Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

*Nat Rev Nephrol*. Author manuscript; available in PMC 2020 June 19.

Published in final edited form as:

*Nat Rev Nephrol*. 2019 October ; 15(10): 599–612. doi:10.1038/s41581-019-0184-x.

# Molecular nephrology: types of acute tubular injury

**Beatriz Desanti De Oliveira**[1], **Katherine Xu**[1], **Tian H. Shen**[1], **Miriam Callahan**[1], **Krzysztof Kiryluk**[1], **Vivette D. D'Agati**[1], **Nicholas P. Tatonetti**[1], **Jonathan Barasch**[1], **Prasad Devarajan**[2,*]

[1]Columbia University, New York, NY, USA

[2]Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH, USA

## Abstract

The acute loss of kidney function has been diagnosed for many decades using the serum concentration of creatinine — a muscle metabolite that is an insensitive and non-specific marker of kidney function, but is now used for the very definition of acute kidney injury (AKI). Fortunately, myriad new tools have now been developed to better understand the relationship between acute

* prasad.devarajan@cchmc.org.

Author contributions

B.D.D.O., K.X., T.H.S., K.K., V.D.D., N.P.T., J.B. and P.D. researched data for the article. All authors contributed equally to discussion of the article's content, writing the article and review/editing the manuscript before submission.

Competing interests

J.B. and P.D. are co-inventors on patents (US57766204P; US8592 170; US797710; EP1766395B1; EP1616184) for the use of NGAL in kidney disease.

Peer review information

*Nature Reviews Nephrology* thanks M. Ostermann, J. Prowle and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Thiouracil tagging

Tagging of newly synthesized RNA. The *Uprt* gene is activated in the Rosa locus by segment- specific Cre drivers. 4-Thiouracil is then introduced at the time of choosing and 4 h later, thiouracil-labelled RNA is extracted from the whole organ without the need for cell dissociation.

Warm IRI

Arterial ischaemia–reperfusion injury of the kidney in the setting of normal body temperature (37 °C).

Sterile inflammation

The presence of inflammatory cells (neutrophils, macrophages and lymphocytes) in the absence of overt infection with bacteria or virus.

RNA pulldown

A process of extraction and purification of labelled RNA from an organ.

Partial pressure of oxygen

($pO_2$). The percentage of atmosphere occupied by oxygen gas, multiplied by the total atmospheric pressure.

Fine mapping

The use of high-resolution microscopy, whereby single cells and cellular details are microscopically discernible.

Siderophore

An organic chemical that binds with high affinity to iron. Bacteria create many different types of complex siderophores (catecholates, hydroxamates, carboxylates) to capture host iron from serum, urine and cells. Metabolic fragments, such as catecholates, can serve as siderophores in mammals. NGAL protein binds catechols and catecholate siderophores enterochelin with high affinity.

Nutritional immunity

A process of sequestering critical nutrients needed for bacterial growth. This includes the capture of iron-bound siderophores by NGAL and more generally the capture of iron by transferrin and lactoferrin.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

tubular injury and elevation in serum creatinine (SCr). These tools include unbiased gene and protein expression analyses in kidney, urine and blood, the localization of specific gene transcripts in pathological biopsy samples by rapid in-situ RNA technology and single-cell RNA-sequencing analyses. However, this molecular approach to AKI has produced a series of unexpected problems, because the expression of specific kidney-derived molecules that are indicative of injury often do not correlate with SCr levels. This discrepancy between kidney injury markers and SCr level can be reconciled by the recognition that many separate subtypes of AKI exist, each with distinct patterning of molecular markers of tubular injury and SCr data. In this Review, we describe the weaknesses of isolated SCr-based diagnoses, the clinical and molecular subtyping of acute tubular injury, and the role of non-invasive biomarkers in clinical phenotyping. We propose a conceptual model that synthesizes molecular and physiological data along a time course spanning from acute cellular injury to organ failure.

Although there is little debate about the myriad life-sustaining functions of the kidney, much controversy surrounds the very definition and nomenclature of states of acute kidney dysfunction. More than 200 years ago, William Heberden described such a state of renal failure accompanied by oliguria as *ischuria renalis*[1,2]. One hundred years later, William Osler described it as acute Bright's disease after Richard Bright, who first described acute kidney damage with rising blood urea[3], and in 1951, Homer W. Smith termed it acute renal failure (ARF)[4]. Although the term ARF has been widely used over the past 70 years, the lack of uniform diagnostic or clinical criteria has made it challenging for researchers to study the incidence, prevalence and clinical relevance of ARF. Largely in response to such challenges, a group of experts published the Risk, Injury, Failure, Loss and End-stage (RIFLE) criteria (2004)[5], which were quickly followed by the AKI network (AKIN)[6] criteria, then by the Kidney Disease Improving Global Outcomes (KDIGO) guidelines in 2012 (REF.[7]). The guidelines define and stratify kidney injury on the basis of increases in serum creatinine (SCr) level and/or decreases in urine output. A new term — acute kidney injury (AKI) — was applied. Although this term acknowledges the existence of cellular injury, its diagnosis is still only defined by the reduced excretory function of the kidney[7] (BOX 1), that is, by the measurement of kidney excretory function based on the amount of creatinine accumulated in the blood or by the amount of water excreted (urine volume).

The standardized RIFLE, AKIN and KDIGO guidelines have aided both epidemiological studies and clinical trials, especially since any elevation of SCr is likely indicative of clinically important disease. For example, in an analysis of 3.8 million patients of whom 61,726 met the KDIGO SCr definitions of AKI, electrolyte abnormalities were evident even after a single day of elevated SCr[8] (FIG. 1), potentially contributing to the excess morbidity and demise of these patients. In addition, morbidity and mortality correlate with the amplitude and duration of SCr increase, demonstrating that SCr can be used to stratify patient outcomes[9]. Taken together, these findings confirm that even brief defects in excretory function of the kidney reflected by an elevation of SCr level can have critical clinical consequences.

However, despite the utility of SCr-based AKI diagnosis, problems remain with regard to its definition and its implications for clinical practice. These problems derive at least in part

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

from a mismatch between SCr level — the steady-state marker of excretory function — and its application as a measurement of excretory function in the absence of a steady state or as a measure of cell damage. To mollify these mismatches, 'qualifiers' and 'descriptors' have been added to AKI definitions, not only to provide information about the kinetics of SCr excretion but also to re-focus SCr definitions on situations in which cellular injury is most likely present. These revised terms include 'subclinical AKI' (whereby AKI is demonstrated by molecular markers in the absence of elevated SCr level)[10–13], 'subacute AKI' (whereby SCr elevations develop over several days)[14], 'transient AKI' (referring to AKI occurring in the context of a brief, transient elevation in SCr), 'sustained AKI' (whereby SCr is elevated for long periods of time)[15–19] and 'severe AKI' (whereby SCr is markedly elevated)[20,21] (TABLE 1). Hence, although the creation of RIFLE, AKIN and KDIGO stages may have initially provided unity and simplicity to the definition of AKI, it has become clear that these definitions do not capture many attributes of acute damage, necessitating the development of additional terminology.

In this Review, we summarize the problems in detecting and understanding AKI using SCr-based definitions, and the cellular and molecular pathophysiology of some of the most common clinical subtypes of acute tubular injury. We also discuss the properties of ideal biomarkers of tubular damage and how they can be combined with SCr-based AKI definitions to provide greater insights into the processes underlying acute tubular injury. We use the term acute tubular injury to refer to molecular and cellular responses of the nephron to injurious stimuli, and the common clinical term AKI to refer to non-steady-state SCr-based diagnoses of excretory dysfunction that might result from tubular injury or from many other processes affecting the kidney.

## SCr: a problematic marker of renal injury

Nephrology is one of the few fields in the biomedical sciences that largely utilizes a single test — in fact a single chemical analyte — for the identification and the stratification of the acutely ill patient. This analyte, SCr, is the commonly used marker of kidney function in the steady state. The steady state has two requirements. First, the production of creatinine by the metabolism of creatinine phosphate in muscle cells must be constant. Second, glomerular filtration and urinary flow must not change so that creatinine is excreted at a constant rate. The mass-transfer equation:

$$\text{Urine Cr} \times \text{volume of urine ml/min} = \text{plasma Cr} \times \text{glomerular filtration rate}$$

governs the analysis of kidney function, and is only valid at steady state. Yet, SCr is used as a measure of kidney function even when its rate of excretion (as in different forms of AKI) or its rate of production (as in sepsis or rhabdomyolysis)[22–28] is not at steady state. In these contexts, the use of a single measurement of SCr as an assessment of kidney function is problematic, particularly when a patient is first assessed, for reasons outlined below.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

### SCr is a delayed marker of dysfunction

First, SCr is a delayed marker of kidney dysfunction. Changes in SCr level that do not reach KDIGO criteria for AKI may nonetheless represent loss of function, but the process of creatinine accumulation in blood to levels sufficient to define an abnormal test result generally takes 24–48 h from the point of injury, depending on a patient's muscle mass and volume of distribution. The delay in reaching a detectable SCr threshold affects acute patient care, as nephrotoxins such as antibiotics or contrast materials are often given to patients immediately following presentation to an emergency department despite uncertainties regarding kidney excretory function. The presence of excretory dysfunction in the absence of an initial diagnostic rise in SCr level (that is, where SCr elevations develop over several days) is called 'subacute AKI' and cannot be diagnosed without multiple measurements of SCr over several days and retrospective analysis of SCr changes. However, subacute AKI is an independent risk factor for hospital mortality[12], and is completely missed at the time of initial presentation using current KDIGO criteria for AKI.

### SCr is an insensitive marker of injury

Second, changes in SCr level may not detect acute tubular injury incurred in less than 50% of nephrons. Infarction or obstruction of one kidney may not induce a doubling of SCr level, and in some cases SCr might not even be elevated[29,30]. Even kidney donation, which results in an immediate loss of 50% of nephrons, might result in a mean SCr of just 1.2 mg/dl (106 pmol/l; that is, the upper limit of normal) after 30 years[31]. Moreover, the insensitivity of SCr as a marker of renal tubular injury limits its diagnostic accuracy in routine practice, since most forms of tubular injury are characterized by focal areas of damage[32]. Injury without a diagnostic rise in SCr might be due to the compensatory effect of the remaining nephrons — that is, through reliance on the renal reserve[33]. This state, which is called 'subclinical AKI', cannot be diagnosed by SCr criteria, yet is independently associated with poor kidney and overall outcomes[9–11].

### SCr is non-specific

Third, elevated SCr is not a specific marker of renal injury. An abnormal SCr level may or may not represent tubular damage because a rise in SCr can occur in many different settings including ischaemic, toxic, obstructive, as well as rapidly reversible volume depletion. A survey of patient records in the USA[8] and in Australia[9] demonstrate wide differences in SCr kinetics between patients, with SCr levels resolving in approximately 33%, 50% and 75% of patients within 1, 2 or 3 days of hospital admission, respectively (FIG. 1), and the remaining 25% of patients demonstrating prolonged increases in SCr (slow resolvers). These patients probably received widely dissimilar therapies despite all being diagnosed as having AKI. Fluid therapy, for instance, might benefit rapid resolvers who experience transient episodes of increased SCr, whereas this therapy might be harmful to slow resolvers who experience 'sustained AKI'. Hence, the measurement of SCr at the time of initial patient encounter neither provides a disease-specific phenotype nor suggests a disease-specific therapy.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

### Many variables affect the rise of SCr

Fourth, the magnitude of elevated SCr can be influenced by many confounding variables. RIFLE and KDIGO staging depends on the magnitude of SCr rise above baseline. Yet, extra-renal and intra-renal confounding factors can change the relationship between excretory failure and the magnitude of SCr rise. Extra-renal con- founders include body composition and metabolism (for example, muscle mass[34], muscle damage[22,23], nutritional status[35], body surface area and volume of distribution), which affect the extent and rate at which SCr rises. Intra-renal confounders such as chronic kidney disease (CKD) and medications modify the balance between glomerular and tubular pathways of SCr clearance. CKD[36–42], for example, differentially affects the glomerular filtration and tubular transport of SCr, such that the ratio of creatinine-to-inulin clearance can vary by as much as 2.5-fold[43]. Medications such as cimetidine, trimethoprim, pyrimethamine and salicylates, can also block tubular excretion of SCr, thereby raising SCr level without inducing cell injury. Thus, the mechanism of SCr amplification and its relationship to injury is highly context-dependent.

