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Biomarkers of Nephrotoxic Acute Kidney Injury

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Abstract

Acute kidney injury (AKI) is a common condition with significant associated morbidity and mortality. Epidemiologic data suggest that a significant proportion of AKI cases are at least partially attributable to nephrotoxin exposure. This is not surprising given intrinsic renal susceptibility to toxicant-induced injury, a consequence of the unique physiologic and biochemical properties of the normally functioning kidney. A number of pathophysiologic mechanisms have been identified that mediate toxic effects on the kidney, resulting in a variety of clinical syndromes ranging from subtle changes in tubular function to fulminant renal failure. Unfortunately, standard metrics used to diagnosis and monitor kidney injury, such as blood urea nitrogen and serum creatinine, are insensitive and nonspecific, resulting in delayed diagnosis and intervention. Considerable effort has been made to identify biomarkers that will allow the earlier diagnosis of AKI. Further characterization of these candidate biomarkers will clarify their utility in the setting of acute nephrotoxicity, define new diagnostic and prognostic paradigms for kidney injury, facilitate clinical trials, and lead to novel effective therapies.

Introduction

The kidney normally performs a variety of functions essential to the regulation of a constant extracellular environment and the maintenance of metabolic homeostasis. In order to sustain

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Disclosure Statement

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glomerular filtration and renal metabolism, the renal vascular bed receives a disproportionately large blood flow, averaging 20 – 25% of resting cardiac output. As a result, cells of the renal vasculature, glomerulus, tubules, and interstitium are exposed to high volumes of blood borne toxicants. The tubular epithelium is especially susceptible to injury, a result of (1) solute and water reabsorption along the nephron, producing greater concentrations of filtered toxicants in the tubular fluid than that seen in the general circulation; (2) transport processes resulting in high intracellular concentrations of toxicants and their metabolites; and (3) high energy requirements necessary to support epithelial cell metabolism and solute transport.

The effects of toxicants on the kidney are protean, with a multitude of different compounds and varying mechanisms of injury implicated. Injury may occur secondary to altered renal hemodynamics, direct cellular damage to the tubular epithelium, tubular obstruction of urinary flow due to the precipitation of toxins or their metabolites, interstitial inflammation, and/or thrombotic microangiopathy (see Figure 1). Initially, injury is often manifest by subtle changes in tubular function, including altered urine concentrating ability and/or electrolyte handling. In the absence of judicious monitoring, injury may evade detection until a significant decline in renal functional capacity occurs.

Acute Kidney Injury (AKI) has been reported to complicate 1 – 7% (Hou *et al.*, 1983; Shusterman *et al.*, 1987; Edwards *et al.*, 1994; Chertow *et al.*, 2001; Nash *et al.*, 2002; Liangos *et al.*, 2006) of all hospital admissions and 1 – 25% of intensive care unit (ICU) admissions (Chertow *et al.*, 1998; de Mendonca *et al.*, 2000). Over the last 50 years, mortality rates have remained essentially unchanged for adult and pediatric patients at 50 – 70% and 30% respectively. The true incidence of acute nephrotoxicity is difficult to estimate, owing to study variability of the patient population surveyed as well as the definition used to designate kidney injury. There is widespread agreement, however, that nephrotoxins represent an important etiologic factor in hospital-acquired AKI, contributing to 8 – 60% of cases (Hou *et al.*, 1983; Liano *et al.*, 1998; Nash *et al.*, 2002; Hui-Stickle *et al.*, 2005; Schetz *et al.*, 2005; Uchino *et al.*, 2005). In a recent large multicenter prospective epidemiological survey of critically ill patients, Uchino *et al.* (2005) reported that drug nephrotoxicity contributed to 19% of AKI cases. In addition, there is evidence that the incidence of nephrotoxic AKI in hospitalized patients has increased over the last two decades (Hou *et al.*, 1983; Nash *et al.*, 2002). Epidemiologic data in the ambulatory setting is less well defined; however, there is one report to indicate that 20% of hospital admissions for AKI have been attributed to community-acquired drug-induced nephrotoxicity (Nolin *et al.*, 2005).

