

Clearance and beyond: the complementary roles of GFR measurement and injury biomarkers in acute kidney injury (AKI)

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Endre ZH, Pickering JW, Walker RJ. Clearance and beyond: the complementary roles of GFR measurement and injury biomarkers in acute kidney injury (AKI). *Am J Physiol Renal Physiol* 301: F697–F707, 2011. First published July 13, 2011; doi:10.1152/ajprenal.00448.2010.—Acute kidney injury (AKI) is a common and frequently fatal illness in critically ill patients. The reliance on daily measurements of serum creatinine as a surrogate of glomerular filtration rate (GFR) not only delays diagnosis and development of successful therapies but also hinders insight into the pathophysiology of human AKI. Measurement of GFR under non-steady-state conditions remains an elusive gold standard against which biomarkers of renal injury need to be judged. Approaches to the rapid (near real-time) measurement of GFR are explored. Even if real-time GFR was available, absent baseline information will always limit diagnosis of AKI based on GFR or serum creatinine to a detection of change. Biomarkers of renal cellular injury have provided new strategies to facilitate detection and early intervention in AKI. However, the diagnostic and predictive performance of urinary biomarkers of injury vary, depending on both the time after renal injury and on the preinjury GFR. Progress in understanding the role of each novel biomarker in the causal pathways of AKI promises to enhance their diagnostic potential. We predict that combining rapid measures of GFR with biomarkers of renal injury will yield substantive progress in the treatment of AKI.

acute renal failure; biomarkers; creatinine clearance; glomerular filtration rate

ACUTE KIDNEY INJURY (AKI) is a common and frequently fatal syndrome. The incidence varies according to definition, ranging from 4 to 20% of hospitalized patients (76, 117) and from 35 to 70% in critically ill patients in the intensive care unit (ICU) (2, 24, 57, 58, 102). In the 4–5% of ICU patients with severe AKI requiring renal replacement therapy (RRT), hospital mortality rates are typically ~50–60% (8, 58, 79, 117). Even mild severity grades and transient AKI are associated with increased in-hospital mortality (20, 115, 116).

AKI has many different etiologies. Many are associated with renal hypoperfusion or ischemia, for example, AKI-complicating blood loss, cardiogenic shock, abdominal surgery or after renal transplantation (1). In contrast, AKI-complicating sepsis may be accompanied by systemic hypotension and intrarenal vasoconstriction (106) or with a hyperdynamic circulation accompanied by renal hyperemia and intrarenal vasodilatation (22, 60, 121) without a significant fall in blood pressure (50). Direct nephrotoxic renal injury causing AKI represents a further etiology with multiple mechanisms and differing preferred renal cellular targets of injury (104). Heterogeneity of etiology obscures any common pathway of injury and highlights that the time frame over which AKI develops will vary widely between

etiologies. Thus detection of AKI is complicated by heterogeneity of etiology and onset and further complicated by heterogeneity of disease severity and comorbidity.

The Rise and Fall of Creatinine as a Surrogate for GFR

Using the increase in “serum” creatinine (usually measured in plasma) to define AKI is a logical extension of the use of creatinine clearance to estimate GFR, a technique introduced more than 80 years ago (101). Creatinine is formed nonenzymatically from creatine in muscle, has a molecular weight of 113.12 Da, is freely filtered at the glomerulus, and is completely cleared by renal excretion when renal function is normal. The proximal tubules secrete creatinine, which accounts for 10–20% of the excreted load, and results in overestimation of GFR when measured by creatinine clearance (89, 107). The contribution of tubular creatinine secretion to clearance may reach 50%, when GFR is reduced, but is highly variable among individuals (89). In contrast, the tubules reabsorb creatinine in some clinical settings such as decompensated heart failure and uncontrolled diabetes (68, 89).

The current consensus definition (RIFLE: risk, injury, failure, loss, end-stage) of AKI requires at least a 33% decline in GFR resulting in at least a 50% increase in plasma creatinine, although a 25% increase is traditionally accepted for diagnosis of contrast-induced AKI (9, 37, 92). As serum creatinine increases slowly in response to a single-step alteration in GFR,

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a new steady-state creatinine-based estimate of GFR will take from 24 to 72 h (3–5 times the new creatinine half-life, which increases from 4 h at normal GFR by the same extent that GFR declines). Obviously, the extent of increase in creatinine, which is determined by the extent of initial decrease in GFR and creatinine production, may allow diagnosis of AKI before the time needed to reach steady state (Fig. 1). However, the limited precision of creatinine measurement and the extent of inpatient variation mean that a minimum 10% change in creatinine has traditionally been required by clinicians to demonstrate measurably significant change. In addition, in the ICU fluid loading may dilute creatinine concentrations, thereby further delaying diagnosis (69). Since there is an inverse relationship between serum creatinine and GFR, it is easy to forget that a small increase in serum creatinine represents a substantial decline in GFR. Even if an increase is observed, the delay required for diagnosis creates uncertainty regarding the timing of the renal insult leading to AKI. While a 33% step decreasing GFR could lead to a creatinine-based diagnosis of AKI within 10 h (Fig. 1), measurement uncertainty, lack of frequent measurement, and a host of confounding influences on the rate of creatinine increases preclude definitive early diagnosis. Furthermore, the clinical scenario is often not a simple or single GFR step decrease resulting in a predictable increase in creatinine but may involve a slow incremental decline in GFR, including periods of recovery (73). The poorly understood paradigm of reversible “prerenal” AKI further delays accurate

