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Phenotyping of Acute Kidney Injury: Beyond Serum Creatinine

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Abstract

Acute kidney injury (AKI) is a common complication in hospitalized patients and is associated with adverse short- and long-term outcomes. AKI is diagnosed by SCr-based consensus definitions that capture an abrupt drop in glomerular filtration rate associated with AKI. However, SCr-based AKI definitions lack sensitivity and specificity for diagnosing structural kidney injury. Moreover, AKI is a heterogeneous condition consisting of distinct phenotypes based on its etiology, prognosis, and molecular pathways, and potentially require different therapies. SCr-based AKI definitions provide no information on these AKI phenotypes. This review highlights traditional and novel tools that overcome the limitations of SCr-based AKI definitions to improve AKI phenotyping.

Keywords

AKI; Biomarkers; Creatinine; Cystatin C; NGAL; IL-18; KIM-1; L-FABP; IL-6; Furosemide; Urine Microscopy; urinary casts

Introduction

Acute Kidney Injury (AKI) complicates one in five hospital admissions and is associated with adverse short- and long-term outcomes, and increased healthcare expenditure.^{1–4} The current gold-standard for AKI diagnosis is through consensus definitions such as AKI Network (AKIN) or Kidney Disease: Improving Global Outcomes (KDIGO).^{5,6} By using an increase in serum creatinine (SCr) and/or a decrease in urine output, these AKI definitions

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attempt to capture an abrupt drop in glomerular filtration rate (GFR) that is often associated with AKI. This standardization has facilitated research, clinical care, and healthcare management by allowing uniform comparisons across studies, cohorts, and hospitals. However, there is an unmet need to enhance the SCr-based definition to improve phenotyping of AKI for clinical and research purposes. This review covers the limitations of SCr-based AKI definitions and evaluates tools for AKI phenotyping beyond SCr.

1. Limitations of serum creatinine(SCr)-based AKI definition

SCr-based AKI definition is limited in two ways: a) lack of accuracy for diagnosis of structural kidney injury and b) in those with structural kidney injury, it provides no information on AKI etiology, prognosis, molecular pathways, or responses to treatment.⁷ For example, a 0.3 mg/dl or 50% rise in serum creatinine, which is the current definition of SCr-based AKI in KDIGO classification, reflects vastly different phenotypes. This rise in SCr in a patient in the intensive care unit (ICU) with sepsis, hypotension, and granular casts on urine sediment examination indicates structural kidney injury, reflects poor prognosis, and requires aggressive management. In contrast, this rise in SCr in a patient with decompensated congestive heart failure started on diuretic therapy, in a patient with diabetic kidney disease started on renin-angiotensin aldosterone system (RAAS) inhibitors, or in a patient with long-standing hypertension started on intensive blood pressure control likely reflects a hemodynamic rise in SCr without structural kidney injury, and is not associated with poor prognosis.^{8,9} Similarly, this rise in SCr in a person with advanced chronic kidney disease (CKD) with stable health may merely reflect laboratory or biological variation in creatinine, and does not indicate structural kidney injury.¹⁰

a) SCr-based AKI and structural kidney injury

A conceptual model of the limitations of SCr-based AKI definitions is presented in Figure 1.¹¹ The goal of SCr-based AKI definitions is to correctly identify all possible cases of structural kidney injury (Figure 1, box D), while also simultaneously excluding all cases without structural kidney injury (Figure 1, box A). However, rise in SCr denotes reduced *glomerular* filtration rate (GFR), does not directly capture tubulointerstitial injury, and lacks accuracy for diagnosis of structural kidney injury.

Kidney injury without rise in SCr (Figure 1, box B)—Kidney injury can occur in the absence of a rise in SCr, which represents an emerging condition called “subclinical AKI.”^{11–13} Subclinical AKI occurs when the effects of tubular injury and reduced GFR in some nephrons are compensated for by other non-injured and functioning nephrons via a phenomenon called “renal reserve.” Subclinical AKI is thought to be an early step in the spectrum of kidney injury and is associated with poor outcomes.¹³ Animal studies show development of renal fibrosis and abnormal gene expression profiles in kidneys after an episode of kidney injury despite return of SCr to normal values.^{11,14,15} An analysis of a biopsy series found that 21% cases of biopsy-proven acute tubular injury (ATI) cases did not meet the KDIGO SCr-based AKI definition.¹⁶

Moreover, SCr rises to its peak level 48–72 hours after kidney injury. This delay is undesirable in clinical care as patients experience significant exposure to nephrotoxic agents

as clinical AKI has not yet been recognized. The delay is also undesirable in research as potential therapies for AKI may need to be administered early after kidney injury before irreversible damage occurs. Finally, structural kidney injury may also be missed as SCr fails to rise from the dilutional effects of fluid administration and decreased SCr production due to low muscle mass and sepsis.