The Acute Kidney Injury Network (AKIN) was recently formed in an effort to facilitate improved care of patients with or at risk for AKI. In recognition of increasing data suggesting that even small changes in serum creatinine are associated with poorer outcome as measured by mortality (Lassnigg *et al.*, 2004; Chertow *et al.*, 2005), the AKIN committee proposed uniform standards for defining, diagnosing, and classifying AKI. This group defined AKI as “functional or structural abnormalities or markers of kidney damage including abnormalities in blood, urine, or tissue tests or imaging studies present for less than three months (Mehta *et al.*, 2006).” The committee set diagnostic criteria for AKI as

“an abrupt (within 48 hours) reduction in kidney function currently defined as an absolute increase in serum creatinine of either 0.3 mg/dl or a percentage increase of 50% or a documented reduction in urine output (documented oliguria of < 0.5 ml/kg/h for > 6 hours (Mehta *et al.*, 2006).”

Current clinical definitions, diagnostic criteria, and classification schemes for AKI are highly dependent on changes in serum creatinine. In addition, serum creatinine remains the metric of choice in preclinical animal studies of kidney injury, although histopathology is also commonly used. Unfortunately, creatinine is a suboptimal marker following injury since levels are often not reflective of glomerular filtration rate (GFR) due to a number of renal and non-renal influences (Star, 1998). In the setting of AKI, the time relationship between changes in serum creatinine and changes in GFR inhibits the ability to accurately estimate timing of injury and severity of dysfunction following injury (Moran and Myers, 1985). As dictated by the laws of mass balance, a sudden fall in GFR to a constant low level causes a gradual increase in serum creatinine until a new steady state between generation and excretion is achieved. The rate of rise following AKI is dependent on many factors including the new GFR, rate of tubular secretion, rate of generation, and volume of distribution (Moran and Myers, 1985; Star, 1998). As a result, large changes in GFR may be associated with relatively small changes in serum creatinine in the first 24 – 48 hours following AKI, resulting not only in delayed diagnosis and intervention, but also in underestimation of the degree of injury (Star, 1998). In addition, there is considerable variability in the relationship between changes in serum creatinine and baseline GFR, functional renal reserve, and creatinine synthesis rates. As a result, a renal injury of comparable magnitude may result in disparate alterations in creatinine concentration in different individuals (Bellomo *et al.*, 2004).

The insensitivity and non-specificity of serum creatinine as well as other traditionally used markers of renal injury, including blood urea nitrogen, urine sediment, and urinary indices (fractional excretion of sodium, urine osmolality, etc.), have been major obstacles in assessing for nephrotoxicity as well as developing strategies to ameliorate injury. Drug development has suffered due to suboptimal strategies in the determination of product benefit and safety. In addition, clinical trials suggesting inefficacy of putative therapies of AKI are by definition confounded by delayed diagnosis and/or treatment. This paradigm is analogous to the initiation of therapy in patients with myocardial infarction or 48 hours after the onset of cardiac ischemia (Vaidya and Bonventre, 2006). Such a delay in diagnosis and intervention likely predisposes to increased morbidity and mortality as well as irreversible injury resulting in chronic kidney dysfunction.

Biomarkers of Acute Kidney Injury

Intensive investigative efforts have led to the identification and evaluation of many urinary and serum proteins as potential biomarkers of AKI (Emeigh Hart, 2005; Trof *et al.*, 2006; Vaidya and Bonventre, 2006). In general, the urine has yielded the most promising candidates for the early detection of AKI and further characterization of these biomarkers is expected to qualify their utility in the setting of acute nephrotoxicity as well as other modes of injury. Table 1 provides a general overview of candidate biomarkers with appropriate

references for further review. Detailed information regarding the most promising biomarkers and a description of the knowledge garnered to date follows.