### Duration versus level of SCr rise

Fifth, the duration of SCr elevation might provide an alternative metric not only of excretory dysfunction but perhaps also of tubular injury. Several studies[16,44,45] have demonstrated that the duration of SCr elevation is more strongly associated with morbidity and mortality than the absolute rise in SCr[9,45,46]. This difference might be because the magnitude of SCr elevation is subject to AKI, acute kidney injury, according to SCr and urine output; RIFLE, Risk, Injury, Failure, Loss and End-stage; KDIGO, Kidney Disease Improving Global Outcomes; Cr, creatinine; SCr, serum creatinine; ATN: acute tubular necrosis. confounding factors, as described above, whereas the duration of SCr elevation probably reflects the intrinsic timing of tubular injury repair, which relies on various cell-autonomous factors, including induction of the cyclin-dependent kinase inhibitor $p21^{CIP1}$ (REFS[47–49]).

Together, these findings show that SCr lacks the kinetic properties necessary for real-time measurements of kidney dysfunction, the sensitivity to diagnose tubular injury before excretory failure develops, and the specificity to distinguish tubular injury from other causes of elevated SCr level. These issues probably contributed to the finding that different SCr-based definitions of AKI diagnose different patients, and that false-positive rates of AKI diagnosis are as high as 30% in some studies[50,51].

Multiple measurements of SCr over several days clarify some issues such as those associated with the different kinetics of transient and subacute AKI. Repetitive measurements can also capture slow rises in SCr, even in patients with limited muscle mass. In addition, confounding factors such as volume depletion and medication interference can be accounted for by measuring SCr over several days. Importantly, multiple measurements permit a calculation of the duration of elevated SCr, which, as mentioned above, has independent diagnostic power. However, despite the benefits of multiple SCr measurements, this approach is still unsuitable for the diagnosis of subclinical AKI or for the timely diagnosis of acute tubular injury.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Given the complexity and subtleties of AKI definitions, it is not at all surprising that SCr dissociates from histological findings on kidney biopsy[52–54] and from molecular analyses (see below). These shortcomings of SCr affect clinical decision-making, particularly in acute settings when multiple measurements of SCr over time are not possible.

## AKI subtypes: a continuum or a collection?

The conceptual problems of current SCr-based AKI definitions raise the question as to whether damaged kidneys express distinct clinical, physiological, cellular and molecular patterns of acute injury or whether all instances of elevated SCr level refer to a common final pathway. This question is fundamental to the use of SCr-based scales of excretory dysfunction as surrogates of cellular damage, and as a guide for the targeted use of specific therapies, such as fluids and approaches to enhancing cardiac output, that in the future will target specific signal transduction pathways.

By current KDIGO definitions, AKI is present in all cases in which SCr rises above a certain level. In other words, this definition defends a conceptual framework in which acute tubular injury occurs as a continuum that includes rapidly reversible entities such as mild volume depletion at one end of the spectrum and severe, sustained ischaemic disease at the other end. If this continuum exists, then volume depletion and arterial ischaemia due to vascular clamping should induce the expression of similar genes in the same segment of the nephron, although perhaps at different levels of intensity. We have examined this hypothesis using two mouse models of AKI: one characterized by severe volume depletion and the other by mild arterial ischaemia. Importantly, both models had equivalent increases in SCr level, allowing us to determine whether different stimuli generated different responses at the same SCr elevation, thereby testing the specificity of SCr to characterize kidney disease. Using two different methods — namely by RNA sequencing of kidney domains (glomeruli, cortex, outer medulla and inner medulla) isolated by laser capture microscopy[6,55] and thiouracil tagging to enable RNA sequencing of specific kidney segments within intact mice at specific time points (T. Shen and J. Barasch, unpublished work) — we showed that the two models shared very few genetic pathways, with fewer than 10% of expressed genes shared between the two models.

Volume depletion activated metabolic pathways consistent with starvation (such as gluconeogenesis, lipid metabolism) as well as many anti-inflammatory molecules (for example, SOCS proteins). By contrast, ischaemic clamping of the kidney pedicle activated sets of inflammatory, coagulation and epithelial repair pathways such as the Hippo signalling pathway. Hundreds of genes, including some already known to encode biomarkers of tubular damage, were markedly upregulated in ischaemic compared with healthy kidney (for example, *Havcr* (also known as *Kim1*) was induced 536-fold; *Il6* was induced 468-fold; *Cxcll* 219-fold; and *Lcn2* (also known as *Ngal*) was induced 214-fold), whereas others unexpectedly decreased (*B2m* was expressed at levels 0.45-fold that of healthy control kidneys; *Timp2* 0.43- fold; and *Igfbp7* 0.6-fold). By contrast, these genes were unchanged in the volume depletion model compared with control samples, suggesting that elevated SCr in the volume-depleted mouse might not be equally diagnostic of tubular damage. These conclusions are supported by earlier scriptomic responses[56] and assessment of physiological

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

responses to different stimuli, which showed that tubular cell energetics were dysregulated by ischaemic vascular clamp[57] but not by volume-induced elevations in SCr.

The differences observed in volume depletion and ischaemia-reperfusion injury (IRI) models of AKI are also evident in clinical studies. Studies that analysed acutely ill patients[15,58], those admitted for management of cirrhosis[59,60], and those admitted to the ICU[61] found that urinary concentrations of tubule injury biomarkers neutrophil gelatinase-associated lipocalin (NGAL, also known as LCN2), kidney injury molecule 1 (KIM1, also known as HAVCR) and IL-18 were low in patients with rapidly reversible elevations of SCr (that is, transient AKI lasting <72 h), but >10-fold higher in patients with prolonged elevations of SCr (that is, patients with sustained AKI lasting ≥7 days), than in patients presenting to the emergency department without RIFLE- defined elevations in SCr (AKI). In fact, one study showed that patients with acute heart failure who were undergoing volume depletion with diuretics and diagnosed with AKI as demonstrated by elevated cystatin C and SCr level (up to 1 mg/dl (88.4 μmol/l)), did not express the tubular injury biomarkers *N*-acetyl-β-D-glucosaminidase (NAG), KIM1 or NGAL[62]. Together, these data demonstrate that volume and haemodynamic challenges that affect the excretion of SCr do not induce the expression of the same genes as ischaemic nephropathy.

These findings also support our understanding that different aetiologies of excretory dysfunction elicit divergent responses at the molecular, cellular and functional level. Clinicians have long recognized that myriad causes of AKI exist. In fact, AKI is often multifactorial, with overlapping components. In addition to volume depletion and ischaemic injury as outlined above, hypoxia, sepsis, nephrotoxins, inflammation, obstruction and primary kidney diseases can induce elevations in SCr level. Numerous basic and translational studies have consistently identified distinct genomic, proteomic, metabolomic, structural, functional and repair responses to each of these stimuli.

In summary, although SCr might be elevated in a variety of kidney diseases, the molecular and cellular responses that accompany different stimuli – even stimuli such as volume depletion and ischaemia that have in the past been considered part of the same disease spectrum — do not indicate that these diseases are a continuum or reflect a single common final pathway. In this context, the redefinition of tubular injury by use of the functional marker SCr alone is a misleading oversimplification of the disease.

## Molecular anatomy of AKI subtypes

Elucidating the molecular changes that occur within the injured tubule is key to understanding the processes that underlie kidney damage. In order to understand these underlying processes, we need to determine whether the intensity of the stimulus, the type of the stimulus or the time course of the response to the stimulus determines the overall pattern of the kidney response, or rather, whether the kidney generates a single stereotyped response that is independent of environmental variables. For example, a severe stimulus might damage different regions of the kidney, or different nephrons or even different segments than a weaker stimulus. A stimulus might activate the same or different genes in different segments of the nephron, whereas a second stimulus might replicate one of these patterns or

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

it might induce the expression of an entirely distinct set of genes in a different location. Additionally, a stimulus might activate different sets of genes over the disease course, either as a result of intrinsic mechanisms that induce gene expression or as a result of secondary effects such as the recruitment of immune cells to the kidney.

Our understanding of the molecular responses to kidney injury will continue to develop with the application of tools including histopathology, single cell analyses, novel methods of RNA capture and urinary biomarkers, which despite marked discrepancies when compared with SCr[63], already indicate that distinct, segment-specific responses occur throughout the tubule in response to different injuries. For example, we found that different stimuli induce distinct responses in distinct regions. Volume depletion predominantly affected the inner medulla, whereas ischaemia targeted the outer strip of the outer medulla. In addition to these dominant regions, these stimuli also induce responses in non-dominant segments; for example, ischaemia-induced RNA expression of *Havcr* in the proximal tubule and *Lcn2* in the distal nephron[8,64] (FIG. 2). Hence, rather than a common final pathway and a singular readout in response to injury, the experimental data to date suggest that specific insults might lead to anatomically distinct patterns of responses reflecting changes in the underlying segmental physiology of the kidney. We predict that future studies will lead to the development of a 'wiring diagram' that outlines how the responses of one segment to a particular injury affects the responses of other segments[65,66], allowing for greater diagnostic accuracy.

## Anatomical evidence for injury subtypes

Whether common clinical diseases also result in anatomically distinct patterns of injury is less clear. In some cases of clinical tubular injury, the principal targeting mechanism and response to injury can be identified. For example, there is little debate concerning the target of injury in scenarios involving drug-induced nephrotoxicity and myoglobinuria. However, the target of injury in many of the major syndromes treated by nephrologists, such as sepsis, ischaemia and volume depletion, has not been fully elucidated.

**Toxin-induced tubular injury—**Several toxins injure very specific segments of the nephron. For instance, myoglobin, a highly nephrotoxic protein, is released into the circulation during rhabdomyolysis, from where it is filtered through the glomerulus and reabsorbed from the ultrafiltrate via the endocytic receptors megalin and cubilin in the proximal tubule, leading to death of the epithelia[67]. Similarly, cadmium, a widespread environmental pollutant, is reabsorbed from glomerular ultrafiltrate via megalin and injures the proximal tubule[68]. Likewise, aminoglycosides, a class of nephrotoxins frequently used in clinical practice, can accumulate within proximal epithelia via megalin[69], causing cell death[70,71]. This targeted injury was initially observed by histology, but was also closely linked to the discovery, development and use of biomarkers of tubular injury in humans. In rats with cadmium-induced injury, for instance, urine KIM1 expression rose before the onset of cell death and 4–5 weeks before the onset of proteinuria[72]. Likewise, gentamicin also elevated levels of urinary biomarkers, especially KIM1, even in the absence of changes in SCr[73], suggesting that KIM1 might be more useful than SCr for the timely diagnosis of gentamicin-induced acute tubular injury.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

These observations led to the identification of further biomarkers of drug-induced kidney injury[74,75]. In 2008, the Food and Drug Administration (FDA)[76] and European Medicines Agency[77] approved a panel of seven safety biomarkers that could be used with traditional markers of kidney excretory dysfunction in toxicity studies of medications using rodents, and this roster was extended in 2014 (REFS[78,79]) and 2016 (REFS[80,81]) to include osteopontin and NGAL. In 2018, the FDA approved a safety biomarker panel comprising six biomarkers (clusterin, cystatin-C, KIM1, NAG, NGAL and osteopontin) to aid the detection of kidney tubular injury in healthy volunteers participating in phase I clinical trials[82].

**Sepsis-associated tubular injury—**Although the localized effect of several toxins is now known, the pathogenesis and anatomical characterization of sepsis-associated tubular injury has not yet been elucidated. Some segments of the nephron are likely to respond to the events of sepsis before others. For instance, lipopolysaccharide (LPS) injection in rats leads to accumulation of the endotoxin strictly within proximal tubules within 60 min, without uptake of LPS in distal tubules[83]. The uptake of LPS correlates with changes in the distribution of Toll-like receptors (TLRs) in response to injury. Immunofluorescence microscopy shows that TLR4 is primarily expressed in the distal tubules of healthy mice, but its expression is dramatically increased in proximal tubular cells following colon puncture[83,84]. Similarly, sepsis induced the expression of the TLR4 co-receptor CD14, in a segment-specific manner in rats. CD14 expression was significantly upregulated in proximal tubules during sepsis, whereas little if any CD14 was detected in distal tubules[83].