Rise in SCr without kidney injury (Figure 1, box C)—Elevations in SCr may not always represent kidney injury. For example, hemodynamic reduction in renal blood flow and glomerular filtration in cardiorenal and hepatorenal syndromes causes a rise in SCr in the absence of discernible structural kidney injury.^{7,10,17} RAAS inhibitors can also lead to increases in SCr due to reduced glomerular filtration rate, despite the potential absence of kidney injury. In fact, SCr may be misleading in the setting of RAAS inhibitors as improved patient outcomes are noted with these drugs despite SCr-based AKI.¹⁸ SCr may increase due to medications such as trimethoprim that inhibit tubular secretion of SCr without a reduction in GFR.

b) Phenotypes of SCr-based AKI

Arguably the biggest limitation of the current SCr-based AKI definitions is their failure to provide detailed phenotypes of AKI in those with structural kidney injury (Figure 1, box D). Recognizing this limitation of SCr-based AKI definition, researchers have turned their attention to discover, test, and validate novel biomarkers to improve AKI phenotyping. Since AKI is a highly heterogeneous condition, no single biomarker has successfully overcome all of the limitations of SCr in all settings. However, some novel biomarkers and repurposed, traditional, non-SCr based biomarkers have reliable evidence in specific areas overcoming the deficits of SCr.

2. Traditional and novel biomarkers for AKI phenotyping

The FDA defines a “biomarker” as “a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to therapeutic intervention.”¹⁹ In the past five years, over 700 publications evaluated biomarkers in AKI in clinical settings.²⁰ These biomarkers fall under the following broad categories:

a) Markers of decreased glomerular filtration

These are small molecules that are produced by the body at a constant rate, filtered by the glomeruli, and, ideally, neither reabsorbed nor secreted by the tubules. The two examples of GFR markers in clinical use are *SCr* and *Cystatin C*. SCr is freely filtered and a small amount is secreted by tubules. In the setting of AKI with decreased glomerular filtration, SCr increases due to decreased filtration and back leak through damaged proximal tubular cells.²¹ The use of SCr as a marker of glomerular filtration is supported by an extensive body of evidence that has evaluated both its strengths and weaknesses in many settings. However, newer filtration markers have been evaluated in specific settings to overcome some of the limitations of SCr. The most promising among the filtration markers is serum Cystatin C.²² In a study from the Translational Research Investigating Biomarker Endpoints in AKI

(TRIBE-AKI) cohort, AKI defined by SCr or Cystatin C did not differ in their ability to predict subsequent mortality or dialysis requirement. However, combination of the two markers provided better risk prediction for short-and long-term mortality and dialysis than either biomarker alone.^{23,24} The association of SCr with GFR becomes less accurate in cirrhosis due to issues with fluctuating total volume and decreased production from low muscle mass. One study found that, in patients with cirrhosis, changes in Cystatin C were more closely associated with subsequent dialysis and mortality than changes in SCr.²⁵

b) Tubular function

Structural kidney injury can lead to loss in tubular function and tests such as *fractional excretion of sodium* (FENa) and “*Furosemide Stress Test*” aim to detect this loss in tubular function. FENa captures the ability of the nephron to reabsorb filtered sodium. In structural kidney injury, the ability of the injured tubules to reabsorb sodium is impaired resulting in higher loss of sodium in urine and a higher FENa. In contrast, when SCr rises due to hemodynamic causes, this ability to reabsorb sodium is intact and results in low FENa values. A newer functional test called “*Furosemide Stress Test*” challenges the renal tubular function by administering a high dose of the diuretic, furosemide.²⁶ This test measures the urine output response to furosemide, an inhibitor of sodium potassium chloride cotransporter in the Loop of Henle, and provides an indirect measure of functioning nephrons.²⁷