Urinary Proteins with Enzymatic Activity

Urinary enzymes have been extensively studied in a number of pathologic conditions predisposing to acute and chronic renal injury, including nephrotoxicant exposure, diabetic nephropathy, hypertension, renal ischemia, renal transplantation, and glomerulopathies. In the setting of injury, enzymes normally present in the tubular epithelial cell may be released into the urine secondary to leakage from damage or secondary to intensified enzyme induction during the repair and regeneration process (D'Amico and Bazzi, 2003; Emeigh Hart, 2005). Urinary enzymes have been found to be sensitive biomarkers of AKI with dose-dependent increases in urinary enzymatic activity correlated to the degree of tissue damage present (Westhuyzen *et al.*, 2003; Emeigh Hart, 2005). In addition, preclinical and clinical studies have led to extensive knowledge regarding segmental nephron distribution and cellular origin (brush border, lysosome, or cytoplasm) of tubular enzymes. As a general rule, brush border enzymuria is indicative of a less severe injury than lysosomal or cytosolic enzymuria (D'Amico and Bazzi, 2003; Westhuyzen *et al.*, 2003; Emeigh Hart, 2005). Thus, enzyme detection in the urine potentially provides valuable information not only pertinent to the site of tubular injury (proximal vs. distal tubule) but also the severity of injury. It should be noted, however, that despite the theoretical advantage of identifying the primary site of nephron injury, thus far enzymatic markers have not proven effective in differentiating between predominate proximal or distal tubular involvement (D'Amico and Bazzi, 2003).

N-Acetyl- β -glucosaminidase (NAG), a proximal tubule lysosomal enzyme, is among the most extensively studied and best characterized urinary enzymes. It has proven to be a sensitive and robust indicator of AKI. Increased NAG levels have been reported with nephrotoxicant exposure (D'Amico and Bazzi, 2003; Emeigh Hart, 2005), delayed renal allograft function (Mukhopadhyay *et al.*, 2004), chronic glomerular disease (Bazzi *et al.*, 2002), diabetic nephropathy (Ikenaga *et al.*, 1993), as well as following cardiopulmonary bypass procedures (Ascione *et al.*, 1999). Westhuyzen *et al.* (2003) reported that urinary NAG levels (in addition to other tubular enzymes) were highly sensitive in detecting AKI in a population of critically ill adult patients, preceding increases in serum creatinine by 12 hours to 4 days. Chew *et al.* (1993) reported a poorer outcome (death in hospital, requirement for long term renal replacement therapy) in patients with higher urinary NAG levels on admission to a renal care unit, indicating a dose response by injury. The greater the urinary NAG in patients already diagnosed using AKI clinical criteria, the greater the incidence of the combined end point of dialysis or death (Liangos *et al.*, 2007). However, urinary NAG activity has been found to be inhibited by endogenous urea (Bondiou *et al.*, 1985) as well as a number of industrial solvents and heavy metals (Vaidya and Bonventre, 2006). In addition, increased NAG levels have been reported in a variety of conditions in the absence of clinically significant AKI, including rheumatoid arthritis (Iqbal *et al.*, 1998), impaired glucose tolerance (Fujita *et al.*, 2002), and hyperthyroidism (Tominaga *et al.*, 1989). As a result, insensitivity and nonspecificity may limit the use of NAG as a biomarker of nephrotoxicant induced AKI in some settings.

The glutathione S-transferases (GST) are a family of enzymes that hold promise as biomarkers of AKI. In animal and human studies, increased urinary alpha GST (α -GST), a proximal tubule cytosolic enzyme, has been reported in response to Compound A, a nephrotoxic metabolite of sevofluorane (Kharasch *et al.*, 1997; Goldberg *et al.*, 1999). Pai *et al.* (2005) reported increased urinary levels of α -GST and pi-GST, a distal tubule cytosolic enzyme, in male patients but not female patients following amphotericin B administration. In addition, Westhuyzen (2003) found both α - and pi GST isoforms to be sensitive and specific predictors of AKI in critically ill patients. Urinary quantification of GST isoenzymes requires a specific stabilization to ensure appropriate quantification.