**Ischaemia–reperfusion injury—**Similar to sepsis- associated acute tubular injury, IRI induces primary and secondary events that affect the kidney. Although researchers generally agree that the vast majority of clinical IRI develop focal and limited histological patterns of tubule injury[63], the nephron segment(s) that is/are most directly injured by an ischaemic insult remain(s) a matter of inquiry[85]. Most studies have focused on the last (S3) segment of the proximal tubule and on the medullary thick ascending limb (mTAL). Both segments are particularly susceptible to ischaemic insults[85], since they are located in the medulla, a region that is hypoxic even under physiological conditions. Different forms of ischaemia drive different patterns of injury: warm in generates a proximal (S3) pattern of injury, whereas the so-called ‘distal nephron models’, which are triggered by cold ischaemia, vasoconstrictors, contrast agents, Gramnegative sepsis, myoglobinuria and obstruction, result in mTAL injury[63]. Moreover, differences in patterns of injury occur as a result of segment-specific differences in cellular properties. For instance, the proximal tubule relies on aerobic oxidative metabolism and therefore requires a continuous supply of oxygen[86], whereas the mTAL is capable of some degree of anaerobic metabolism[87]. When hypoxic injury disrupts the delicate balance between oxygen supply and demand, the energy-consuming process of transport activity has been shown to be the major factor governing mTAL damage. Thus, transport inhibition might afford protection to this segment[88].

In addition, segmental variations in the activation of mitogen-activated protein kinases in response to IRI correlate with differences in segmental survival. Epithelial cell survival during oxidative stress is dependent on the balance between the activation of c-Jun N-terminal kinase (JNK) and extracellular regulated kinase (ERK)[47,89]. During IRI, only JNK

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

is activated in the cortex (where the proximal tubule predominates), whereas both JNK and ERK pathways are activated in the outer medulla (where mTAL predominates), where ERK contributes to cellular survival. Hence, segment- specific differences in gene expression can generate different epithelial behaviours.

Although use of gene expression technologies provides insights into the anatomical pattern of damage induced by IRI, the secondary effects of IRI complicate the interpretation of findings. Secondary effects are mediated by the hypoxia-induced expression of genes regulated by hypoxia-inducible factor (HIF) that stimulate local microcirculation, such as those that encode erythropoietin, vascular endothelial growth factor (VEGF) and nitric oxide synthase[90]. Secondary effects are also mediated by the hypoxia-induced release of vasoactive mediators (for example, adenosine, endothelin and angiotensin II), which redirect medullary and cortical blood flow. Tertiary effects include a reduction in glomerular filtration rate (GFR) via vasoconstriction, inflammation and tubular obstruction, epithelial and endothelial injury due to hypoxia and ATP depletion, and increased susceptibility to microvascular thrombosis via endothelial damage[91,92], followed by an inflammatory response involving leukocyte infiltration and the up- regulation ofchemokines and cytokines in the kidney[93] — a process sometimes called sterile inflammation[93].

One approach to distinguishing primary from secondary changes in gene expression in response to IRI is to analyse tissue gene expression and urinary biomarkers in the presence and absence of leukocyte populations and specific chemokines[94]. This approach has been used to determine that NGAL is produced by epithelial cells of the tubule as a primary response to IRI rather than a secondary consequence of neutrophil activation. For example, ablation of neutrophils prior to IRI did not alter the cellular patterning of *Ngal* expression in response to injury[95]. Similarly, chemotherapeutic immunosuppression reduced levels of serum NGAL in patients without AKI, but did not prevent the expression of NGAL in immunosuppressed patients with AKI[94]. Although these studies represent preliminary findings, they indicate that primary and secondary responses to injury can be devolved and organized into signalling networks with currently available technology.

**Extracellular fluid volume depletion—**Finally, extracellular fluid volume (ECFV) depletion is thought to affect many segments of the kidney secondary to activation of the renin-angiotensin-aldosterone-antidiuretic hormone systems, but we know from RNA pulldown experiments that changes in gene expression induced by ECFV depletion are most prominent in the inner medulla, where final salt and water balances are achieved[8,56]. ECFV expansion in sheep affected kidney oxygenation, leading to a sustained increase in the medullary partial pressure of oxygen ($pO_2$) with no changes in cortical perfusion and $pO_2$ (REF[96]) Consequently, the therapeutic value of ECF expansion as a prophylaxis against many different nephrotoxic agents such as radiological contrast material might in part lie in its segment-specific increase in oxygen delivery. Additional studies attempting to document medullary oxygenation in other animal models and in humans are ongoing using blood oxygen level-dependent MRI or urinary $pO_2$ measurements[97–100].

**Secondary effects of injury—**Undoubtedly, many events that occur as a consequence of injury, including tissue damage and microcirculatory changes secondarily affect multiple

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

areas of the kidney[101,102] and modulate the patterning of gene expression. For example, infiltrating leukocytes might release reactive oxygen species, proteases, elastases and other enzymes that directly cause tissue damage and change the initial distribution of epithelial responses to tubular injury[103] while simultaneously driving changes in microcirculatory flow (reviewed elsewhere[104]). Capillary occlusion at sites of cellular and fibrin aggregation[105] and conversely increased vascular permeability in damaged capillaries might follow. Localized oedema, which, in the kidney — an encapsulated organ — might alter transmural pressures, aggravate venous congestion and contribute to deteriorating microcirculatory perfusion[106]. A final outcome of vascular damage is intra-renal shunting and the modulation of perfusion in the renal medulla and cortex[107].

Perhaps the new focus on mitochondrial dysfunction will provide insights into the effects of sepsis on the kidney and clarify our understanding of changes that occur in vascular flow. Structural changes in mitochondria are the predominant findings on histopathological examination of autopsy samples from patients who died from sepsis[108]. Similarly, histopathological examination of a mouse model of septic AKI revealed mitochondrial swelling and disruption of cristae[109]. Mitochondrial respiratory chain dysfunction in the setting of sepsis can also reduce oxygen utilization, leading to reduced ATP production and bioenergetic failure[110]. Notably, mitochondrial structural as well as bioenergetic alterations occur before the rise in SCr, suggesting that damage to this organelle might be an early response to an injurious stimulus[109,111]. Furthermore, the expression of PPARγ co-activator-1α (PGC-1α), a major regulator of mitochondrial biogenesis and metabolism, is downregulated during sepsis, and its rebound during resuscitation is critical to the functional recovery from septic tubular injury in mice[109]. Not surprisingly, given the energy demands of the proximal tubule, the majority of these studies point to the proximal tubule and, to a lesser degree, the mTAL as being the nephron segments with the greatest density of mitochondria and the main sites of mitochondrial dysfunction during sepsis.

## Diagnosis of injury by biomarkers

Given the issues related to the use of SCr as a diagnostic marker of tubular injury in addition to its utility as a marker of excretory dysfunction, it is important to consider the minimum requirements for an authentic KIM1, kidney injury molecule 1; NGAL, neutrophil gelatinase-associated lipocalin. biomarker of tubular injury (TABLE 2) and consider how a biomarker might correct the shortcomings of the SCr-based diagnostic system. Here, we discuss a number of biomarkers, but focus mainly on NGAL, which is secreted primarily from cells of the collecting duct and mTAL, and is the most widely studied urinary biomarker of tubular damage in animal models and in humans.

### Ease of measurement

Use of a biomarker for AKI requires that it can be accurately and reliably quantified. This requires identification of the gene product rather than its metabolic products by use of immunoblotting or enzyme-linked immunosorbent assays (ELISAs) with specific antibodies, and demonstration that the gene product is stable during collection and processing. For example, characterizing KIM1, NGAL, IGFBP7 and TIMP2 proteins in urine with

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

immunoblots identified many candidate molecular weights rather than a single gene product, suggesting that the original translated protein might have been post-translationally processed[8,112]. In the case of NGAL, we identified a 23–25-kDa kidney-specific 'monomer' translated from kidney cell mRNA, as well as products representative of NGAL homo- and heterodimers, including NGAL-NGAL, NGAL-MMP9 and NGAL bound to polymeric immunoglobulin receptor sequences linked by a human-specific unpaired cysteine residue[113,114]. Given this complexity, all NGAL- related diagnoses of tubular injury in patients should be made using 'monomer'-specific antibodies by ELISA or immunoblotting. Similar proteomic analyses of KIM1 metabolic products have identified the shed extracellular domain in the urine[115]. Such proteomics studies are essential for the identification of suitable disease biomarkers in order to identify the most clinically relevant immune-reactive species for the diagnosis of tubular damage[8].

## 'Rapid on' and 'rapid off kinetics

The *Ngal* promoter is characterized by an open chromatin structure (W. Yu and J. Barasch, unpublished work) and multiple activating histone marks, suggesting that it can be transcribed rapidly. Indeed, the gene is transcribed within 30 min of NF-kB activation by LPS and newly synthesized NGAL protein can be detected in urine or plasma within 2–3 h after an injurious signal, such as LPS exposure or IRI in animal models and in humans[95,116]. Work in mice has shown that the extent to which the increase in NGAL levels can be reversed following injury depends on the severity of the stimulus. For example, brief ischaemia induced peak NGAL levels within 12 h and recovery by 24 h[95], whereas stronger stimulation resulted in prolonged time courses[95]. Studies in human neonates demonstrated rapid recovery of urine NGAL levels after discontinuation of gentamicin[73]. Thus, NGAL RNA and urine protein levels rise with injury and are reversed with relief of the stimulus — a pattern that contrasts with the failure of SCr to measure injury in kinetically active disease.

Additional work is necessary to document the kinetics of other biomarkers, particularly to resolve their relationship with primary and secondary causes of damage (discussed earlier). Moreover, although the temporal pattern of biomarker expression involving upregulation of NGAL followed by IL-18, L-FABP, TIMP2-IGFBP7 and lastly KIM1 has been reproduced by a number of studies of ischaemic injury[64,117,118], it remains to be seen whether this temporal pattern is universal across different forms of acute tubular injury. This temporal pattern has great potential diagnostic value that has not yet been tested in the same way that displacement of cardiac markers, for example, troponin, creatinine phosphokinase and lactate dehydrogenase, has been characterized and utilized to time the initiation of cardiac injury before patient presentation. However, use of temporal changes in biomarker expression to assess tubular injury requires additional evaluation of the expression of biomarkers throughout the acute and resolution phases of injury, which has been inadequately addressed to date.

## Quantitative assessment of damage

A critical requirement of an injury biomarker is that it exhibits a dose-dependent response to damage. Using NGAL reporter mice as well as reverse transcriptase- polymerase chain reaction (RT-PCR) and immuno- blots, we found that graded doses of ischaemia, LPS or

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

nephrotoxins generated graded expression levels of NGAL protein and mRNA in kidneys and urine, with a dynamic range of ~1,000-fold[95,119]. In severe disease, the expression level of tubular *Ngal* mRNA even surpassed that of β-actin RNA. Combined examination of urine and plasma protein together with mRNA in the kidney revealed that the ischaemic dose delivered to the kidney directly correlated with the level of detectable NGAL monomer protein in plasma and urine and with kidney *Ngal* mRNA levels. In a multicentre prospective cohort study of thousands of patients presenting to the emergency department, the level of urinary NGAL at the time of presentation correlated in a logarithmic fashion with the maximum RIFLE score achieved over the course of hospitalization[15]. Other studies in mice and humans have shown that bilateral vascular ischaemia or bilateral obstruction to urine flow induces levels of NGAL that are twice as high as those induced by unilateral vascular ischaemia or unilateral urinary obstruction[29,119]. Interestingly, such quantitative readouts are not achieved for other biomarkers of tubular kidney injury because they seem to saturate at lower levels of injury[15].