c) Markers of tubular Injury

Urine sediment microscopy, used to detect sloughed off renal tubular epithelial cells and casts, is a time-tested tool that is supported by rigorous evidence in its ability to phenotype AKI in terms of its etiology and prognosis.²⁸ However, the utilization of this tool remains low outside academic medical centers due to lack of available equipment outside a hospital, lack of training, and perhaps lack of reimbursement for this time-consuming test. Another traditional marker for tubular injury is *urine microalbumin*. Long considered a marker of glomerular injury, albumin reabsorption in the proximal tubule is reduced in ATI and its expression is upregulated in AKI making it a promising marker of structural kidney injury. While its universal availability and low cost of measurement certainly make urine albumin an attractive marker, its inability to separate glomerular from tubular processes, lack of specificity for tubular segments, and unreliability in preexisting albuminuric CKD are the major limitations.

Several proteins are released from specific segments of the nephron, and are detectable in the blood and urine. For example, *Kidney Injury Molecule-1* (KIM-1) is upregulated in the proximal tubule in response to injury, modulates immune responses in kidney injury, and protects the kidney from immune injury when released early after injury.²⁹ *Neutrophil Gelatinase-Associated Lipocalin* (NGAL) is produced from distal tubular cells in response to injury, sequesters iron, and protects the tubular epithelial cells from cell death by upregulating heme oxygenase-1.³⁰ *Liver Fatty Acid Binding Protein* (L-FABP) is localized in the proximal tubule and is shed in the urine on kidney injury. *Tissue inhibitor of metalloproteinases-2* (TIMP2) and *insulin-like growth factor-binding protein 7* (IGFBP7) are expressed in epithelial cells and cause cell cycle arrest in AKI.

d) Other markers of AKI: Inflammation

The activation of inflammatory pathways within the kidneys and recruitment of inflammatory cells to the site of injury is an early response to kidney injury. **Interleukin (IL)-18**, a member of the interleukin 1 superfamily, is generated after cleavage of pro-IL-18 by caspase 1, is an important mediator of ischemic kidney injury, and its major source is believed to be the proximal tubule. **Monocyte chemotactic protein-1** (MCP-1, also known as chemokine C-C motif ligand 2) is produced by a plethora of cells in response to injury and attracts CCR-2 expressing monocytes to the kidneys during injury.³¹ **IL-6** expression is increased in AKI and leads to accumulation of neutrophils after AKI.³² **Repair:** Several novel biomarkers that contribute to kidney repair after injury have also been discovered. **YKL-40** is a product of chitinase 3-like 1 gene, and protects animals from death after ischemia/reperfusion.³³

3. Biomarkers identify AKI before rise in SCr

SCr rise occurs gradually after kidney injury often meeting the AKI criteria 48–72 hours after kidney injury. In the TRIBE-AKI study, which included patients with a well-timed kidney injury as part of cardiac surgery, the median (interquartile range) time from kidney injury to first evidence of AKI was 3 (2–4) days.^{34,35} Several biomarkers have overcome this limitation of SCr kinetics. In a cohort of 71 children who underwent cardiac surgery, urine NGAL levels measured 2 hours after surgery predicted subsequent development of AKI with an AUC of 0.998.³⁶ In the TRIBE-AKI study of 1,219 adults who underwent cardiac surgery, urine IL-18, urine NGAL, and plasma NGAL from samples collected within 6 hours after surgery predicted subsequent development of AKI with AUCs of 0.74 (0.04), 0.67 (0.04) and 0.7 (0.04), respectively.³⁴ Similarly, IL-6 measured within 6 hours after cardiac surgery was associated with higher risk of subsequent AKI.³⁷ In samples obtained at ICU admission, cell cycle arrest markers TIMP2 and IGFBP7 predicted subsequent development of AKI.³⁸ In the ICU setting with AKI prevalence of 17–23% and at a cut-off of 0.30 ng/ml*1000, the test had remarkable negative predictive value of 0.88–0.97, but a low PPV of 0.26–0.31.