A number of potential pitfalls have been noted with respect to the use of enzymuria in the setting of AKI. Although highly sensitive for renal injury, the utility of urinary enzymes has been clouded by the low threshold for release in response to physiologic conditions that may not precede clinically significant AKI (D'Amico and Bazzi, 2003). In such cases it is not clear whether this represents decreased specificity (enzymuria in the absence of injury) or increased sensitivity (enzymuria that indicates mild injury that does not elicit a change in serum creatinine, the existing reference standard). Perhaps more important in terms of practicality is the instability of many urinary enzymes and the specific processing necessary to ensure integrity in samples (Emeigh Hart, 2005). For example, the brush border enzymes alanine aminopeptidase (AA), alkaline phosphatase (AP), and γ -glutamyl transpeptidase (γ -GT) are stable for only 4 hours after urine collection and samples require gel filtration to eliminate interfering substances (Vaidya and Bonventre, 2006).

In general, the utility of urinary enzyme excretion as diagnostic or predictive biomarkers for nephrotoxic AKI remains an area that warrants additional investigation. Further characterization is needed to determine threshold levels indicative of clinically significant injury and if the theoretic advantage of identifying the primary site of tubule injury will translate to a clinically important advantage in terms of guiding intervention.

Filtered low-molecular weight proteins

Increased urinary excretion of filtered low-molecular weight proteins (β_2 -microglobulin [β_2 M], α_1 -microglobulin [α_1 M], retinol-binding protein [RBP], and cystatin-C [Cys-C]) is reflective of a primary defect in proximal tubular uptake, which may occur in the setting of cellular damage or overload. Produced at different extra-renal sites, these proteins are freely filtered by the glomerulus and reabsorbed but not secreted by proximal tubular cells. Injury to the proximal tubular epithelium leads to diminished microvillus surface area of the brush border membrane and associated reduced reabsorptive capacity. The resultant tubular proteinuria has been extensively studied as an index of tubular injury.

β_2 M is an 11.8 kilodalton (kDa) protein that is the light chain of the major histocompatibility class (MHC) I molecule expressed on the cell surface of all nucleated cells. β_2 M dissociates from the heavy chain in the setting of cellular turnover and enters the circulation as a monomer (Tolkoff-Rubin *et al.*, 1988). β_2 M is typically filtered by the glomerulus and almost entirely reabsorbed and catabolized by the proximal tubular cells (Miyata *et al.*, 1998), a process that is impeded with AKI. Increased urinary β_2 M excretion has been observed to be an early marker of tubular injury in a number of settings including

nephrotoxicant exposure (Schentag and Plaut, 1980), cardiac surgery (Fernandez *et al.*, 1988; Dehne *et al.*, 1995), and renal transplantation (Schaub *et al.*, 2005), preceding rises in serum creatinine by as much as 4 – 5 days (Tolkoff-Rubin *et al.*, 1988). Unfortunately, the utility of β_2 M as a biomarker has been limited by its instability in the urine, with rapid degradation observed at room temperature and in urine with a pH less than 6.0. In addition, it should also be noted that although β_2 M may serve as an early biomarker for AKI, it has been found to be poorly predictive of severe injury requiring renal replacement therapy (Herget-Rosenthal *et al.*, 2004).

α_1 M is a 27 – 33 kDa protein synthesized by the liver with approximately half of the circulating protein complexed to IgA. The free form is filtered by the glomerulus and reabsorbed by proximal tubule cells. Unlike β_2 M, urinary α_1 M is stable over the range of pH found in routine clinical practice (Yu *et al.*, 1983; Emeigh Hart, 2005). It has been found to be a sensitive biomarker for proximal tubular dysfunction even in the early phase of injury when no histologic damage is observable (Wolf and Boldt, 2007). Herget-Rosenthal *et al.* (2004) reported α_1 M to be an early indicator of unfavorable outcome (requirement for RRT) in a heterogeneous population of patients with non-oliguric AKI. Although sensitive immunoassays have been developed for the quantification of α_1 M, standardization is lacking. In addition, a number of conditions have been associated with altered plasma/serum levels including liver disease and HIV (Penders and Delanghe, 2004); therefore, urinary specificity and sensitivity for AKI may be suboptimal in these settings.