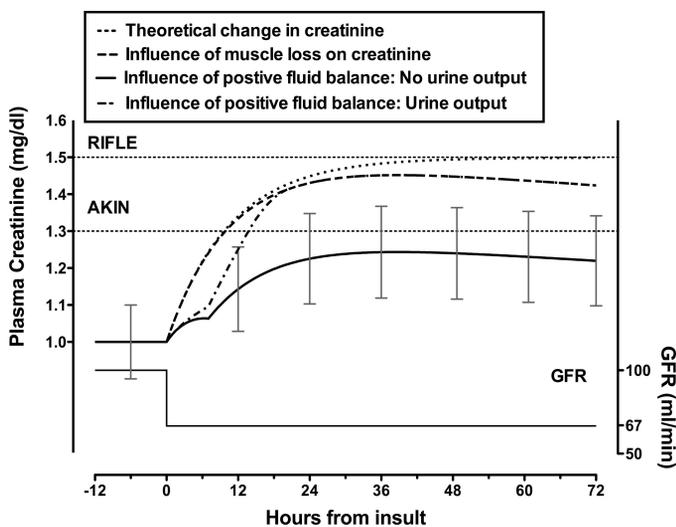


Fig. 1. Delay in diagnosis of acute kidney injury (AKI) following a decrease of glomerular filtration rate (GFR) by 33% at *time 0* in a 70-kg subject with baseline creatinine of 1 mg/dl. Theoretically, creatinine should asymptote toward 1.5 mg/dl (dotted curve). Creatinine increases by >0.3 mg/dl within ~ 10 h allowing for diagnosis of AKI by the Acute Kidney Injury Network (AKIN) criteria although the severity of the insult would only be known after ~ 36 –48 h. In practice, muscle loss, fluid loading, and measurement error delay detection of the real increase in creatinine and further delay early investigation and intervention. In this scenario, the patient received intensive fluid therapy over the first 7 h, resulting in an initial positive fluid balance of 7 liters. Muscle mass was assumed to decrease at 2%/day (dashed curve). The solid line represents the achieved plasma creatinine if this positive balance is maintained in the presence of anuria. The achieved plasma creatinine would only exceed the 0.3 mg/dl boundary in the presence of laboratory error (a 10% error in creatinine measurement is shown by the error bars). If the excess fluid was excreted within 12 h (beginning from initiation of fluid therapy), the diagnosis would be delayed but not prevented (dot-dashed line). RIFLE: risk, injury, failure, loss, end-stage.

diagnosis of AKI although there is no information on whether renal injury is evolving, possibly transiently, during this phase of AKI. This uncertainty in diagnosis represents a lost opportunity to investigate and identify the underlying pathophysiology of AKI in humans. The recognized imprecision in determining change in GFR and the importance of a small absolute change in creatinine led the Acute Kidney Injury Network (AKIN) to remove GFR from the consensus classification of AKI and include a small increase in creatinine (≥ 0.3 mg/dl) as sufficient to diagnose AKI. Oliguria remains in the definition as an alternative to creatinine change (71) although oliguria alone is not a validated marker of AKI (cf. Ref. 110).

An alternative surrogate to plasma creatinine is plasma cystatin C. With one-third the volume of distribution of creatinine, this freely filtered endogenous extracellular marker will reach a new steady state three times more rapidly than creatinine (15) and is often proposed as a replacement for creatinine (29, 111). Consequently, increases in plasma cystatin C precede plasma creatinine increases following contrast injury and other forms of AKI (15, 54, 78). Herget-Rosenthal et al. (54) in a grouped analysis reported a 50% increase in cystatin C preceded that of creatinine by 1.5 days (54). While this study relied on blood samples taken daily, that of Nejat et al. (78), where sampling was performed every 12 h on the first day, observed only a 5.8-h difference in a paired analysis. Since changes in cystatin C should occur more rapidly than 24 h, further studies with more frequent sampling, e.g., 6 hourly, are needed to validate the temporal profile of cystatin C vs. creatinine. Cystatin C is less influenced by variables that affect creatinine, including age, diet, and muscle mass (6, 66, 113) although glucocorticoids, thyroid status, and cancer may influence baseline status (43, 103, 123). While the production rate of cystatin C appears constant in non-acutely ill patients (109), the affect of acute illnesses on production rate has not been determined. There is evidence of substantial nonrenal clearance of cystatin C (~ 21 ml \cdot min $^{-1}$ \cdot 1.73 m $^{-2}$) (109) that suggests that extrapolating from cystatin C overestimates true GFR. The limitations and delays caused by fluid loading discussed above in relation to creatinine will also affect other plasma biomarkers including cystatin C. The more complex assay methods, the lack of widespread availability, and high costs remain impediments to acceptance of cystatin C as a substitute for creatinine. Limited data on the affects of ethnicity and age remain additional stumbling blocks. Nevertheless, the advantages of cystatin C over creatinine remain, and we support further application in clinical trials.