4. Biomarkers of “subclinical AKI”

Subclinical AKI refers to a condition where structural kidney injury occurs without a rise in SCr. Subclinical AKI was identified by studies that demonstrated adverse long-term kidney outcomes in patients with elevated kidney injury biomarker levels, but without increases in SCr levels. For example, in a meta-analysis, patients with elevated NGAL concentrations but without a rise in SCr subsequently required renal replacement therapy more frequently and experienced greater mortality compared with patients without elevations in NGAL or SCr.³⁹ In a multicenter cohort of emergency room patients, urine NGAL and KIM-1 predicted initiation of dialysis or in-hospital death in patients without increases in SCr.⁴⁰ In the TRIBE-AKI study, we demonstrated that elevated urinary IL-18 and KIM-1 were independently associated with higher 3-year mortality in those who did not develop SCr-based AKI.⁴¹ Finally, in a study of 581 deceased kidney donors biopsied at organ procurement, we noted that over half of the cases of histological ATI occurred in donors

without SCr-based AKI. In this group of donors without SCr-based AKI, urine NGAL concentrations were significantly higher with increasing severity of histological ATI (Figure 3; manuscript under revision).

5. Distinguishing hemodynamic rise in SCr from ATI

The SCr-based AKI definition cannot differentiate between two common forms of AKI: (a) **hemodynamic rise in SCr** without kidney injury (Figure 1, box C), which includes pre-renal azotemia (PRA), hepatorenal and cardiorenal syndromes, and rise in SCr accompanied by RAAS inhibition and (b) **rise in SCr accompanied by ATI** (Figure 1, box D). Distinguishing between these two forms of AKI is desirable as it has significant implications on management and prognosis. Conditions causing hemodynamic rise in SCr are rapidly reversible upon restoration of hemodynamics. For example, hemodynamic rises in SCr are expected to recover rapidly when hemodynamic derangement is corrected by administration of intravenous fluids (IVF) to those with volume depletion, vasopressor therapy to those with hepatorenal syndrome, and inotropic agents to those with cardiorenal syndrome. In contrast, ATI typically takes longer to recover and its management includes supportive care, along with temporary renal replacement therapy. Moreover, since there is very little overlap between the activated pathways in ATI and the hemodynamic causes of SCr rise, it is expected that therapies will be specific to each of these conditions.⁴² While no specific therapy for ATI presently exists, it is expected that if such a therapy were to be developed, it would be important to differentiate between PRA and ATI to improve the power of such trials.⁴³ Figure 4 (adapted from ref.⁴³) demonstrates that, if patients with PRA are inadvertently enrolled in a clinical trial of a therapy for ATI, it may show null results despite it being effective for ATI. Unfortunately, the distinction between ATI and hemodynamic rise in SCr is made retrospectively based on clinical course, duration of SC rise, and response to therapy. Biomarkers can help prospectively differentiate between hemodynamic rise in SCr from ATI in specific situations as described below.

a) Cirrhosis

Cirrhosis is an important clinical setting where ATI must be distinguished from rise in SCr from hemodynamic causes such as PRA and hepatorenal syndrome. In cirrhosis, PRA is treated by increasing volume by administering albumin or withholding diuretics. Hepatorenal syndrome is reversed with vasoconstrictor therapy and subsequent liver transplantation. In contrast, patients with ATI are treated with supportive care and dialysis.

The current paradigm suggests diagnosing hepatorenal syndrome using clinical criteria.⁴⁴ However, many of the currently available clinical parameters are unreliable in cirrhosis. First, SCr is falsely lower in cirrhosis due to higher volume of distribution and lower muscle mass. Second, FENa<1% is a universal finding regardless of the diagnosis and does not reliably differentiate PRA and hepatorenal syndrome from ATI. To overcome these limitations, Belcher et. al. reevaluated traditional markers such as FENa and urine albumin and evaluated novel biomarkers for differentiating these conditions (Table 1).⁴⁵ They noted that urine electrolytes, urine microalbumin, and novel biomarkers (NGAL and IL-18) successfully differentiated ATI, PRA, and hepatorenal syndrome. A future trial investigating

therapies for AKI in cirrhosis can use these results to enroll participants. For example, a study investigating novel vasoconstrictive therapy for hepatorenal syndrome can enroll desired participants with low urine NGAL and very low FENa and randomize these patients to receive therapy drug or placebo.