RBP is a 21 kDa protein that is hepatically synthesized and responsible for transporting vitamin A from the liver to other tissues. It is freely filtered by the glomerulus and subsequently reabsorbed and catabolized by the proximal tubule. Bernard *et al.* (1987) monitored patients with tubular injury from various etiologies including antibiotic and environmental toxicant exposure and found urinary RBP to be a highly sensitive indicator of renal tubule dysfunction, preceding urinary NAG elevation. In addition, they reported a favorable stability profile for RBP in acidic urine when compared to β_2 M (Bernard *et al.*, 1987). It should be noted that serum RBP levels are depressed in vitamin A deficiency and urinary levels may theoretically yield a false negative result in this setting (Tolkoff-Rubin *et al.*, 1988).

Cys-C is a 13 kDa protein that is believed to be one of the most important extracellular inhibitors of cysteine proteases. Serum concentrations appear to be independent of sex, age, and muscle mass. Cys-C is freely filtered by the glomerulus, reabsorbed and catabolized, but not secreted by the tubules. Over the last decade, serum Cys-C has been extensively studied and found to be a sensitive serum marker of GFR and a stronger predictor of risk of death and cardiovascular events in older patients than serum creatinine (Shlipak *et al.*, 2005). Urinary Cys-C levels have been found to be elevated in individuals with known tubular dysfunction (Uchida and Gotoh, 2002; Conti *et al.*, 2006). In addition, Herget-Rosenthal *et al.* reported that elevated urinary Cys-C levels were highly predictive of poor outcome (requirement for RRT) in a heterogeneous group of patients with initially non-oliguric AKI (Herget-Rosenthal *et al.*, 2004).

Microalbuminuria

Microalbuminuria, the pathologic excretion of urinary albumin at levels below the threshold of detection by conventional urinary dipstick (30 – 300 mg/L), is widely recognized as a useful marker of the development and progression of renal disease. Historically, microalbuminuria has been thought to herald the onset of altered glomerular structure and function. Recent evidence in rats, however, suggests that the normal glomerular filter actually leaks albumin at much higher levels than previously thought and albuminuria may reflect alterations in the proximal tubule cell retrieval pathway (Russo *et al.*, 2007). Therefore, microalbuminuria may prove to be a useful marker of AKI and concomitant proximal tubular cell damage. Microalbuminuria has previously been reported with short-term and long-term administration of nephrotoxic chemotherapeutics (Koch Nogueira *et al.*, 1998; Kern *et al.*, 2000) and antibiotics (Tugay *et al.*, 2006). Microalbuminuria, however, may also occur in the setting of vigorous exercise, hematuria, urinary tract infection, dehydration, fever, and poor glycemic control. Therefore, lack of specificity for AKI may compromise utility.

Kidney injury molecule-1 (KIM-1)