While there is a great deal of information about a large number of cellular (tubular and endothelial) events and the autonomic, inflammatory, and renal vascular responses in experimental ischemia-reperfusion injury (28, 50), there is little corroborative and time-relevant clinical pathophysiological data. Usually, the clinical diagnosis of ischemic AKI is a diagnosis of exclusion. Thus the potential delay imposed by reliance on creatinine is further delayed by investigations (e.g., exclusion of urinary outflow obstruction) or interventions (e.g., fluid loading to treat or exclude underlying “prerenal” AKI); these are designed to exclude other causes rather than confirm the diagnosis of ischemic AKI. Evidence of preserved tubular transport function in the presence of an increased serum creatinine suggests potential reversibility. A low fractional excretion of sodium (FE_{Na}) and/or urea (FE_{urea}) (19, 87) are often

used to define prerenal AKI, although diuretics and sepsis make interpretation of the FE_{Na} difficult (7) and aging and sepsis alter FE_{urea} (31, 75). While muddy brown or epithelial cell casts, renal tubular cells, and trace hematuria and pyuria are common in both septic and nonseptic AKI, and their presence in patients with an intermediate pretest probability of AKI is potentially helpful (88), these biomarkers are neither definitive of a type of AKI, nor ubiquitous in AKI (see Ref. 4). In fact, the time evolution of most traditional AKI biomarkers (like FE_{Na} and microscopy) is poorly documented; their use is further confounded by the fact that they sometimes influence the diagnosis of AKI used in studies, e.g., an $FE_{Na} > 2\%$ as the criterion for ATN (4), although there is little correlation with renal histopathology (96). Other investigations such as measurement of global or parenchymal renal blood flow, or renal biopsy, which might provide insight into human AKI pathophysiology are delayed (often for weeks), are difficult to interpret in the absence of baseline data, and are, in any case, rarely performed.

Since diagnosis is delayed, it is not surprising that there has been failure of pharmacological intervention in clinical trials, which are largely based on experimental interventions to prevent rather than treat AKI (59). The failure to translate apparently effective pharmacological preventive measures in animal models into clinical practice suggests that the delay in diagnosis because of reliance on daily measurements of serum creatinine merely complements a lack of fundamental understanding of renal pathophysiology in human AKI. Unsurprisingly, then, pharmacological prevention of AKI has also largely failed, even when an apparent etiology is known, e.g., following parenteral administration of iodinated radiocontrast, and treatment is administered before injury (42, 80, 126). Failure of prevention must therefore reflect our incomplete understanding of clinical AKI. Various factors have contributed to this knowledge deficit, including inadequate or unrepresentative experimental models (56). Trial design is also a critical factor in the failure of some intervention and prevention trials. Most rely on changes in creatinine as an outcome, yet few measure creatinine for the 7 days required to establish AKI according to the RIFLE definition (37). Many trials are underpowered because the incidence of AKI is low, and patient numbers are insufficient. Patient heterogeneity increases the effective underpowering, with potential mismatching of severity and treatment particularly in the presence of the underlying chronic kidney disease (CKD) that presents in 30–50% of AKI (5, 39), and difficulty in accounting for the effect of patient comorbidity on the sensitivity to and course of AKI.

We need to explore the early stages of loss of renal function in humans to understand the relevant pathophysiology of clinical AKI. We can conceptualize this as an evaluation of the evolution of the injury phase which leads to loss of GFR and the early time window after GFR decrease (Fig. 2). Detection and characterization of this very early phase in humans appear essential for progress and highlight the need for rapid assessment of renal function. In particular, we need to consider methods for assessing GFR under non-steady-state conditions, rapid methods of measuring GFR, and how biomarkers of renal status other than creatinine can be utilized to assess AKI. Current evaluation of novel biomarkers of renal injury is largely confined to diagnosis or prediction of serum creatinine-

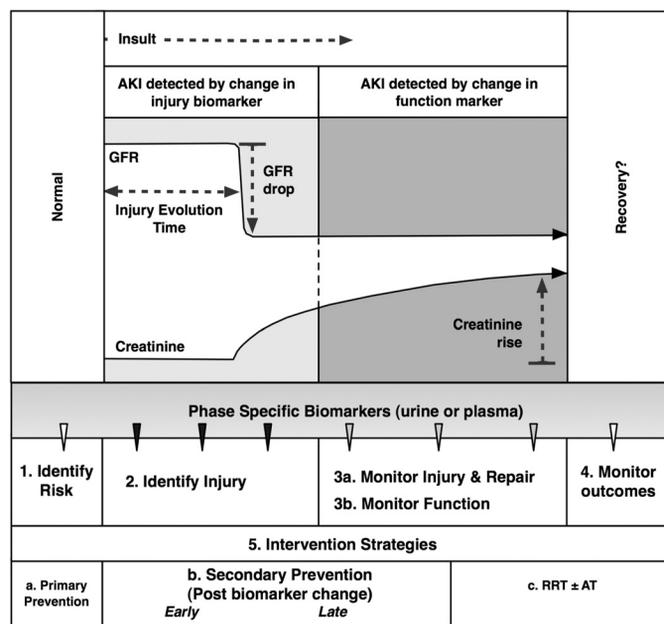


Fig. 2. Paradigm of secondary prevention (from Ref. 93, used with permission).

based categorical definitions of AKI. We now need to correlate these biomarkers with the pathophysiology and phase of injury.