b) Hospitalized patients

Urine microscopy was shown to be a reliable tool in differentiating PRA from ATI in hospitalized patients with AKI. In patients evaluated by the nephrology consult service at Yale, a high urine microscopy score, indicated by at least 1 renal tubular epithelial cell per high power field or at least 1 granular cast per low power field, was associated with 74 (17, 329)-fold higher odds of ATI as compared with absence of these findings.⁴⁶ In another study, hospitalized patients with ATI also had significantly higher urine IL-18 level as compared with healthy controls (644 pg/mg creatinine vs. 16 pg/mg, $P<0.0001$).⁴⁷ In a study of 161 hospitalized patients, urine NGAL discriminated between clinically-adjudicated ATI from PRA with AUC of 0.87.⁴⁸ KIM-1 levels were higher in patients with ischemic ATI compared with other forms of AKI and CKD.⁴⁹ While most studies have examined clinically adjudicated cases of ATI, in one of the only studies examining histological ATI, deceased donors with increasing severity of ATI had higher urine NGAL levels (Severe ATI, mild ATI, vs. no ATI: 201.5 (34.4–530.3), 103.8 (14.7–370.2), vs. 45.2 (15–172.1), $P<0.001$). (Manuscript under revision)

6. Phenotyping AKI by prognosis

Determining the prognosis in a patient at the time of an AKI event is desirable in order to decide best management approaches. Knowing the prognosis at the time of AKI can also facilitate enrolling patients at highest risk of adverse outcomes in clinical trials to reduce sample size and cost.⁴³ Biomarkers have been evaluated to prognosticate various outcomes such as progression to higher stage of AKI, duration of AKI, and mortality.

a) Markers of short-term prognosis after AKI

An important aspect of AKI phenotyping is the ability to prognosticate progression of an AKI case to higher stage AKI, particularly to a stage requiring renal replacement therapy. An important and underutilized test of short-term prognosis is urine microscopy. In a cohort of patients on the nephrology consult service, a high urine microscopy score was associated with worsening of AKI stage, dialysis requirement, and death during hospitalization.⁵⁰ Among patients with urine microscopy score 3, which indicated presence of at least 6 renal tubular epithelial cells per high power field and/or at least 6 granular casts per low power field, 64% had worsening of AKI stage and 31% required dialysis. In contrast, among patients with urine microscopy score of 0, only 9% had worsening of their AKI stage and 2% required dialysis. Another marker of short-term prognosis in AKI patients is the “Furosemide Stress Test.” In a study by Koyner et. al., urine output within 2-hours of administration of furosemide reliably predicted progression to stage 3 AKI (AUC: 0.87 (0.09)) and for requirement of renal replacement therapy (AUC: 0.86(0.08)).²⁶ Finally, patient with elevated plasma NGAL on the day of AKI had higher risk of AKI progression and plasma NGAL showed a net reclassification improvement of 0.69.⁵¹

b) Markers of long-term outcomes after an AKI event

The occurrence of an AKI event is now recognized as a risk factor for adverse long-term outcomes.⁵² AKI is associated with increased risk of CKD, ESRD, cardiovascular disease, and mortality. Findings at the time of an AKI event can predict these long-term adverse outcomes. For example, in a study by Kellum et. al., patients with higher stage of AKI (by either SCr or urine output) had a higher likelihood of developing end stage renal disease or death at one year.⁵³ Several novel biomarkers measured at the time of kidney injury can predict long-term mortality. We found that urine NGAL, IL-18, KIM-1, and L-FABP,] were independently associated with 3-year mortality after AKI.⁴¹ Similarly, MCP-1, a marker of inflammation was also associated with higher long-term mortality after an AKI event.⁵⁵ In contrast, plasma NGAL was not independently associated with long-term mortality independent of changes in SCr.⁵⁴

A special case of ischemia reperfusion injury-mediated AKI is seen in deceased organ transplant. Donors often experience kidney insults during their initial hospital admissions. Donors with AKI at the time of their hospital admission had higher NGAL, IL-18, KIM-1 and L-FABP in urine samples collected at organ procurement. However, only elevated NGAL was associated with increased risk of subsequent delayed graft function in the recipients. Higher NGAL and L-FABP also correlated with lower 6-month recipient eGFR, but this effect was only seen in those with delayed graft function.⁵⁶