KIM-1 is a 36 kDa (unglycosylated) type I cell membrane glycoprotein containing a unique six-cysteine immunoglobulin-like domain and a mucin domain in its extracellular portion (Ichimura *et al.*, 1998). Rat and human cDNAs encoding KIM-1 (Kim-1 in the rat) were initially identified by our group using representational difference analysis, a polymerase-chain reaction-based cDNA subtraction analysis designed to identify genes with differential expression between normal and regenerating kidneys following ischemia/reperfusion (I/R) injury (Ichimura *et al.*, 1998). KIM-1 is found to be expressed at low to undetectable levels in the normal adult rat kidney but dramatically upregulated in the post-ischemic kidney (Ichimura *et al.*, 1998). Our group later reported that the KIM-1 ectodomain was shed from cells *in vitro* (Bailly *et al.*, 2002) and into the urine *in vivo* in rodents and humans after proximal tubular injury of varying etiology (Ichimura *et al.*, 2004; Humphreys *et al.*, 2005; Prozialeck *et al.*, 2007; Zhou *et al.*, 2007). In animal and human studies, KIM-1 has been found to be an early indicator of AKI that compares favorably to a number of conventional biomarkers and tubular enzymes (Han *et al.*, 2002; Vaidya *et al.*, 2006). In the setting of cisplatin exposure as well as renal ischemia/reperfusion (I/R) in rats, Kim-1 was found to have superior sensitivity for detecting AKI than serum creatinine and BUN or urinary NAG, glycosuria, and proteinuria (Vaidya *et al.*, 2006). Adjusted for age, gender, and length of time delay between insult and sampling, a one-unit increase in normalized KIM-1 was associated with a greater than 12-fold increase in the presence of acute tubular necrosis (ATN) (Han *et al.*, 2002). Ongoing studies have shown KIM-1 to be a sensitive, specific, and stable biomarker of AKI in both cross-sectional and prospective adult clinical studies (Vaidya & Waikar, unpublished data).

Neutrophil gelatinase-associated lipocalin (NGAL)

Human NGAL is a 25 kDa protein initially identified bound to gelatinase in specific granules of the neutrophil. NGAL is synthesized during granulocyte maturation in the bone marrow (Borregaard *et al.*, 1995), but also may be induced in epithelial cells in the setting of

inflammation or malignancy (Nielsen *et al.*, 1996). Later, NGAL expression was found to be upregulated in the post-ischemic rat kidney (Mattheus *et al.*, 2001). Using cDNA microarray screening techniques, Devarajan and colleagues reported NGAL to be one of seven genes whose expression was upregulated > 10-fold within the first few hours after ischemic renal injury in a mouse model (Mishra *et al.*, 2003). Immunohistochemistry studies demonstrated minimal NGAL expression in control mouse kidneys, but marked upregulation in proximal tubules within 3 hours of ischemia (Mishra *et al.*, 2003). Increased NGAL levels were readily detected in the urine of rodents following I/R injury and after cisplatin exposure, far preceding changes in serum creatinine as well as the appearance of urinary β_2 M and NAG (Mishra *et al.*, 2003; Mishra *et al.*, 2004). As a result, NGAL has generated much interest as a sensitive early biomarker for diagnosing AKI. A prospective study of pediatric patients undergoing cardiopulmonary bypass for cardiac corrective surgery found urinary NGAL to be a powerful early marker of AKI, preceding any increase in serum creatinine by 1 – 3 days (Mishra *et al.*, 2005). This study, however, specifically excluded patients with known nephrotoxin exposure. A similar study of adult patients showed urinary NGAL levels at 1, 3, and 18 hours after cardiac surgery to be significantly higher in patients who went on to develop clinically significant AKI (Wagener *et al.*, 2006). It should be noted that serum NGAL levels are known to rise in the setting of a number of inflammatory and infective conditions (Xu *et al.*, 1995; Ohlsson *et al.*, 2003). Further studies are required to determine specificity of urinary NGAL for AKI in the setting of sepsis, a condition frequently associated with clinically significant renal injury.

Interleukin-18 (IL-18)