Rapid Determination of GFR in AKI: Demands of Non-Steady-State Conditions

There are no true real-time, i.e., instantaneous, techniques for determining GFR. Some clearance techniques can approximate this (see the radionuclide and fluorescence marker techniques described below). However, conventional clearance methods usually require at least 4 h.

Clearance methods of determining GFR under steady-state conditions (constant production and excretion) quantify urinary excretion of a marker that is ideally excreted solely by glomerular filtration. With constant infusion, or a constant endogenous production rate, the steady-state plasma concentration of the marker is determined by GFR. Marker molecules used include endogenous creatinine (although there is tubular secretion of creatinine) and cystatin C, and infused molecules such as inulin or its highly water soluble analog sinistrin (18), iohalamate, iohexol, and radioactive markers [^{125}I]iothalamate, ^{99m}Tc -DTPA, ^{51}Cr -EDTA (14, 97). In clinical practice, inulin and sinistrin clearance (including fluorescein-labeled forms) (95) are reserved for research studies. Radioactive marker clearances in some jurisdictions, for example, Australia and New Zealand, have become the clinical gold standard, although the burden of safety and regulatory procedures deters many clinicians from using radioactive markers in other jurisdictions such as the United States.

Methods to determine GFR under non-steady-state conditions are based on the elimination kinetics of the marker from plasma after a single bolus injection (14, 97). There is a good correspondence between total ^{51}Cr -EDTA plasma clearance following a single injection as developed by Noslin (81) and renal inulin clearance measured simultaneously by the sustained infusion method (17). The rapid increase in the plasma concentration (the first compartment) of a filtration marker

(tracer) injected intravenously is followed by a decrease usually characterized as two or more exponentials: an early rapid decline followed by a late slow phase. The early decline is assumed to represent both distribution into other compartments (especially interstitium) and renal excretion, while the late phase represents renal clearance alone and assumes equilibration between the interstitial and plasma compartments. While total clearance may overestimate GFR, the rate constant, k , of the terminal phase is an accurate measure of GFR scaled to the extracellular volume; k is easily determined from two or more plasma samples collected between 120 and 360 min after injection. Single-sample methods based on the plasma concentration at 3 or 4 h postinjection have also entered clinical practice (90). Direct measurement of serum or plasma clearance (without urine collection) of radionuclides such as ^{99m}Tc -DTPA, [^{125}I]iothalamate, ^{51}Cr -EDTA, ^{99m}Tc -MAG3 are established single-injection techniques validated against inulin clearance. These methods rely on all compartments being in steady state during the collection interval.

The conventional 24-h creatinine clearance using endogenous creatinine can provide an adequate approximation for clinical purposes under steady-state conditions (10), especially if tubular creatinine secretion is allowed for or inhibited, e.g., by prior treatment with cimetidine (51, 52, 63, 112). This is not a useful time frame for detecting AKI. Multiple strategies have been used to obtain rapid estimates of GFR. Brief (2–8 h) collection studies have shown that 2-h collections are closely correlated with 24-h clearance measurements (55), reproducible (53), and superior to Cockcroft-Gault or eGFR (55). While well-performed studies are few and limited to single centers, the practicality and apparent reliability of 2-h clearance measurements have resulted in brief clearance methods being adopted into practice in some centers. Reliability was not affected by variation in urine flow rates (55). Collections for less than 2 h are of uncertain value, suggesting that the 2-h collection for clearance may be a practical lower limit for urine collection requiring clearance methods. Our own experience of up to nine 4-h collection periods for clearance over 7 days from 528 patients at ICUs in two centers demonstrated that the method was practical, with an error rate $<3\%$ from failure to determine a measured clearance (39) and better at detecting change in GFR than plasma creatinine (Pickering JW, Endre ZH, unpublished observations). Thus, although clearance of endogenous creatinine requires the assumption of steady state, the lack of measurable change in serum creatinine over this time frame suggests that a 2- to 4-h measured clearance is a useful approximation of the GFR.

Brief-duration measurements of GFR have also been developed which use the single-compartment plasma clearance method described by Bröchner-Mortenson (16) and do not require urine collection. By using a shielded detector attached to a patient's arm (an ambulatory renal monitor; ARM), Rabito et al. (100) were able to monitor extracellular excretion of a radionuclide attached to a marker cleared exclusively by glomerular filtration, ^{99m}Tc DTPA, for 12–24 h after a single injection. Radionuclide activity and the slope of the activity-time relationship were determined every 15 s from the preceding 5 min of activity; a linear relationship between log activity (corrected for decay) and time was consistent with clearance from a single-compartment system. Slope was related to urine output, e.g., it was zero in anuric patients and increased before