Thus, the level of biomarker expression at the time of a patient encounter is a consequence of the intensity of the injurious signal and the maximum SCr level achieved during the subsequent period of hospitalization. Differences between the biomarkers may be useful as diagnostic tools to judge the intensity of the response to a particular stimulus.

## Tubular origin of candidate biomarkers

In order for a biomarker to directly reflect tubular damage, its RNA message must be expressed at sites of tubular damage. This absolute requirement of anatomical and biological plausibility has been demonstrated for only a few biomarkers (FIG. 3). In the case of secreted biomarkers, such as NGAL, their tubular origin must be demonstrated by detecting RNA or by gene knockout technology rather than by detecting protein because of the confounding role of megalin in capturing biomarkers that are generated outside of the kidney or recycled within the kidney.

NGAL, IGFBP7 and TIMP2 are the urinary biomarkers that have been most extensively characterized. Application of RNA detection techniques have shown that ischaemia of a polar branch of the renal artery generates tubular casts and induces NGAL expression in the ischaemic artery and in damaged tubules (that is, in those containing casts, cysts or other forms of epithelial damage), but not in neighbouring tubules[95,120]. fine mapping in paraffin-embedded mouse and human kidneys subjected to ischaemia revealed *NGAL* mRNA in the thick ascending limb of the loop of Henle and collecting ducts of the kidney (Fig. 2), a distribution that been confirmed in mice by cell-specific RNA labelling techniques (T. Shen, K. Xu and J. Barasch, unpublished work) using segment- specific gene knockouts, and in humans by new in-situ technology[95,121,122] Deletion of intercalated cells from the collecting duct or deletion of *Ngal* throughout the kidney reduced urine NGAL levels[121]. Hence, urinary NGAL is derived from distal segments of damaged tubules, which is the site at which *NGAL* RNA is upregulated in response to different forms of kidney damage (Fig. 3).

IGFBP7 and TIMP2 have also been detected by immunocytochemistry at the luminal brush-border of the proximal tubule and intracellularly within distal tubule epithelia, respectively[123]. Unexpectedly, however, RT-PCR, gene arrays and in-situ hybridization

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

studies in the mouse kidney show that *Igfbp7* and *Timp2* mRNA expression either declines or remains unchanged in the setting of kidney damage[8,48,124], highlighting the difficulty of studying secreted proteins, that may, in part, derive from outside the kidney. Comparing the site of *Igfbp7* and *Timp2* RNA synthesis with the distribution of their encoded proteins (using immunocytochemistry) in different forms of kidney damage will shed light on the metabolism of these clinically useful biomarkers.

## Distinguishing types of tubular injury

In contrast to SCr, the levels of which can rise for various reasons, biomarkers of kidney damage do not respond equally to all forms of noxious stimuli. For example, *Ngal, Havcr, Clusterin, Timp1, Spp1, Cxcl1, Il6* and hundreds of other genes are not induced by even extreme volume depletion in mice[8.] Extensive animal studies using NGAL reporter mice, laser-capture RNA-Seq, RNA labelling and RNA pulldown studies (T. Shen and J. Barasch, unpublished work), as well as extensive studies of patients arriving in the emergency department or ICUs or being treated for cirrhosis, showed that levels of NGAL increase by ~2-fold at the most in cases of 'transient AKI' (constituting ~75% of all AKI patients), whereas its expression increases as much as 10–100-fold in cases of 'sustained AKI'[15,44,45,58,59,61,125]. Consequently, biomarkers at the time of patient encounter are a better reflection of the degree of epithelial injury than SCr.

However, it is not yet clear whether any of the currently known biomarkers can be uniquely matched to a specific form of tubular damage. As mentioned earlier, many of the biomarkers rise together in a defined temporal sequence suggesting linkage between different segments of the nephron in which these molecules are expressed. Nonetheless, some evidence suggests that KIM1 is particularly important for the detection of proximal tubule nephrotoxins[126], and conversely, that NGAL is particularly expressed in response to sepsis-induced tubular injury and urinary obstruction[127–133] implying the possibility of stimulus-specific biomarkers. The temporal responses of the different biomarkers in response to different forms of tubular injury are an unexplored area of great interest.

## Biological relevance of biomarkers

Finally, the reproducibility of a biomarker across different patient populations and animal models is tied to the relevance of its role in the disease process. NGAL is a member of the lipocalin superfamily that was initially identified in activated neutrophils and is therefore a component of the innate immune system. It binds strongly to the bacterial siderophore, enterochelin[134], thereby interrupting iron acquisition by bacteria and inducing 'nutritional immunity'. Notably, intercalated cells of the tubule not only secrete NGAL, a bacteriostatic protein, but also protons — which are also bacteriostatic — and IL-18 — a chemoattractant for neutrophils — thereby establishing these cells as immune defence cells. KIM1 is also a critical response protein for the resolution of kidney damage by clearing oxidized lipids and dying cells[135]. Hence, the most reproducible biomarkers are not only reporters but also critical components of the disease process itself.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

## Apples and oranges: biomarkers and SCr

As previously mentioned, in contrast to other fields of medicine such as cardiology, gastroenterology, rheumatology and oncology, which use multiple analytes to diagnose acute clinical disease, nephrology has relied largely on a single assay. The novelty of tubular injury biomarkers such as NGAL and KIM1 is that they provide an assessment of cell stress and damage without measuring the functional state of the kidney, in contrast to SCr and cystatin C, which provide an assessment of steady- state kidney function. Changes in these two parameters — cell viability and kidney function — might occur concurrently, sequentially or might occur in isolation. Loss of kidney function may or may not be the result of kidney injury, and kidney injury may or may not cause loss of kidney function. This paradigm is analogous, for example, to the use of troponins and echocardiograms in cardiology: a myocardial infarction that leads to a rise in serum troponin may not necessarily cause congestive heart failure demonstrated on an echocardiogram (FIG.4). Perhaps the best way to incorporate both types of assessment is to view them in a sequence because kidney injury must occur in stages. Damaging stimuli (for example, sepsis, obstruction or nephrotoxins) induce a sequence of events that must include cellular responses (that could be measured by assessing metabo- lomic responses) and potentially cellular damage (that could be assessed by measurement of transcriptomic responses and biomarkers). These events are potentially followed by widespread organ damage and only then by organ dysfunction (as determined by changes in steady- state markers of kidney function). As discussed above, evidence suggests that different stimuli (for example, volume depletion and ischaemia) can activate different response pathways — an observation that is in line with the different response of these injuries to different clinical treatments. This model, in which we consider the series of events, does not substitute SCr for biomarkers, but rather incorporates the information provided by each analyte (SCr as a marker of excretory dysfunction; biomarkers as indicators of injury) to improve our understanding of disease (FIG. 5). This kinetic version of a previously proposed framework for the evaluation of AKI using biomarkers[136] considers situations in which the expression of injury biomarkers increases without a rise in SCr (for instance, in the initial phase of damage, or in situations in which damage is not sufficiently widespread to cause loss of excretory function); in which a rise in SCr occurs without increased expression of kidney injury biomarkers (for instance, following activation of a pathway that reduces kidney excretion without changing known tubular injury biomarkers, such as in the context of reversible volume depletion); or in which the expression of biomarkers is accompanied by a rise in SCr level (for example, in the context of tubular damage that is sufficient to cause loss of excretory activity). Terminology such as ‘subacute AKI’ and ‘subclinical AKI’ does not have a place in this scheme because the biomarkers are simply expressed more rapidly and are more sensitive indicators of injury than is SCr. In addition, the description of different causes of elevated SCr as ‘subtypes of AKI’ is also inappropriate, as it is understood that different injurious stimuli initiate different biological processes that may or may not be related to each other and that may or may not cause functional loss.

Since biomarkers provide information that is distinct from that provided by SCr, combined analysis of biomarkers and SCr can have clinical benefits. Studies from the past few years

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

have demonstrated that different combinations of NGAL and SCr predicted with graded risk the need for renal replacement, intensive care, length of hospital stay and mortality[137]. Similarly, the combination of either NGAL or KIM1 with SCr predicted the need for renal replacement or mortality within 7 days of presentation in 15% of cases, whereas use of a biomarker or SCr alone identified only 5% of such patients[15]. Furthermore, combining NGAL and cystatin C predicted 'severe AKI' and simultaneously ruled out the possibility of 'transient AKI'[125]. Conversely, the elevation of a functional marker without the finding of urinary NGAL was an excellent predictor of 'transient AKI'[138].

Thus, the discovery and translation of the first generation of biomarkers have finally enabled SCr to be placed into a biological framework of tubular kidney injury (FIG. 5) that accounts for the diverse and variable intensity of environmental challenges to this extraordinarily complex organ. This scheme enables the diagnosis of acute tubular injury to be categorized into two potentially simple ideas: first, evidence of injured cells derived from non-invasive biomarkers, and second, evidence of loss of kidney excretory function based on changes in SCr and/or urine output.

## Conclusions and perspectives

The era of personalized and predictive kidney medicine is well upon us, ushered in by some of the remarkable genomic and proteomic advances that have contributed to our understanding of kidney health and disease, as described in this Review. Biomarkers are indispensable for the implementation of personalized medicine. Current AKI criteria, although originally designed to standardize definitions and thus aid research efforts, have been applied to guide individual therapy and have fallen short of this intention. The historic reliance on a single steady-state marker of excretory function — SCr — as a measure of acute cellular injury continues to cripple our ability to comprehend, diagnose and manage AKI.

Kidneys exhibit myriad exceptionally complex physiological, cellular, molecular and clinical consequences of acute injury. Our understanding of these processes may be improved in the future by combining firstgeneration damage biomarkers and functional criteria into a rational biological algorithm. For immediate implementation, we propose cataloguing the injury with two metrics: first, evidence of injured cells derived from non-invasive damage biomarkers, and second, evidence of loss of organ excretory function based on changes in SCr and/or urine output.

We envision a number of refinements to this concept in the near future. We predict the emergence of a generation of functional markers and tests to more accurately reflect excretory failure and to replace SCr, including real-time monitoring of GFR[139] and new filtration markers with intrinsic kinetic characteristics that better reflect the timing and severity of kidney injury than does SCr, such as pro-enkephalin. The institution of such metrics requires a better understanding of the sites of production and metabolism of these filtration markers as well as further clinical testing in patients with acute tubular damage[140,141]. We also predict the emergence of a generation of stimulus-specific urinary biomarkers that will help to discern the primary aetiology of the injury, since the molecular

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

response of the kidney to various stimuli (for example, volume depletion, IRI, sepsis and nephrotoxins) is known to differ dramatically. The temporal rise and fall of specific biomarkers will help to determine the timing of the initiating injury and to predict progression of the ensuing damage. Available evidence suggests that biomarkers such as NGAL and KIM1, TIMP2 and IGFBP7 are co-expressed; thus, it is necessary to understand how the expression of these biomarkers is linked in different segments of the nephron. Whether this co-expression occurs because of shared homology in promoter sequences or because of intra-nephron communication between cells that express these biomarkers remains to be determined. We expect that distinct biomarkers will be identified that differentiate damage from repair, enabling the early identification of a recovering kidney. Finally, we forecast the emergence of biomarkers that predict the transition from acute damage to chronic injury, enabling early identification of a primary contributor to the global epidemic of CKD.

The NIH-funded Kidney Precision Medicine Project is expected to provide further insights into the molecular changes underlying tubular kidney injury by comprehensively mapping gene expression in patients identified by elevated SCr plus a urinary biomarker. The identification of injury biomarkers stratified by aetiology, timing and segment specificity will revolutionize our understanding and management of kidney injury, particularly in complex cases in which multiple stimuli may be involved. Together, these data will test the age-old concept that multiple ‘hits’ can summate in the kidney to produce tubular damage[142–151]. They will also enable us to rationally tailor interventions, based on specific molecular pathways.

## Acknowledgements

J.B. is supported by NIH 1U54DK104309, NIH 2R01DK073462, UG3 DK114926, T32-DK108741 and a Columbia Precision Medicine Pilot Award. P.D. is funded by NIH P50 DK096418, NIH R01HL133695, NIH 1R01HL132551.