IL-18, formerly known as interferon- γ -inducing factor, is produced as a 24 kDa inactive precursor that is cleaved by caspase-1 to generate its mature, biologically active form (Ghayur *et al.*, 1997; Gu *et al.*, 1997; Gracie *et al.*, 2003). IL-18 has been found to have broad immunomodulatory properties and appears to play a critical role in host defense against a number of infections (Gracie *et al.*, 2003). Parikh *et al.* reported increased levels of IL-18 in patients with AKI of varying etiology, especially those with delayed renal allograft function and ischemic ATN (Parikh *et al.*, 2004). Following transplantation, a rapid decline in urinary IL-18 levels was predictive of a steeper decline in serum creatinine concentrations postoperative days 0 – 4 (Parikh *et al.*, 2004). Immunohistochemical staining of renal transplant protocol biopsies revealed constitutive IL-18 expression in the distal tubular epithelium. There was strong positive immunoreactivity in the proximal tubules of patients with acute rejection. There was also strong immunoreactivity in infiltrating leukocytes, and endothelium, suggesting upregulation in the setting of immunopathological reactions (Striz *et al.*, 2005). In a study of critically ill adult patients with acute respiratory distress syndrome (ARDS), increased urinary IL-18 was found to be an early marker of AKI, preceding changes in serum creatinine by 1 – 2 days, and was an independent predictor of death (Parikh *et al.*, 2005). Faubel *et al.* reported increased levels of kidney IL-18 in mice with cisplatin – induced AKI (Faubel *et al.*, 2007); however, the role of IL-18 as a potential urinary marker of nephrotoxic AKI remains largely unexplored.

Fatty acid binding protein (FABP)

The fatty acid binding proteins (FAPB) are small cytoplasmic proteins abundantly expressed in tissues with an active fatty acid binding metabolism. The primary function of FABP is the facilitation of long-chain free fatty-acid transport from the plasma membrane to sites for oxidation (mitochondria, peroxisomes) (Oyama *et al.*, 2005; Pelsers *et al.*, 2005). Two types of FABP have been identified in the human kidney, liver-type FABP (L-FABP) in the proximal tubule and heart-type FABP (H-FABP) in the distal tubule (Maatman *et al.*, 1991; Maatman *et al.*, 1992). H-FABP levels have been found to be a sensitive marker for aminoglycoside induced kidney injury in rats (Pelsers *et al.*, 2005). In addition, higher levels of H-FABP, as well as other biomarkers, in kidney perfusates were determined to be useful adjuncts to routine indicators (donor age, donor medical history, macroscopic appearance, warm ischemic time, and ex-vivo perfusion) of suitability of non-heart beating donor kidneys for transplantation (Gok *et al.*, 2003a; Gok *et al.*, 2003b; Pelsers *et al.*, 2005). Clinical studies into the utility of H-FABP as a urinary biomarker in more conventional human models of AKI are lacking.

Urinary L-FABP has been studied extensively in preclinical and clinical models and has been found to be a potential biomarker in a number of pathologic conditions, including chronic kidney disease, diabetic nephropathy, IgA nephropathy, and contrast nephropathy. Kamijo *et al.* reported increasing L-FABP levels with deterioration of renal function in those with non-diabetic CKD (Kamijo *et al.*, 2006). In addition, Nakamura *et al.* have reported that urinary L-FABP may serve as a non-invasive biomarker to discriminate between IgA nephropathy and thin basement membrane disease (Nakamura *et al.*, 2005) as well as a potential predictive marker for contrast induced nephropathy (Nakamura *et al.*, 2006). Yamamoto *et al.* recently reported urinary L-FABP to be highly correlated with ischemia time as well as hospital stay in a small cohort of living related donor renal transplant recipients (Yamamoto *et al.*, 2007). In addition, we have performed preliminary studies that suggest that L-FABP is an early indicator of AKI in the setting of aminoglycoside administration, preceding changes in serum creatinine (Ferguson *et al.*, 2007).

Urinary L-FABP may be non-specific for AKI in the setting of acute liver injury. Although Kamijo *et al.* reported urinary L-FABP levels in patients with liver disease to be similar to levels in healthy volunteers, it is unclear if these patients suffered from acute or chronic liver injury (Kamijo *et al.*, 2006). This will be important to investigate as AKI and acute liver injury commonly co-occur in the critically ill population.