recovery of urine output. A decline in slope (activity) could be measured within 5–10 min in patients with normal or moderately decreased renal function or 10–15 min in patients with a GFR <30 ml/min. This change preceded or occurred simultaneously with a decline in urine output and changes in plasma creatinine or short creatinine clearance studies in most but not all cases. In some, this allowed both timing and monitoring of the response to successful intervention. Only rarely was a transient decline in slope followed by a decline in creatinine clearance. These human observations were ratified in a primate study which demonstrated that large reductions in GFR due to ischemia or ureteral obstruction were detected within minutes and that recovery was also predicted by changes in the rate constant well in advance of changes in serum creatinine (49). A recent follow-up study of 50 patients with varying degrees of renal function showed a correlation of $r^2 = 0.97$ with [^{125}I]iothalamate clearance which was subsequently validated in 80 prospective kidney donors. The ARM measurement protocol to determine GFR took 15 min and had a coefficient of variation of 6.9% (99). The current monitor records data, which need to be downloaded to make calculations, increasing the time to determine GFR to 30 min. However, at least theoretically, if the monitor was coupled directly to a computer during the measurement process (as in earlier experiments) (100), the only barrier to monitoring GFR in near real-time is the rate of sampling (every 10–15 s) and computer processing time. This monitor is now being readied for commercialization.

Magnetic resonance imaging (MRI) can be used to measure GFR directly and in as little as 4 min following injection of gadolinium-chelate contrast agents (e.g., Refs. 44 and 61). Unfortunately, the development of nephrogenic systemic fibrosis in a small number of patients with a GFR <15 ml/min, including some with AKI or recent transplant dysfunction (e.g., Ref. 35), has highlighted a risk associated with Gd-contrast administrations in high-risk patients, namely, those with severe renal insufficiency, including those with AKI, where GFR is <15 ml/min. Development of safer contrast agents with exact molecular sizing and structure such as dendrimers (e.g., Ref. 25) may facilitate GFR measurement. However, the physical difficulty of imaging critically ill patients in the bore of a high-field magnet creates additional barriers to the use of whole-body MRI in detecting AKI.

Alternative strategies using fluorescent markers have been tried in experimental animals. Fluorescence of a single injected marker has been used to determine GFR (21, 32, 98). As some of the fluorescent markers are chelates of europium with DTPA (e.g., Refs. 21, 32, and 98), some caution will be required in clinical use of these in AKI, since free lanthanides are nephrotoxic (36) and may also rarely cause nephrogenic systemic fibrosis in subjects where GFR is low (35), including patients with AKI. Yu et al. (125) developed a ratiometric approach, which involved using intravital two-photon microscopy to monitor the ratio in renal capillaries of a filterable to that of a nonfilterable fluorescent marker following simultaneous intravenous injection of both. Since the low molecular weight (MW; FITC-inulin, 3–5 kDa) and the high MW (500 kDa, Texas red-dextran) fluoresce yellow and red, respectively, intensity maps of the capillaries changed from green to red over a period of 244 s with filtration of the low MW marker and retention of the high MW marker. By examining plasma clearance under various conditions, including uni- and bilateral

nephrectomy, the rate constant for extrarenal clearance due to nonspecific tissue distribution was determined and the overall strategy for determining GFR was validated (125). The investigators also demonstrated that imaging blood vessels in skin (rodent genitalia was chosen to eliminate problems with hair fluorescence and need for skin flaps) yielded the same values for clearance. This technique has recently been enhanced by a two-compartment model (122). However, the simple elegance, reduced noise, and greater accuracy obtained by comparing the elimination kinetics of a filtered and retained marker may also be a disadvantage of the technique, since repetition is presumably restricted until the high MW marker is cleared by extrarenal pathways (days). In contrast, the transcutaneous measurement of skin (extravascular) fluorescence after bolus injection of FITC-sinistrin (developed to overcome the poor water solubility of FITC-inulin) using a small-animal imager, allowed 5-min images to be collected up to 120 min, and determination of the GFR from exponential fitting to the concentration time curve between 45 min and 120 min using a single-compartment model (105). Overestimation of GFR results from using the single-compartment method (see Ref. 16) and needs to be corrected for against a renal clearance technique based on blood and urine sampling. However, the established safety profile of fluorescein and sinistrin and repeatability of the technique (albeit after 2 or more hours) suggest potential clinical utility. Fluorescence techniques could be advantageously applied to humans, and results of human trials are eagerly awaited.

The rapidity with which clearance can be assessed by the various proposed methods depends on factors including marker clearance rate, the precision desired, the accuracy of tracer concentration measurements, the assumed distribution and mixing of the tracer in body fluid compartments, potential changes in the volume of these compartments, and time required for measurement and analysis of tracer concentrations. We propose that clinically useful measurements should be repeatable, at hourly or shorter intervals. Following the initial setup, the ability of both the ARM method and some fluorescent studies to monitor renal function every 5–15 min is rapid enough to be clinically significant. However, the precision with which these techniques can monitor small changes in GFR is yet to be determined. For example, the minimum percent change in GFR detectable and the minimum time following change in which detection can occur are unknown. A clinically significant time may be <1 h. Given that even a small change in creatinine is a significant risk factor, a relatively small change in GFR (say 10–20%) may also be significant. We suggest that for these techniques to be clinically useful they would need to meet these or similar goals. The accuracy to which the tracer concentrations can be measured, the influence of physiological changes over the period of measure, as well as the time required for measurement will all need to be determined in further studies. Furthermore, for the ARM to be accepted in routine clinical use, safety issues around the routine use of a radioactive marker in settings such as the ICU would need to be addressed, and the clinical benefit of a rapid GFR measurement would need to be shown to outweigh any additional risks to either patients or staff.