## References

1. Heberden W Commentaries on the History and Cure of Diseases (Wells and Lilly, 1818).
2. Eknoyan G Emergence of the concept of acute renal failure. Am. J. Nephrol. 22, 225–230 (2002). [PubMed: 12097745]
3. Cameron JS Bright’s disease today: the pathogenesis and treatment of glomerulonephritis-I. Br. Med. J. 4, 87–90 (1972). [PubMed: 4562073]
4. Smith HW The Kidney: Structure and Function in Health and Disease (Oxford University Press, 1951).
5. Bellomo R et al. Acute renal failure — definition, outcome measures, animal models, fluid therapy and information technology needs: the second international consensus conference of the acute dialysis quality initiative (ADQI) group. Crit. Care 8, R204–212 (2004). [PubMed: 15312219]
6. Mehta RL et al. Acute kidney injury network: report of an initiative to improve outcomes in acute kidney injury. Crit. Care 11, R31 (2007). [PubMed: 17331245]
7. Khwaja A KDIGO clinical practice guidelines for acute kidney injury. Nephron Clin. Pract. 120, c179–c184 (2012). [PubMed: 22890468]
8. Xu K et al. Unique transcriptional programs identify subtypes of AKI. J. Am. Soc. Nephrol. 28, 1729–1740 (2017). [PubMed: 28028135]
9. Uchino S, Bellomo R, Bagshaw SM & Goldsmith D Transient azotaemia is associated with a high risk of death in hospitalized patients. Nephrol. Dial. Transpl. 25, 1833–1839 (2010).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript


10. Vanmassenhove J, Van Biesen W, Vanholder R & Lameire N Subclinical AKI: ready for primetime in clinical practice? J. Nephrol. 32, 9–16 (2018). [PubMed: 30523562]
11. Haase M, Kellum JA & Ronco C Subclinical AKI — an emerging syndrome with important consequences. Nat. Rev. Nephrol. 8, 735–739 (2012). [PubMed: 23007617]
12. Ronco C, Kellum JA & Haase M Subclinical AKI is still AKI. Crit. Care 16, 313 (2012). [PubMed: 22721504]
13. Huen SC & Parikh CR Molecular phenotyping of clinical AKI with novel urinary biomarkers. Am. J. Physiol. Ren. Physiol. 309, F406–F413 (2015).
14. Fujii T, Uchino S, Takinami M & Bellomo R Subacute kidney injury in hospitalized patients. Clin. J. Am. Soc. Nephrol. 9, 457–461 (2014). [PubMed: 24311710]
15. Nickolas TL et al. Diagnostic and prognostic stratification in the emergency department using urinary biomarkers of nephron damage: a multicenter prospective cohort study. J. Am. Coll. Cardiol. 59, 246–255 (2012). [PubMed: 22240130]
16. Mehta S et al. The prognostic importance of duration of AKI: a systematic review and meta-analysis. BMC Nephrol. 19, 91 (2018). [PubMed: 29673338]
17. Au V, Feit J, Barasch J, Sladen RN & Wagener G Urinary neutrophil gelatinase-associated lipocalin (NGAL) distinguishes sustained from transient acute kidney injury after general surgery. Kidney Int. Rep. 1, 3–9 (2016). [PubMed: 27610421]
18. Freda BJ, Knee AB, Braden GL, Visintainer PF & Thakar CV Effect of transient and sustained acute kidney injury on readmissions in acute decompensated heart failure. Am. J. Cardiol. 119, 1809–1814 (2017). [PubMed: 28395891]
19. Moriyama N et al. Early development of acute kidney injury is an independent predictor of in-hospital mortality in patients with acute myocardial infarction. J. Cardiol. 69, 79–83 (2017). [PubMed: 26917196]
20. Basu RK, Kaddourah A, Goldstein SL & Investigators AS Assessment of a renal angina index for prediction of severe acute kidney injury in critically ill children: a multicentre, multinational, prospective observational study. Lancet Child. Adolesc. Health 2, 112–120 (2018). [PubMed: 30035208]
21. Yang X et al. Urinary matrix metalloproteinase-7 predicts severe AKI and poor outcomes after cardiac surgery. J. Am. Soc. Nephrol. 28, 3373–3382 (2017). [PubMed: 28698269]
22. Efstratiadis G et al. Rhabdomyolysis updated. Hippokratia 11, 129–137 (2007). [PubMed: 19582207]
23. Walid MS Blood urea nitrogen/creatinine ratio in rhabdomyolysis. Indian J. Nephrol. 18, 173–174 (2008). [PubMed: 20142932]
24. Doi K et al. Reduced production of creatinine limits its use as marker of kidney injury in sepsis. J. Am. Soc. Nephrol. 20, 1217–1221 (2009). [PubMed: 19389851]
25. Vanholder R, Sever MS, Erek E & Lameire N Rhabdomyolysis. J. Am. Soc. Nephrol. 11, 1553–1561 (2000). [PubMed: 10906171]
26. Schetz M, Gunst J & Van den Berghe G The impact of using estimated GFR versus creatinine clearance on the evaluation of recovery from acute kidney injury in the ICU. Intensive Care Med. 40, 1709–1717 (2014). [PubMed: 25266132]
27. Ravn B, Prowle JR, Martensson J, Martling CR & Bell M Superiority of serum cystatin C over creatinine in prediction of long-term prognosis at discharge from ICU. Crit. Care Med. 45, e932–e940 (2017). [PubMed: 28614196]
28. Wilson FP, Sheehan JM, Mariani LH & Berns JS Creatinine generation is reduced in patients requiring continuous venovenous hemodialysis and independently predicts mortality. Nephrol. Dial. Transpl. 27, 4088–4094 (2012).
29. Sise ME et al. Urine neutrophil gelatinase- associated lipocalin identifies unilateral and bilateral urinary tract obstruction. Nephrol. Dial. Transpl. 26, 4132–4135 (2011).
30. Decoste R, Himmelman JG & Grantmyre J Acute renal infarct without apparent cause: a case report and review of the literature. Can. Urol. Assoc. J. 9, E237–239 (2015). [PubMed: 26085895]
31. Ramcharan T & Matas AJ Long-term (20–37 years) follow-up of living kidney donors. Am. J. Transpl. 2, 959–964 (2002).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript


32. Molitoris BA Therapeutic translation in acute kidney injury: the epithelial/endothelial axis. J. Clin. Invest. 124, 2355–2363 (2014). [PubMed: 24892710]
33. Sharma A, Mucino MJ & Ronco C Renal functional reserve and renal recovery after acute kidney injury. Nephron Clin. Pract. 127, 94–100 (2014). [PubMed: 25343829]
34. Moretti C et al. Androgens and body composition in the aging male. J. Endocrinol. Invest. 28, 56–64 (2005).
35. Kimmel PL, Lew SQ & Bosch JP Nutrition, ageing and GFR: is age-associated decline inevitable? Nephrol. Dial. Transpl. 11,85–88 (1996).
36. Musso CG et al. Creatinine reabsorption by the aged kidney. Int. Urol. Nephrol. 41,727–731 (2009). [PubMed: 19115077]
37. Sjostrom PA, Odlind BG & Wolgast M Extensive tubular secretion and reabsorption of creatinine in humans. Scand. J. Urol. Nephrol. 22, 129–131 (1988). [PubMed: 3206217]
38. Gault MH & Cockcroft DW Letter: creatinine clearance and age. Lancet 2, 612–613 (1975).
39. Cockcroft DW & Gault MH Prediction of creatinine clearance from serum creatinine. Nephron 16, 31–41 (1976). [PubMed: 1244564]
40. Inker LA et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. N. Engl. J. Med. 367, 20–29 (2012). [PubMed: 22762315]
41. Schaeffner ES et al. Two novel equations to estimate kidney function in persons aged 70 years or older. Ann. Intern. Med. 157, 471–481 (2012). [PubMed: 23027318]
42. Sutherland SM et al. AKI in hospitalized children: comparing the pRIFLE, AKIN, and KDIGO definitions. Clin. J. Am. Soc. Nephrol. 10, 554–561 (2015). [PubMed: 25649155]
43. Toto RD Conventional measurement of renal function utilizing serum creatinine, creatinine clearance, inulin and para-aminohippuric acid clearance. Curr. Opin. Nephrol. Hypertens. 4, 505–509 (1995). [PubMed: 8591059]
44. Coca SG et al. First post-operative urinary kidney injury biomarkers and association with the duration of AKI in the TRIBE-AKI Cohort. PLOS ONE 11, e0161098 (2016). [PubMed: 27537050]
45. Coca SG, King JT Jr, Rosenthal RA, Perkal MF & Parikh CR The duration of postoperative acute kidney injury is an additional parameter predicting long-term survival in diabetic veterans. Kidney Int. 78, 926–933 (2010). [PubMed: 20686452]
46. Brown JR, Kramer RS, Coca SG & Parikh CR Duration of acute kidney injury impacts long-term survival after cardiac surgery. Ann. Thorac. Surg. 90, 1142–1148 (2010). [PubMed: 20868804]
47. Safirstein RL Acute renal failure: from renal physiology to the renal transcriptome. Kidney Int. Suppl, S62–S66 (2004). [PubMed: 15461706]
48. Johnson ACM & Zager RA Mechanisms underlying increased timp2 and igfbp7 urinary excretion in experimental AKI. J. Am. Soc. Nephrol. 29, 2157–2167 (2018). [PubMed: 29980651]
49. Johnson AC & Zager RA Plasma and urinary p21: potential biomarkers of AKI and renal aging. Am. J. Physiol. Ren. Physiol. 315, F1329–F1335 (2018).
50. Garner AE, Lewington AJ & Barth JH Detection of patients with acute kidney injury by the clinical laboratory using rises in serum creatinine: comparison of proposed definitions and a laboratory delta check. Ann. Clin. Biochem. 49, 59–62 (2012). [PubMed: 22130632]
51. Lin J et al. False-positive rate of AKI using consensus creatinine-based criteria. Clin. J. Am. Soc. Nephrol. 10, 1723–1731 (2015). [PubMed: 26336912]
52. Labban B et al. The role of kidney biopsy in heart transplant candidates with kidney disease. Transplantion 89, 887–893 (2010).
53. Bergler-Klein J et al. The long-term effect of simultaneous heart and kidney transplantation on native renal function. Transplantion 71, 1597–1600 (2001).
54. Moledina DG et al. Performance of serum creatinine and kidney injury biomarkers for diagnosing histologic acute tubular injury. Am. J. Kidney Dis. 70, 807–816 (2017). [PubMed: 28844586]
55. Gay L et al. Mouse TU tagging: a chemical/genetic intersectional method for purifying cell type-specific nascent RNA. Genes Dev. 27, 98–115 (2013). [PubMed: 23307870]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript


56. Yuen PS, Jo SK, Holly MK, Hu X & Star RA Ischemic and nephrotoxic acute renal failure are distinguished by their broad transcriptomic responses. Physiol. Genomics 25, 375–386 (2006). [PubMed: 16507785]
57. Zager RA Alterations of intravascular volume: influence on renal susceptibility to ischemic injury. J. Lab. Clin. Med. 108, 60–69 (1986). [PubMed: 3711726]
58. Nickolas TL et al. Sensitivity and specificity of a single emergency department measurement of urinary neutrophil gelatinase-associated lipocalin for diagnosing acute kidney injury. Ann. Intern. Med. 148, 810–819 (2008). [PubMed: 18519927]
59. Belcher JM et al. Kidney biomarkers and differential diagnosis of patients with cirrhosis and acute kidney injury. Hepatology 60, 622–632 (2014). [PubMed: 24375576]
60. Verna EC et al. Urinary neutrophil gelatinase- associated lipocalin predicts mortality and identifies acute kidney injury in cirrhosis. Dig. Dis. Sci. 57, 2362–2370 (2012). [PubMed: 22562534]
61. Singer E et al. Urinary neutrophil gelatinase- associated lipocalin distinguishes pre-renal from intrinsic renal failure and predicts outcomes. Kidney Int. 80, 405–414 (2011). [PubMed: 21412214]
62. Ahmad T et al. Worsening renal function in patients with acute heart failure undergoing aggressive diuresis is not associated with tubular injury. Circulation 137, 2016–2028 (2018). [PubMed: 29352071]
63. Heyman SN, Rosenberger C & Rosen S Experimental ischemia-reperfusion: biases and myths- the proximal vs. distal hypoxic tubular injury debate revisited. Kidney Int. 77, 9–16 (2010). [PubMed: 19759527]
64. Liu J et al. Cell-specific translational profiling in acute kidney injury. J. Clin. Invest. 124, 1242–1254 (2014). [PubMed: 24569379]
65. Lautrette A et al. Angiotensin II and EGF receptor cross-talk in chronic kidney diseases: a new therapeutic approach. Nat. Med. 11, 867–874 (2005). [PubMed: 16041383]
66. Azroyan A et al. Renal intercalated cells sense and mediate inflammation via the P2Y14 receptor. PLOS ONE 10, e0121419 (2015). [PubMed: 25799465]
67. Gburek J et al. Renal uptake of myoglobin is mediated by the endocytic receptors megalin and cubilin. Am. J. Physiol. Ren. Physiol. 285, F451–458 (2003).
68. Prozialeck WC & Edwards JR Mechanisms of cadmium-induced proximal tubule injury: new insights with implications for biomonitoring and therapeutic interventions. J Pharmacol. Exp. Ther. 343, 2–12 (2012). [PubMed: 22669569]
69. Schmitz C et al. Megalin deficiency offers protection from renal aminoglycoside accumulation. J. Biol. Chem. 277, 618–622 (2002). [PubMed: 11700326]
70. Servais H et al. Gentamicin-induced apoptosis in LLC- PK1 cells: involvement of lysosomes and mitochondria. Toxicol. Appl. Pharmacol. 206, 321–333 (2005). [PubMed: 16039943]
71. Servais H et al. Renal cell apoptosis induced by nephrotoxic drugs: cellular and molecular mechanisms and potential approaches to modulation. Apoptosis 13, 11–32 (2008). [PubMed: 17968659]
72. Prozialeck WC et al. Expression of kidney injury molecule-1 (Kim-1) in relation to necrosis and apoptosis during the early stages of Cd-induced proximal tubule injury. Toxicol. Appl. Pharmacol. 238, 306–314 (2009). [PubMed: 19371613]
73. McWilliam SJ et al. Mechanism-based urinary biomarkers to identify the potential for aminoglycoside- induced nephrotoxicity in premature neonates: a proof-of-concept study. PLOS ONE 7, e43809 (2012). [PubMed: 22937100]
74. Blank M, Thompson A, Hausner E & Rouse R Biomarkers of drug-induced acute kidney injury: a regulatory perspective. Expert. Opin. Drug. Metab. Toxicol. 14, 929–936 (2018). [PubMed: 30099912]
75. Matheis KA et al. Cross-study and cross-omics comparisons of three nephrotoxic compounds reveal mechanistic insights and new candidate biomarkers. Toxicol. Appl. Pharmacol. 252, 112–122 (2011). [PubMed: 21081137]
76. Woodcock J & Jenkins J Review submission of the qualification of seven biomarkers of drug-induced nephrotoxicity in rats. Department of Health and Human Services https://c-path.org//wp-content/uploads/2014/09/PSTC-NWG-20080414-FDA-FinalConclusion.pdf (2008).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

77. European Medicines Agency. Final report on the pilot Joint EMEA/FDA VXDS experience on qualification of nephrotoxicity biomarkers. EMA https://c-path.org//wp-content/uploads/2014/09/PSTC-NWG-20080523-EMA-FinalConclusion.pdf (2008).
78. Rasi G Letter of support for PSTC translational drug-induced kidney injury (DIKI) biomarkers. European Medicines Agency https://c-path.org//wp-content/uploads/2014/11/letter-of-support-for-PSTC-translational-drug-induced-kidney-injury-DIKI-biomarkers.pdf (2014).
79. Woodcock J Biomarker letter of support. FDA https://c-path.org//wp-content/uploads/2014/09/PSTC-NWG-2014820-FDA-LetterOfSupport.pdf (2014).
80. Rasi G Letter of support for drug-induced renal tubular injury biomarker(s). European Medicines Agency https://www.ema.europa.eu/en/documents/other/letter-support-drug-induced-renal-tubular-injury-biomarkers_en.pdf (2016).
81. Woodcock J Letter of Support for Drug-Induced Renal Tubular Injury Biomarker(s). FDA http://www.fda.gov/media/102623/download (2016).
82. Leptak C & Stockbridge N Qualification determination letter. FDA https://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/BiomarkerQualificationProgram/UCM618888.pdf (2018).
83. El-Achkar TM et al. Sepsis induces changes in the expression and distribution of Toll-like receptor 4 in the rat kidney. Am. J. Physiol. Ren. Physiol. 290, F1034–F1043 (2006).
84. Krivan S et al. Increased expression of Toll-like receptors 2, 3, 4 and 7 mRNA in the kidney and intestine of a septic mouse model. Sci. Rep. 9, 4010 (2019). [PubMed: 30850654]
85. Lieberthal W & Nigam SK Acute renal failure. I. relative importance of proximal vs. distal tubular injury. Am. J. Physiol. 275, F623–F631 (1998). [PubMed: 9815122]
86. Epstein FH Oxygen and renal metabolism. Kidney Int. 51,381–385 (1997). [PubMed: 9027710]
87. Bagnasco S, Good D, Balaban R & Burg M Lactate production in isolated segments of the rat nephron. Am. J. Physiol. 248, F522–F526 (1985). [PubMed: 3985159]
88. Brezis M, Rosen S, Silva P & Epstein FH Transport activity modifies thick ascending limb damage in the isolated perfused kidney. Kidney Int. 25, 65–72 (1984). [PubMed: 6727130]
89. di Mari JF, Davis R & Safirstein RL MAPK activation determines renal epithelial cell survival during oxidative injury. Am. J. Physiol. 277, F195–F203 (1999). [PubMed: 10444573]
90. Schofield CJ & Ratcliffe PJ Oxygen sensing by HIF hydroxylases. Nat. Rev. Mol. Cell Biol. 5, 343–354 (2004). [PubMed: 15122348]
91. Sharfuddin AA & Molitoris BA Pathophysiology of ischemic acute kidney injury. Nat. Rev. Nephrol. 7, 189–200 (2011). [PubMed: 21364518]
92. Ratliff BB, Rabadi MM, Vasko R, Yasuda K & Goligorsky MS Messengers without borders: mediators of systemic inflammatory response in AKI. J. Am. Soc. Nephrol. 24, 529–536 (2013). [PubMed: 23349311]
93. Kalogeris T, Baines CP, Krenz M & Korthuis RJ Cell biology of ischemia/reperfusion injury. Int. Rev. Cell Mol. Biol. 298, 229–317 (2012). [PubMed: 22878108]
94. Kanda J et al. An AKI biomarker lipocalin 2 in the blood derives from the kidney in renal injury but from neutrophils in normal and infected conditions. Clin. Exp. Nephrol. 19, 99–106 (2015). [PubMed: 24599361]
95. Paragas N et al. The Ngal reporter mouse detects the response of the kidney to injury in real time. Nat. Med. 17, 216–222 (2011). [PubMed: 21240264]
96. Lankadeva YR et al. Alterations in regional kidney oxygenation during expansion of extracellular fluid volume in conscious healthy sheep. Am. J. Physiol. Regul. Integr. Comp. Physiol. 315, R1242–R1250 (2018). [PubMed: 30332304]
97. Evans RG et al. Urinary oxygen tension: a clinical window on the health of the renal medulla? Am. J. Physiol. Regul. Integr. Comp. Physiol. 306, R45–R50 (2014). [PubMed: 24226029]
98. Lankadeva YR, Kosaka J, Evans RG, Bellomo R & May CN Urinary oxygenation as a surrogate measure of medullary oxygenation during angiotensin ii therapy in septic acute kidney injury. Crit. Care Med. 46, e41–e48 (2018). [PubMed: 29077618]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript


99. Sgouralis I et al. Bladder urine oxygen tension for assessing renal medullary oxygenation in rabbits: experimental and modeling studies. Am. J. Physiol. Regul. Integr. Comp. Physiol. 311, R532–R544 (2016). [PubMed: 27385734]
100. Zhu MZL et al. Urinary hypoxia: an intraoperative marker of risk of cardiac surgery-associated acute kidney injury. Nephrol. Dial. Transpl. 33, 2191–2201 (2018).
101. Kellum JA & Prowle JR Paradigms of acute kidney injury in the intensive care setting. Nat. Rev. Nephrol. 14, 217–230 (2018). [PubMed: 29355173]
102. Angus DC & van der Poll T Severe sepsis and septic shock. N. Engl. J. Med. 369, 840–851 (2013). [PubMed: 23984731]
103. Brown KA et al. Neutrophils in development of multiple organ failure in sepsis. Lancet 368, 157–169 (2006). [PubMed: 16829300]
104. Gomez H et al. A unified theory of sepsis-induced acute kidney injury: inflammation, microcirculatory dysfunction, bioenergetics, and the tubular cell adaptation to injury. Shock. 41,3–11 (2014).
105. Ince C et al. The endothelium in sepsis. Shock 45, 259–270 (2016). [PubMed: 26871664]
106. Prowle JR & Bellomo R Sepsis-associated acute kidney injury: macrohemodynamic and microhemodynamic alterations in the renal circulation. Semin. Nephrol. 35, 64–74 (2015). [PubMed: 25795500]
107. Post EH et al. Changes in kidney perfusion and renal cortex metabolism in septic shock: an experimental study. J. Surg. Res. 207, 145–154 (2017). [PubMed: 27979471]
108. Takasu O et al. Mechanisms of cardiac and renal dysfunction in patients dying of sepsis. Am. J. Respir. Crit. Care Med. 187, 509–517 (2013). [PubMed: 23348975]
109. Tran M et al. PGC-1alpha promotes recovery after acute kidney injury during systemic inflammation in mice. J. Clin. Invest. 121,4003–4014 (2011). [PubMed: 21881206]
110. Brealey D & Singer M Mitochondrial dysfunction in sepsis. Curr. Infect. Dis. Rep. 5, 365–371 (2003). [PubMed: 13678565]
111. Parikh SM et al. Mitochondrial function and disturbances in the septic kidney. Semin. Nephrol. 35, 108–119 (2015). [PubMed: 25795504]
112. Bailly V et al. Shedding of kidney injury molecule-1, a putative adhesion protein involved in renal regeneration. J. Biol. Chem. 277, 39739–39748 (2002). [PubMed: 12138159]
113. Nickolas TL et al. NGAL (Lcn2) monomer is associated with tubulointerstitial damage in chronic kidney disease. Kidney Int. 82, 718–722 (2012). [PubMed: 22695331]
114. Yan L, Borregaard N, Kjeldsen L & Moses MA The high molecular weight urinary matrix metalloproteinase (MMP) activity is a complex of gelatinase B/MMP-9 and neutrophil gelatinase- associated lipocalin (NGAL). Modulation of MMP-9 activity by NGAL. J. Biol. Chem. 276, 37258–37265 (2001). [PubMed: 11486009]
115. Ichimura T et al. Kidney injury molecule-1 (KIM-1), a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain, is up-regulated in renal cells after injury. J. Biol. Chem. 273, 4135–4142 (1998). [PubMed: 9461608]
116. Mishra J et al. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet 365, 1231–1238 (2005). [PubMed: 15811456]
117. Dong L, Ma Q, Bennett M & Devarajan P Urinary biomarkers of cell cycle arrest are delayed predictors of acute kidney injury after pediatric cardiopulmonary bypass. Pediatr. Nephrol. 32, 2351–2360 (2017). [PubMed: 28755073]
118. Krawczeski CD et al. Temporal relationship and predictive value of urinary acute kidney injury biomarkers after pediatric cardiopulmonary bypass. J. Am. Coll. Cardiol. 58, 2301–2309 (2011). [PubMed: 22093507]
119. Mishra J et al. Identification of neutrophil gelatinase- associated lipocalin as a novel early urinary biomarker for ischemic renal injury. J. Am. Soc. Nephrol. 14, 2534–2543 (2003). [PubMed: 14514731]
120. Paragas N et al. Urinary NGAL marks cystic disease in HIV-associated nephropathy. J. Am. Soc. Nephrol. 20, 1687–1692 (2009). [PubMed: 19628667]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript


