the vast majority of cases. Yet cardiologists have developed the advanced tools necessary to objectively individualize care. Why the nephrology community has been slow in the translation of techniques to clinical studies is a puzzle. The field of nephrology once led the way in the understanding of physiology, yet little effort and funding have been expended to allow for translation of this knowledge to improve patient care in AKI. Only recently have molecular studies to understand human alterations in transplant rejection been utilized,[17] and these same techniques as well as other approaches are needed in AKI. The resulting data will help allow objective individualization of care, rather than the "associations" that have been reported for biomarkers in a population. Only with these approaches and the evolution of structural and functional imagining techniques will the field advance so as to allow promising preclinical therapeutic advances to be translated into the care of patients with AKI.

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See related article, "Tissue Inhibitor Metalloproteinase-2 (TIMP-2)·IGF-Binding Protein-7 (IGFBP7) Levels Are Associated with Adverse Long-Term Outcomes in Patients with AKI," on pages 1747–1754.