Other Renal Transport Functions and Clearance

Since the kidney plays a major role in the excretion of many drugs and/or their metabolites, the clearance of therapeutic agents may be used as an alternative to creatinine. Renally excreted drugs such as gentamicin have shorter half-lives than creatinine; measurement of drug clearance may more accurately reflect early changes in renal function. In a large retrospective analysis of gentamicin dosing, serum creatinine concentration changed up to 6–8 days after a significant change in gentamicin concentration (64). A 20% or greater decrease in gentamicin clearance had a specificity, exceeding 93% in predicting a 20% decrease in creatinine clearance. Changes in gentamicin clearance been used as a predictor of gentamicin-induced nephrotoxicity (64).

It has been suggested that to assess renal pharmacokinetics accurately, the different functions of the nephron need to be assessed with appropriate markers of GFR, renal plasma flow rate, tubular anion and cation secretion, and tubular reabsorption (114). Gross and colleagues (46) demonstrated that a cocktail of agents, sinistrin (for GFR), *p*-aminohippuric acid (for renal plasma flow and tubular anion secretion), pindolol (for cation secretion), and fluconazole (tubular reabsorption), can be used to investigate pathways of renal drug elimination. The extent to which these can be used in renal impairment or under conditions of changing renal function awaits further evaluation. It is interesting to speculate how the changes in tubular function as measured with the above cocktail would correlate with changes in urinary biomarkers in the setting of AKI. It appears unlikely that these would result in real-time data, although some may be superior to endogenous creatinine clearance. Another potential limitation is that some of the agents, e.g., pindolol (65), may alter renal blood flow.

Beyond Clearance: Detecting Injury with Novel Biomarkers

While consensus definitions of AKI and AKI severity have facilitated comparative research and epidemiological studies (9, 37, 71) and highlighted the adverse impact (mortality) of even small increases in serum creatinine (3, 20, 67, 102, 116), the diagnostic delay after renal injury demonstrates the limited utility of creatinine-based methods to monitor change or time intervention (Fig. 2). This has stimulated the search for alternative biomarkers. Many studies have now confirmed the diagnostic and predictive value of novel urinary biomarkers such as kidney injury molecule 1 (KIM-1) and IL-18 (48, 84), and urinary and plasma neutrophil gelatinase-associated lipocalin (NGAL) has achieved meta-analysis status (47). Eventually, a panel of biomarkers should be available to allow timing of injury and facilitate early intervention or stage-specific intervention (34). There have been several comprehensive reviews of biomarkers, covering pathophysiology (118), potential for early diagnosis of AKI (23) and nephrotoxic AKI (23, 41, 118). These have concentrated on NGAL, IL-18, KIM-1, lipid fatty acid binding protein (L-FABP) and cystatin C (26, 40, 85). In addition, there is a comprehensive treatise, which systematically reviews these and other biomarkers of kidney injury (33). Apart from highlighting recent significant advances, we will not repeat the work of these reviewers, but rather concentrate on the problems and potential solutions involved in bringing novel AKI biomarkers to utility.

Redefining the Structure-Function Relationship Using Novel Biomarkers

The change in nomenclature from “acute renal failure” to “acute kidney injury” reflects growing awareness that it is not recognition of renal filtration failure but of prior renal cellular injury that is critical for timely diagnosis and intervention. The term “secondary prevention” has been introduced to highlight that intervention should ideally be introduced before loss of GFR (93). AKI is often compared with myocardial ischemic injury to highlight how delay in diagnosis and intervention is inherent if change in organ function is used to detect injury (e.g., Ref. 34). However, unlike myocardial ischemic injury, AKI is a syndrome not a specific pathological diagnosis and has diverse etiologies (28). This diversity suggests that cellular pathways to filtration failure differ and that successful intervention (secondary prevention) is likely to be disease and/or stage specific (Fig. 2) and may require a multifaceted approach. The use of biomarkers of injury for early detection of AKI should facilitate insight into the pathophysiology of the human syndrome and allow stage-specific intervention.

We will discuss 1) how these biomarkers have been assessed in relation to changes in GFR, which has helped determination of the boundaries of biomarker utility in terms of timing, baseline renal function, and comorbidities; 2) how the absence of a quality gold standard GFR is hindering the interpretation and development of these biomarkers; and 3) the new knowledge being gained concerning the biomarker position and role in the causal pathways leading to AKI and, after AKI, to other outcomes including dialysis, death or CKD.

Biomarker performance: time after injury, baseline GFR, and comorbidity. Biomarker performance varies with time after injury. This is intuitive for biomarkers upregulated after injury, e.g., KIM-1 (119). Analysis of prospectively collected samples has profiled the time course of biomarker increase in patients developing AKI after cardiopulmonary bypass, i.e., for KIM-1, IL-18, and NGAL (48, 72, 84). The profile of several tubular enzymes has been documented in the past (e.g., Ref. 62) for lactate dehydrogenase (LDH) and more recently for γ -glutamyl transpeptidase (GGT) and alkaline phosphatase (39) and *n*-acetyl glucosaminidase (48). Urinary biomarkers can be broadly classified as “performed” in some cellular location typified by the tubular enzymes, or “induced” [i.e., upregulated by some aspect of the injury stress response, e.g., from tubular epithelial cells during dedifferentiation-proliferation-recovery (104), e.g., for KIM-1 (48), or induced in resident or recruited leucocytes, e.g., IL-18 from macrophages (86)]. Performed urinary AKI biomarkers appear immediately in the urine after direct tubular injury (e.g., from brush border in the case of GGT or cytosol in the case of LDH) or because of failure of absorption from the tubular fluid because of injury or competition for transport (e.g., inhibition of megalin-mediated transport in the case of albumin and cystatin C) (77) (Fig. 3).