121. Paragas N et al. alpha-Intercalated cells defend the urinary system from bacterial infection. J. Clin. Invest. 124, 2963–2976 (2014). [PubMed: 24937428]
122. Wang F et al. RNAscope: a novel in situ RNA analysis platform for formalin-fixed, paraffin-embedded tissues. J. Mol. Diagn. 14, 22–29 (2012). [PubMed: 22166544]
123. Emlet DR et al. Insulin-like growth factor binding protein 7 and tissue inhibitor of metalloproteinases-2: differential expression and secretion in human kidney tubule cells. Am. J. Physiol. Ren. Physiol. 312, F284–F296 (2017).
124. Mar D et al. Heterogeneity of epigenetic changes at ischemia/reperfusion- and endotoxin-induced acute kidney injury genes. Kidney Int. 88, 734–744 (2015). [PubMed: 26061546]
125. Basu RK et al. Combining functional and tubular damage biomarkers improves diagnostic precision for acute kidney injury after cardiac surgery. J. Am. Coll. Cardiol. 64, 2753–2762 (2014). [PubMed: 25541128]
126. Vaidya VS et al. Kidney injury molecule-1 outperforms traditional biomarkers of kidney injury in preclinical biomarker qualification studies. Nat. Biotechnol. 28, 478–485 (2010). [PubMed: 20458318]
127. Mori K et al. Endocytic delivery of lipocalin- siderophore-iron complex rescues the kidney from ischemia-reperfusion injury. J. Clin. Invest. 115, 610–621 (2005). [PubMed: 15711640]
128. da Rocha EP et al. Urinary neutrophil gelatinase- associated lipocalin is excellent predictor of acute kidney injury in septic elderly patients. Aging Dis. 9, 182–191 (2018). [PubMed: 29896409]
129. Park HS et al. Urinary neutrophil gelatinase- associated lipocalin as a biomarker of acute kidney injury in sepsis patients in the emergency department. Clin. Chim. Acta 495, 552–555 (2019). [PubMed: 31175848]
130. Srisawat N et al. Neutrophil gelatinase associated lipocalin (NGAL) in leptospirosis acute kidney injury: a multicenter study in thailand. PLOS ONE 10, e0143367 (2015). [PubMed: 26629810]
131. Urbschat A et al. Serum and urinary NGAL but not KIM-1 raises in human postrenal AKI. Eur. J. Clin. Invest. 44, 652–659 (2014). [PubMed: 24837251]
132. Forster CS & Devarajan P Neutrophil gelatinase- associated lipocalin: utility in urologic conditions. Pediatr. Nephrol. 32, 377–381 (2017). [PubMed: 27785626]
133. Kostic D et al. The role of renal biomarkers to predict the need of surgery in congenital urinary tract obstruction in infants. J. Pediatr. Urol. 15, 242.e1–249.e9 (2019). [PubMed: 30979613]
134. Goetz DH et al. The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore- mediated iron acquisition. Mol. Cell 10, 1033–1043 (2002). [PubMed: 12453412]
135. Arai S et al. Apoptosis inhibitor of macrophage protein enhances intraluminal debris clearance and ameliorates acute kidney injury in mice. Nat. Med. 22, 183–193 (2016). [PubMed: 26726878]
136. Murray PT et al. Potential use of biomarkers in acute kidney injury: report and summary of recommendations from the 10th acute dialysis quality initiative consensus conference. Kidney Int. 85, 513–521 (2014). [PubMed: 24107851]
137. Haase M et al. The outcome of neutrophil gelatinase- associated lipocalin-positive subclinical acute kidney injury: a multicenter pooled analysis of prospective studies. J. Am. Coll. Cardiol. 57, 1752–1761 (2011). [PubMed: 21511111]
138. Basu RK et al. Incorporation of biomarkers with the renal angina index for prediction of severe AKI in critically ill children. Clin. J. Am. Soc. Nephrol. 9, 654–662 (2014). [PubMed: 24677554]
139. Molitoris BA & Reilly ES Quantifying glomerular filtration rates in acute kidney injury: a requirement for translational success. Semin. Nephrol. 36, 31–41 (2016). [PubMed: 27085733]
140. Hollinger A et al. Proenkephalin A 119–159 (Penkid) is an early biomarker of septic acute kidney injury: the kidney in sepsis and septic shock (Kid-SSS) study. Kidney Int. Rep. 3, 1424–1433 (2018). [PubMed: 30450469]
141. Denning GM et al. Proenkephalin expression and enkephalin release are widely observed in nonneuronal tissues. Peptides 29, 83–92 (2008). [PubMed: 18082911]
142. Rosen S, Brezis M & Stillman I The pathology of nephrotoxic injury: a reappraisal. Min. Electrolyte Metab. 20, 174–180 (1994).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

143. Heyman SN, Rosen S, Fuchs S, Epstein FH & Brezis M Myoglobinuric acute renal failure in the rat: a role for medullary hypoperfusion, hypoxia, and tubular obstruction. J. Am. Soc. Nephrol. 7, 1066–1074 (1996). [PubMed: 8829123]

144. Alexanian R, Barlogie B & Dixon D Renal failure in multiple myeloma. Pathogenesis and prognostic implications. Arch. Intern. Med. 150, 1693–1695 (1990). [PubMed: 2383164]

145. Cohen DJ, Sherman WH, Osserman EF & Appel GB Acute renal failure in patients with multiple myeloma. Am. J. Med. 76, 247–256 (1984). [PubMed: 6695948]

146. Perazella MA Onco-nephrology: renal toxicities of chemotherapeutic agents. Clin. J. Am. Soc. Nephrol. 7, 1713–1721 (2012). [PubMed: 22879440]

147. Ghane Shahrbaf F & Assadi F Drug-induced renal disorders. J. Ren. Inj. Prev. 4, 57–60 (2015). [PubMed: 26468475]

148. Stacul F et al. Strategies to reduce the risk of contrast-induced nephropathy. Am. J. Cardiol. 98, 59K–77K (2006).

149. Goldfarb S, McCullough PA, McDermott J & Gay SB Contrast-induced acute kidney injury: specialty-specific protocols for interventional radiology, diagnostic computed tomography radiology, and interventional cardiology. Mayo Clin. Proc. 84, 170–179 (2009). [PubMed: 19181651]

150. Schrier RW Nephrology forum: acute renal failure. Kidney Int. 15, 205–216 (1979). [PubMed: 513485]

151. Gines P & Schrier RW Renal failure in cirrhosis. N. Engl. J. Med. 361, 1279–1290 (2009). [PubMed: 19776409]

152. Charlton JR et al. Late onset neonatal acute kidney injury: results from the AWAKEN Study. Pediatr. Res. 85, 339–348 (2018). [PubMed: 30546043]

153. Takaya Y et al. Impact of onset time of acute kidney injury on outcomes in patients with acute decompensated heart failure. Heart Vessel. 31, 60–65 (2016).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

**Box 1 |**

## KDIGO definition of acute kidney injury

The 2012 KDIGO AKI guideline[7] defines acute kidney injury (AKI) using the following criteria:

- An increase in serum creatinine (SCr) of ≥0.3 mg/dl ( ≥26.5 μmol/l) within 48 h, or
- An increase in serum creatinine to ≥1.5 times baseline, which is known or presumed to have occurred within the previous 7 days, or
- A urine volume of <0.5 ml/kg/h for 6 h

Patients are then staged according to the rise in SCr or reduction in urine output to the following categories:[7]

- Stage 1: an increase in SCr level to 1.5–1.9 times baseline, or an increase in SCr by ≥0.3 mg/dl ( ≥26.5 (μmol/l), or a reduction in urine output to <0.5 ml/kg/h for 6–12 h.
- Stage 2: an increase in SCr level to 2.0–2.9 times baseline, or a reduction in urine output to <0.5 ml/kg/h for ≥12 h.
- Stage 3: an increase in SCr level to 3.0 times baseline, or an increase in SCr level to ≥4.0 mg/dl ( ≥353.6 (μmol/l), or a reduction in urine output to <0.3 ml/kg/h for ≥24 h, or anuria for ≥12 h, or the initiation of kidney replacement therapy, or, in patients <18 years of age, a decrease in estimated glomerular filtration rate to <35 ml/min/1.73 $m^2$

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

**Key points**

- Current definitions of acute kidney injury (AKI), based on serum creatinine (SCr) level, focus on loss of kidney function rather than kidney injury.
- AKI definitions cannot provide an acute measurement of loss of function, however, because SCr is a quantitative functional marker only at the steady state.
- Current AKI metrics can neither detect kidney injury in real time nor distinguish dramatically different types of kidney injury.
- Molecular analyses of acutely damaged kidneys have detected cellular and segment- specific responses to injurious stimuli, prior to and distinct from the loss of function as measured by SCr.
- As a result, molecular analyses have detected different types of acute tubular injury and have re-characterized the concept of the kidney response to noxious stimuli into biomarker-positive ‘injury’ and biomarker-negative ‘no injury’.
- A conceptual model places ‘tubular injury’ (biomarkers) upstream of ‘loss of function’ (AKI metrics), providing a unifying ‘injury’ and ‘loss of function’ sequence consistent with biological pathways.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

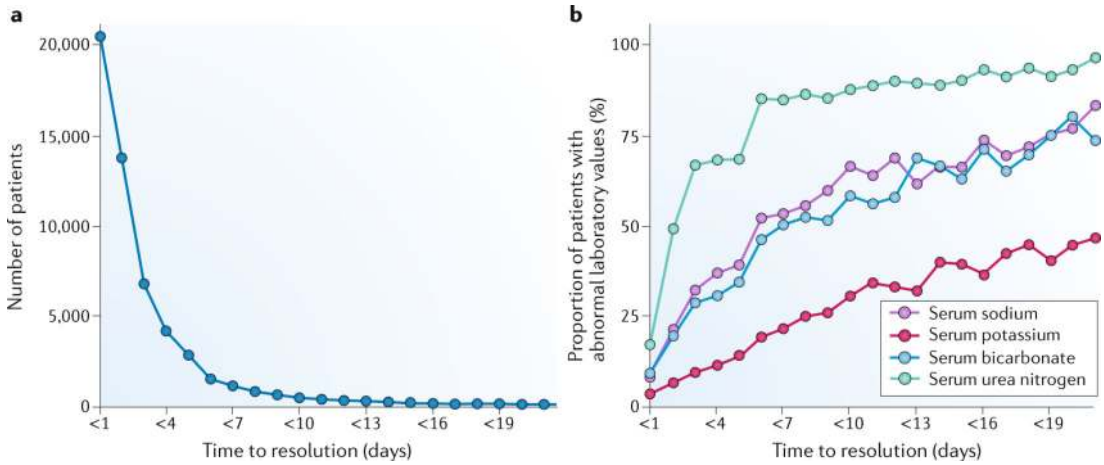


**Fig. 1 |. Rapid resolution of serum creatinine in most patients with AKI.**

**a** | Our study of 61,726 patients who were admitted to the New York Presbyterian Hospital and met Kidney Disease Improving Global Outcomes (KDIGO) criteria for acute kidney injury (AKI) showed that serum creatinine (SCr) resolved rapidly in most patients: ~33% of events resolved within the first days of hospital admission, ~60% within 2 days and ~70% within 3 days. **b** | Assessment of the proportion of patients with electrolyte abnormalities, according to time to resolution of SCr shows that rapid resolvers (that is, those points on the left-side of the graph) were less likely to have electrolyte abnormalities than patients with sustained or unremitting elevation of SCr levels (those on the right-side of the graph). In both **a** and **b**, the difference between two points along the x-axis represents a period of 24 h. Data are taken from Xu et al.[8].