While many biomarkers are associated with adverse long-term outcomes, biomarkers indicating adaptive repair and anti-inflammatory activities are emerging as markers of favorable long-term outcomes. A novel biomarker with strong preclinical data of being cytoprotective in kidney injury is YKL-40.³³ Kidney transplant recipients whose donors had the highest YKL-40 levels (>3.33 ng/ml) had 6.8 ml/min higher GFR at 6-months as compared with those whose donors had the lowest YKL-40 levels (<0.61 ng/ml) after controlling for donors and recipient factors, as well as degree of kidney injury.⁵⁷ Moreover, recipients with the highest donor YKL-40 levels also had 50% lower hazards of graft failure (HR 0.50 (0.27, 0.94)).⁵⁷ YKL-40 has not been studied in other setting of kidney injury and, if validated, may be a promising marker of adaptive repair after kidney injury. An anti-inflammatory marker, IL-10, was shown to be associated with 28% lower risk of 3-year mortality after AKI (HR 0.72 (0.56–0.93)).³⁷

c) Duration of AKI as a marker of long-term prognosis

AKI duration is an independent predictor of long-term outcomes. We examined 4,987 cardiac surgeries and found that as compared with patients without AKI, those with AKI had step-wise increase in long-term mortality with increasing duration of AKI [HR: 1–2 days, 1.66 (1.32–2.09); 3–6 days, 1.94 (1.51–2.49); 7 days, 3.40 (2.73–4.25)].⁵⁸ Similarly, in a study examining >35,000 VA patients, longer duration of AKI was associated with higher HR of death.⁵⁹ Another study of >32,000 patients admitted to the intensive care unit showed that duration of AKI was an independent risk factor for death or dialysis at 1-year regardless of the stage of AKI.⁵³

Duration of AKI, while associated with poor long-term outcomes, is challenging in real-world practice as duration of AKI is unknown at the time of AKI diagnosis. However, duration of AKI is also a potential therapeutic target as therapies to prevent extension of kidney injury and increase repair after an initial insult may be effective at reducing AKI duration. Several biomarkers showed ability to predict duration of AKI at diagnosis. For example, NGAL, IL-18, L-FABP, and KIM-1 obtained within 6 hours of kidney insult (and prior to rise in SCr) all showed significant association with duration of AKI.⁶⁰ Plasma MCP-1 measured before development of AKI was associated with longer duration of AKI.⁵⁵

d) Big data approach to determining prognosis

Given the ubiquity of electronic health records (EHR), their use to improve AKI care is an attractive option. Jannot et. al. used a modified phenome-wide association study (PheWAS) to describe a diagnosis-wide landscape of AKI. They evaluated ICD-10 diagnostic codes to develop a cluster of diagnoses associated with AKI. They noted that codes representing risk factors for AKI such as hemodynamic impairment and surgical procedures, as well as etiologies of AKI such as acute interstitial nephritis were associated with AKI. While not novel in the findings, the principle of using “big data” to identify unique phenotypes is an interesting approach and may be useful while evaluating large datasets.⁶¹ In the hopes of improving clinical care and avoiding iatrogenesis in patients with early AKI or those at-risk for developing AKI, Saly et. al. used big data approaches to predict AKI and developed prognostic models to predict short-term outcomes such as dialysis and death after an AKI event.⁶²

Future directions

AKI is common, is associated with adverse outcomes, and imposes a significant healthcare burden. Yet there is currently no AKI-specific therapy. This is despite significant investment in AKI research by the National Institute of Health (NIH), academic institutions, pharmaceutical companies, and other agencies.⁶³ In fact, in the absence of an ongoing phase III study for AKI treatment, there will be no AKI-specific therapy for another decade (www.clinicaltrials.gov).⁶⁴