Biomarker performance also varies with baseline renal function. In a cohort of patients following cardiopulmonary bypass, McIlroy et al. (70) showed that urine NGAL was only predictive of AKI for patients with normal renal function before surgery. Urine NGAL is increased in patients with CKD and predicts progression (11); a high background NGAL may explain why this did not increase in patients with AKI developing in the presence of CKD. We recently confirmed these

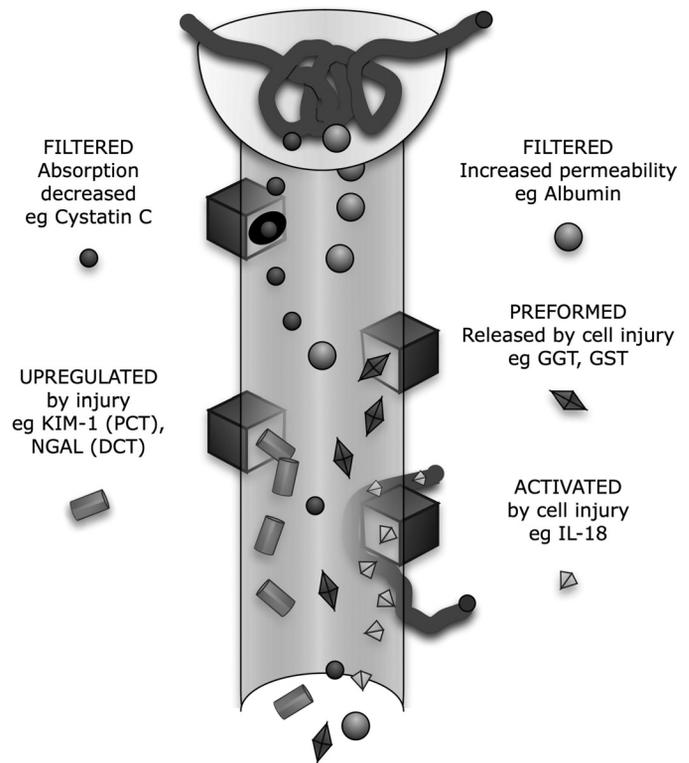


Fig. 3. Biomarker sources. Shown is a schematic of a glomerulus and tubule. PCT, proximal convoluted tubule; DCT, distal convoluted tubule; KIM-1, kidney injury molecule 1; NGAL, neutrophil gelatinase-associated lipocalin; GGT, γ -glutamyl transpeptidase; GST, glutathione *S*-transferase.

observations in a comparison of the performance of 6 novel urinary biomarkers in 529 ICU patients (38). Comparisons were made using the area under the receiver operator characteristic curve (AUC) for diagnosis or prediction of AKI and reassessed after patient stratification by baseline renal function (eGFR) and time after renal insult. All urinary biomarkers performed poorly in this heterogeneous population. When the population was stratified for baseline renal function, all biomarkers still performed relatively poorly early after injury if baseline GFR was <60 ml/min. For example, cystatin C and KIM-1 had AUCs of 0.69 and 0.73, respectively, within 6 h of injury, but between 12 and 36 h cystatin C (0.88), NGAL (0.85), and IL-18 (0.94) had utility. With eGFR >60 ml/min, GGT (0.73), cystatin C (0.68), and NGAL (0.68) had the highest AUCs within 6 h of injury, and between 6 and 12 h all AUCs except alkaline phosphatase were between 0.68 and 0.78. Beyond 12 h, NGAL (0.71) and KIM-1 (0.66) performed best. Thus both duration of injury and baseline renal function must be considered in evaluating biomarker performance to diagnose AKI (38).

Biomarker performance: assessment in the absence of a gold standard. In typical clinical studies of AKI, biomarker performance is judged against creatinine-based definitions of AKI, usually either the RIFLE (9) or AKIN (71) consensus definitions, although other categorical (120) and continuous variables have been proposed (94). For historical reasons, AKI caused by iodinated contrast agents (contrast-induced AKI) has been defined by a different absolute and relative change in creatinine than AKI for other causes; we have suggested that this anomaly be addressed (37). The R, I, and F stages of

RIFLE and comparable *stages 1–3* of AKIN are increasingly used to define AKI severity (37); however, these categorical variables do not have a linear relationship to severity and are therefore less reliable than continuous variables in defining AKI outcome (94). Furthermore, both categorical and continuous variables based on creatinine are critically dependent on an accurate estimate of the patient's baseline creatinine before renal injury.