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

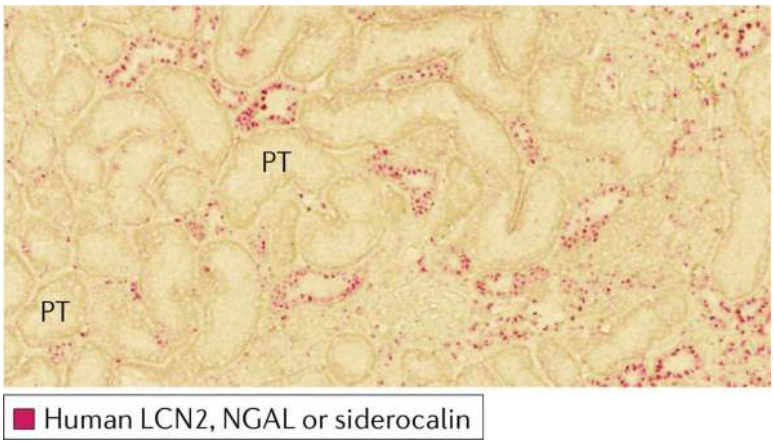


**Fig. 2 |. Renal expression of *NGAL* mRNA.**
In situ hybridization of an ischaemic human kidney explanted by nephrectomy shows *NGAL* expression primarily in distal segments of the nephron. Proximal tubules (PT, large crosssectional profiles) are negative. This patterning is similar to that seen in ischaemic mouse kidneys[8,64].

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

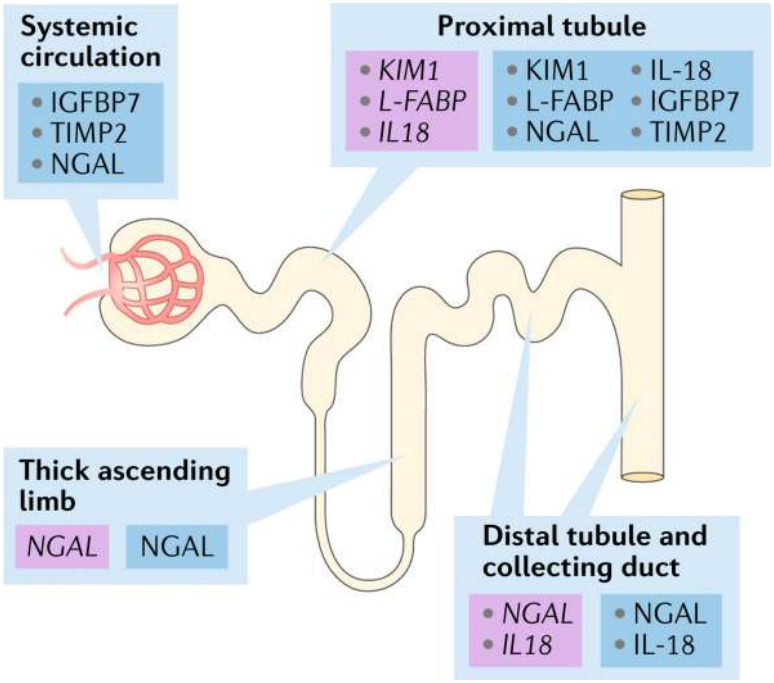


**Fig. 3 |. Sources of AKI biomarker mRNA and protein.**

mRNAs encoding *KIM1, L-FABP* and *IL18* are transcribed in the proximal tubule (pink boxes, italics), whereas *NGAL* mRNA is synthesized primarily in the thick ascending limb and collecting duct. The protein products (blue boxes) are detected primarily at the sites of mRNA transcription, but may also be derived from the systemic circulation. The primary source of *IGFBP7* and *TIMP2* mRNA is unclear, since these genes and their protein products may be expressed ubiquitously within and outside the kidney. Plasma NGAL is freely filtered and largely reabsorbed by the proximal tubule by megalin-dependent pathways, and may be multimeric. Kidney-derived NGAL is monomeric.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

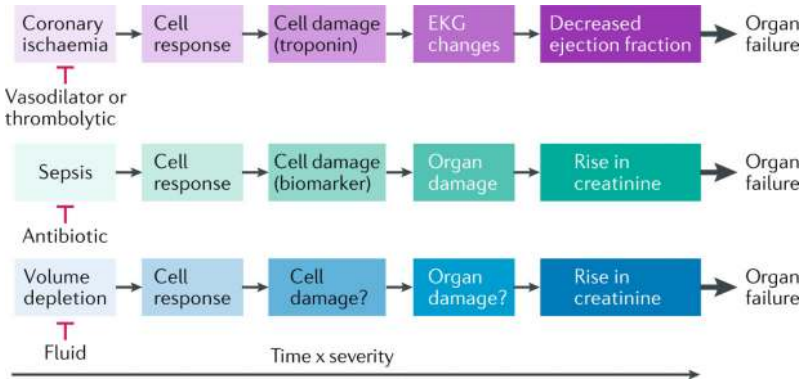


**Fig. 4 |. Biomarkers change the definition of AKI.**

Different stimuli (for example, coronary ischaemia, sepsis or volume depletion) activate different biological pathways in the heart and kidney. The pathways begin with stimulus-dependent cellular responses, which may result in cell damage and subsequently organ damage, if and when the stimulus is sufficiently severe. In the most severe cases of 'organ damage', 'organ failure' occurs. Cellular responses and damage can be detected by biomarkers, but organ failure is currently estimated through the use of functional tests such as an echocardiogram (in the context of heart failure) or serum creatinine (SCr; in the context of kidney injury). Detection of biomarkers without evidence of organ failure represents a milder form of damage (or one detected early in its course) than detection of biomarkers together with evidence of organ failure. Note that radically different therapies apply to these three examples. Also note that volume depletion and ischaemia do not induce the same damage markers: >100 different markers of ischaemic damage are not upregulated by volume depletion, and hence metrics of cell damage, if actually present in volume depletion, are not yet established. EKG, electrocardiogram.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

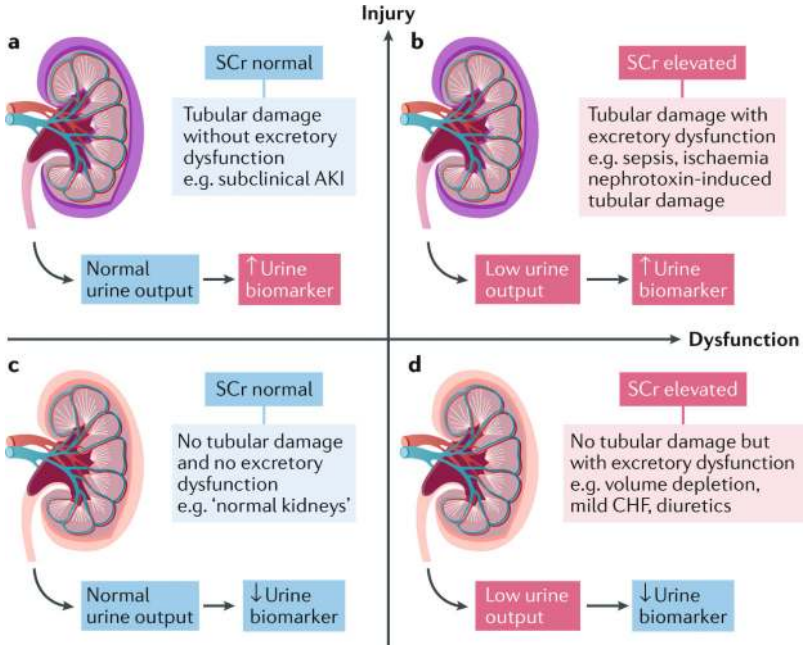


**Fig. 5 |. Combined analysis of biomarker, serum creatinine and urine output for the assessment of kidney injury.**

Biomarkers serve as 'injury markers' of the tubule, providing data regarding cellular injury and responses to noxious stimuli. Rising serum creatinine (SCr) on the other hand is a 'functional marker', indicative of excretory dysfunction. **a |** An insult leads to cellular damage, which can be detected by urine biomarkers. If insufficient numbers of nephrons are injured to cause organ dysfunction, no rise in SCr will be detected. **b |** An insult leads to cellular damage, which can be detected by urine biomarkers. If nephron injury is sufficient to cause organ dysfunction, a rise in SCr is detected. **c |** The absence of urine biomarkers and a normal SCr indicates that no cellular injury has occurred and that the kidney is not challenged by haemodynamic failure. **d |** Excretory dysfunction can occur without biomarker evidence of tubular injury. A rising SCr is indicative of excretory dysfunction, whereas the absence of urine biomarkers indicates sparing of tubular cells, a scenario that challenges the specificity of SCr as a marker of injury. This scenario is common in cases of volume depletion, diuretic use, and mild forms of heart or liver failure. AKI, acute kidney injury (defined according to SCr-based KDIGO Guidelines); CHF, congestive heart failure.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript



**Table 1 |**

Qualifiers and descriptors of AKI

| Current AKI nomenclature | Description | Interpretation | Example references |
|---|---|---|---|
| Subclinical AKI | Tubular damage biomarker indicates damage, yet SCr is not elevated[10] | • SCr is an insensitive marker of tubular damage<br>• Elevation of SCr requires damage to >50% of nephron mass. Damage to a portion of the kidney is not detectable | 10–13 |
| Transient versus sustained AKI | • SCr elevation <3 days versus SCr elevation >3 days[17]<br>• SCr returning to within 10% of baseline versus >72 h of azotaemia[18]<br>• RIFLE-AKI resolved by 72 h versus RIFLE-AKI persisting ≥72 h[15]<br>• Resolving below AKI criteria[19]<br>• SCr approaching 25–50% of baseline versus no recovery at discharge; failing to meet transient criteria[16] | A single measurement of SCr at the time of patient encounter does not provide prospective information about the kinetics of SCr; transient AKI includes volume depletion (prerenal azotaemia), whereas sustained AKI includes ATN | 15–18 |
| Severe AKI | Stages 2–3 AKI KDIGO guidelines | SCr is a valid quantitative tool only in steady state. However, multiple studies have demonstrated that AKI stages 2–3 correlate with biomarkers of tubular injury | 20,21 |
| Subacute AKI | AKI developing over >7 days | Cr, a product of muscle, accumulates slowly in serum. As a result, some patients cannot be diagnosed at the time of patient encounter using SCr criteria | |
| Late onset AKI | • AKI occurring >7 days after birth[152]<br>• AKI occurring ≥5 days from admission[153]<br>• AKI occurring 48 h after admission[19] | Optimal use of SCr requires correlating its values with the clinical course in order to | 15,19,21,53 |

AKI, acute kidney injury, according to SCr and urine output; RIFLE, Risk, Injury, Failure, Loss and End- stage; KDIGO, Kidney Disease Improving Global Outcomes; Cr, creatinine; SCr, serum creatinine; ATN: acute tubular necrosis.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

**Table 2 |**

Characteristics of an ideal biomarker of acute tubular injury

| Property | Explanation |
|---|---|
| Easily measured | Reliable quantification of a biomarker requires that the biomarker is stable during collection and processing and the test distinguishes the gene product from its metabolic product |
| 'Rapid on, rapid off' kinetics | Biomarker expression must be upregulated shortly after the injurious stimulus and downregulated after termination of stimulus |
| Dose-dependent response | The quantity of the biomarker must be proportional to the number of injured nephrons or the severity of the injured nephrons. Hence, a biomarker must be sensitive to the injury of a small number of nephrons but also demonstrate a broad dynamic range to respond to widespread injury |
| Tubular origin | In acute renal failure caused by tubular injury, the biomarker must be expressed at sites of tubular damage |
| Specific | An ideal biomarker should be able to distinguish the injury induced by different types of acute renal failure, such as volume depletion versus tubular damage and potentially proximal tubular from distal tubular injury |
| Essential to homeostasis, injury or repair | An ideal biomarker should reflect the injury process, a property called 'biological plausibility', e.g. KIM1 is needed to remove cellular debris, whereas NGAL defends the urinary system from infection |
| Distinct from functional marker | Analysis with an 'injury' biomarker should interact in a synergistic fashion with analysis by a 'functional' biomarker |

KIM1, kidney injury molecule 1; NGAL, neutrophil gelatinase- associated lipocalin.