A tissue-based study of human AKI is necessary to discover novel therapeutic targets. Human AKI is a complex disorder; it has diverse etiologies, clinical course, histopathological findings, and molecular pathways. Attempting to treat a complex disorder with a single drug is unlikely to be successful. A detailed study of human AKI biopsy tissue is needed to advance our understanding of differential activation of various molecular pathways causing AKI. Although these pathways may look histologically and clinically similar, they occur through different clinical insults, have differing prognoses and potentially differing therapies. Significant progress has been made in breast oncology using tissue-based phenotyping. Identification of tumor signatures via tissue-based research led to personalized care in the form of targeted chemotherapy and improved prognostication. The use of trastuzumab in human epidermal growth factor receptor 2 (HER2) overexpressing tumors led to improved tumor-free survival.⁶⁵ Genomic testing of tumors enabled patient risk-stratification.⁶⁶ A randomized trial found that a 70-gene tumor signature assay could help

identify patients at low risk of metastasis and avoid unnecessary chemotherapy.⁶⁷ Similar approaches in AKI using tissue-based research can identify molecular phenotypes and novel therapeutic targets of AKI.

A tissue-based approach will also lead to biomarker discovery to improve AKI phenotyping. Numerous AKI biomarkers have been tested in large cohorts over the last decade. Despite the desire to diagnose “kidney injury,” they were evaluated via comparisons with SCr as a supposed “gold standard.” As a result of this suboptimal comparison, the performance of the biomarkers has been disappointing. Evaluating these biomarkers using the real gold standard of kidney injury - biopsy - is needed to identify true biomarkers. Creation of a reliable, well-phenotyped kidney tissue atlas with corresponding biorepository of plasma and urine samples will significantly advance our ability to phenotype AKI. In addition to existing biomarkers, novel biomarkers of molecular pathways discovered from such tissue-based research can be tested and validated in such a biorepository. Moreover, biomarkers tied to drug mechanisms can phenotype AKI cases into appropriate drug targets in the hopes of advancing personalized medicine.

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Figure 1. Limitations of SCr-based AKI definition

SCr-based AKI definition may fail to identify individuals with structural kidney injury (“subclinical AKI”) or it incorrectly identifies individuals without structural kidney injury (“Hemodynamic AKI”). Even in individuals with structural kidney injury correctly identified by SCr-based AKI definition, it provides no information on AKI etiology, molecular pathways, therapy, or prognosis. ATI, acute tubular injury; AKI, acute kidney injury; SCr, serum creatinine; RAAS, renin angiotensin aldosterone system

Figure 2. Classification of biomarkers of AKI

SCr, serum creatinine; FENa, fractional excretion of sodium; KIM-1, kidney injury molecule-1; NGAL, neutrophil gelatinase-associated lipocalin; TIMP2, tissue inhibitor of metalloproteinases-2; IGFBP-7, insulin like growth factor binding protein-7; IL, interleukin; MCP-1, monocyte chemotactic protein 1

Figure 3. Subclinical ATI. In deceased donors without SCr-based AKI, NGAL levels were higher with increasing severity of ATI

NGAL, neutrophil gelatinase-associated lipocalin; ATI, acute tubular injury

Figure 4. Observed relative risk approaches null due to accidental enrolment of PRA patients in a trial of drug with true relative risk of 0.7 in ATI patients

Assuming 20% event rate in control and 5% event rate in PRA. Adapted from Parikh et. al. Kidney Int 2016.

ATI, acute tubular injury; PRA, pre-renal azotemia

Table 1

Distinguishing PRA, HRS, ATI using traditional and novel biomarkers

	Urine NGAL (ng/ml)	Urine IL-18 (pg/ml)	FENa (%)	Urine Albumin (mg/g)
PRA	↓↓ 54 (17–180)	↓↓ 15 (15–49)	↓ 0.27 (0.13–0.58)	↓↓ 21 (4–70)
HRS	↓ 115 (51–373)	↓ 37 (15–90)	↓↓ 0.1 (0.02–0.23)	↓↓ 24 (13–129)
ATI	↑↑ 565 (76–1000)	↑↑ 124 (15–325)	↓ 0.31 (0.12–0.65)	↑↑ 92 (44–253)

PRA, pre-renal azotemia; HRS, hepatorenal syndrome; ATI, acute tubular injury; NGAL, neutrophil gelatinase-associated lipocalin; IL-18, interleukin 18; FENa, Fractional excretion of sodium