Baseline creatinine is available in only ~50% of critically ill patients (39). Use of the Modified Diet in Renal Disease formula (assuming a GFR of 100 or 75 ml/min) to back-calculate missing baseline creatinines was suggested (9). Unfortunately, this strategy overestimates the incidence and severity of AKI in critically ill populations (5, 91, 108). For biomarker performance studies where AKI can be determined retrospectively, a better approach is to estimate baseline creatinine from the final or lowest hospital creatinine (91). Nevertheless, none of these approaches is useful in patient management.

Most biomarker studies are too small to provide a realistic assessment of biomarker performance for adverse outcomes consequent to AKI such as dialysis, length of hospital stay, death, or development of CKD. Nevertheless, reporting of such sequelae and adverse events on all trials will enable later meta-analysis.

In experimental nephrotoxic injury, the accepted gold standard is renal histology (13, 119). After meticulous studies involving multiple international centers and major pharmaceutical companies, the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have accepted and ranked multiple rodent urinary and plasma biomarkers as surrogates for renal histology and accepted these for both preliminary evaluation of nephrotoxicity in drug development and for adjunctive monitoring of nephrotoxicity in humans in postmarketing surveillance (13, 30, 82, 119).

These novel biomarkers are designed to measure renal cellular injury, not physiological function. Even induced biomarkers reflect changes occurring soon after injury. Without a gold standard that reflects near real-time change in GFR, there will always be doubt concerning the relationship between injury and functional change. Thus an immediate outcome of a rapidly measurable and repeatable real-time GFR method will be an ability to link changes in GFR to renal cellular events. In the meantime, we recommend that studies be conducted which utilize short (2–4 h) creatinine clearance in conjunction with injury biomarker measurement to assist in temporally linking cellular injury to functional changes.

Biomarker role in causal pathways. Establishing biomarker utility is more difficult than simply discovering an association with AKI or its sequelae. For a biomarker to be a valid surrogate of AKI, the role of the biomarker in the disease process must be understood. Statistical correlation alone cannot establish a cause-effect relationship. Thus ideal biomarkers should be both timely and related to the causal pathway leading to or from renal injury. It is important to understand why the biomarker signifies loss of renal physiological function, for example (but not confined) to loss of GFR or other outcomes that identify the causal pathway to renal failure-induced mortality or other major adverse AKI-dependent outcomes. That is, “the strong foundation provided by detailed understanding of the sensitivity and specificity of a biomarker in various con-

texts of injury is . . . critical to its appropriate use in animals and/or humans” (13).

KIM-1, NGAL, and IL-18 are good examples of novel biomarkers where the role of the biomarker in the causal pathway is at least partly understood. For example, the ectoprotein KIM-1 is highly overexpressed in dedifferentiated proximal tubule cells after ischemic or nephrotoxic AKI and functions as a phosphatidyl serine receptor which allows surviving epithelial cells to recognize and scavenge apoptotic cells (12). It also recognizes oxidized lipoproteins. NGAL is a siderophore which chelates free iron released after cell injury, and in excess NGAL can protect against ischemic injury (27, 74). IL-18 is the product of caspase-1 activation and mediates ongoing injury/inflammation (86, 124). Since cytokines induced by renal injury appear to mediate lung injury, it becomes logical to accept that an increase in IL-18 cannot only predict AKI but also mortality associated with acute respiratory distress syndrome (84). In general, preformed biomarkers, such as the brush-border enzymes GGT, alkaline phosphatase, and the glutathione *S*-transferases, appear to reflect an early phase of injury leading to brush-border and other cellular damage or remodeling. These therefore appear to be useful for timing early injury. While induced biomarkers reflect a potentially later cellular response to injury and highlight processes of repair, these may also appear very rapidly in urine or plasma. For example, 2 h after completion of cardiopulmonary bypass, urinary and plasma NGAL are predictive of subsequent creatinine-based AKI (72).

Involvement of the biomarkers in post-AKI sequelae is a growing area of research. For a biomarker to be a surrogate for this linkage requires that its place in the pathway is defined. A growing body of evidence suggests that the deleterious effects of AKI on lung function is caused in part by dysregulation of lung immune, inflammatory, and soluble mediator metabolism signaled by IL-6 and IL-10 following dysregulation of renal cytokine metabolism (45, 83). It is possible that these cytokines that link the effects in the remote (lung) inflammatory transcriptome to local (renal) inflammation in AKI may be useful biomarkers for predicting these sequelae. Other remote organs where AKI can compromise function include the heart, gastrointestinal tract, bone marrow, and brain (83). Thus the dramatic increase in in-hospital mortality in patients with AKI is not just an association with AKI but is causally related. This represents a significant shift away from the old and disputed adage that “patients die with, but not because of AKI.”

Conclusions

Surrogate measures of clearance have limited utility in the critically ill. More accurate and rapid measures of GFR are still in the experimental stage but show promise. However, they only tell one side, the “functional” side, of the AKI story. Biomarkers of renal injury can diagnose AKI significantly earlier than change in plasma creatinine. However, validation of each biomarker requires an understanding of the dependence on time of injury and baseline function as well as an assessment of sensitivity, specificity, and predictive value with reference to hard outcomes, such as dialysis and mortality. Validation against real-time GFR may identify structure-function relationships in a way never possible with long half-life surrogates such as creatinine. We predict that substantive progress

in the treatment of AKI will become a reality, when real-time methods of measuring GFR are combined with biomarkers of renal injury.

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