Other Biomarkers

A number of other biomarkers of AKI have been proposed, but require further characterization to determine clinical utility. Na⁺/H⁺ exchanger isoform 3 and Fetuin-A, are exosomal associated proteins found to be excreted in the urine of rats and humans in the setting of AKI (du Cheyron *et al.*, 2003; Zhou *et al.*, 2006a; Zhou *et al.*, 2006b). Unfortunately, issues with assay throughput and sensitivity currently limit the quantification of exosomal-associated proteins, limiting their practical use in large-scale studies of AKI. Cysteine-rich protein (Cyr61) was found to be rapidly induced and excreted into the lumen of the proximal tubules following bilateral I/R injury in rats; however, levels fell quickly

despite progression of injury (Muramatsu *et al.*, 2002). Clusterin is a multifaceted glycoprotein that has been studied extensively in a variety of preclinical AKI models, including I/R injury, nephrotoxicant induced injury, and unilateral ureteral obstruction (Rosenberg and Paller, 1991; Witzgall *et al.*, 1994; Chevalier, 1996; Silkensen *et al.*, 1997; Kharasch *et al.*, 2006). There are currently no clinical studies evaluating urinary clusterin as a potential biomarker in AKI. Osteopontin, a potent chemoattractant of mononuclear cells, has been investigated extensively in animals and humans. Immunohistochemical and in situ hybridization studies have demonstrated upregulated expression in a number of conditions associated with AKI, including I/R injury, nephrotoxicant exposure, and renal allograft rejection (Persy *et al.*, 1999; Verstrepen *et al.*, 2001; Xie *et al.*, 2001; Iguchi *et al.*, 2004; Alchi *et al.*, 2005; Basile *et al.*, 2005). There are currently no published clinical studies evaluating urinary osteopontin as a potential biomarker in AKI, although it is known to be excreted in the urine and there are commercially available ELISA kits that may be used for quantification. Renal papillary antigen (RPA-1) has been studied as a marker of collecting duct injury and increased urinary levels have been noted in response to a number of nephrotoxins in rats (Hildebrand *et al.*, 1999); however, human studies are lacking.

Future Directions

Efforts to identify biomarkers to assist with the early diagnosis of AKI have yielded many promising candidates. To date, most studies have emphasized discovery, characterization, and validation of individual biomarkers using a single model of kidney injury. This approach is necessary in the initial stages of biomarker development; however, translation to general clinical applicability requires considerable additional work.

In the clinical setting, an ideal biomarker for the detection of AKI is one that is easily obtained, easily and rapidly measured, sensitive to minor disturbances in normal function, site specific, highly correlated with the degree of injury, and indicative of injury progression and regression. A single biomarker is rarely adequate to clearly define a particular pathologic state (Rifai *et al.*, 2006; Fliser *et al.*, 2007). Given inherent renal heterogeneity and the disparate settings under which kidney injury occurs, a panel of carefully selected biomarkers may prove to be most appropriate. Development of such a panel will require large, well-designed prospective studies comparing multiple biomarkers in the same set of urine samples over extended time courses. Such studies will allow temporal patterns of biomarker elevation to be established, as well as patterns that may be specific to the mechanism of nephrotoxicant induced injury, population of interest (elderly, pediatric, etc.), and/or co-occurring disease states (diabetes, heart disease, sepsis, etc.). Different biomarkers will likely provide different information. Some will reflect systemic influences; others will reflect the location and extent of tubular injury; whereas others will reflect the functional consequences of tubular injury.

Conclusion

Nephrotoxic AKI is a common condition associated with considerable morbidity and mortality. Despite impressive advances in our understanding of the pathophysiology and optimization of care for those with AKI, outcomes have remained little changed over the last

50 years. Urinary proteins have been identified that are highly sensitive and specific for kidney injury. Further investigative efforts will not only define the utility of these proteins as biomarkers, but also enhance our understanding of AKI pathophysiology and aid in the development of targeted interventions to ameliorate injury. Additional characterization and validation of individual biomarkers and biomarker panels in AKI will ultimately result in earlier diagnosis of kidney injury, improved prognosis of outcome, as well as new strategies for stratification of patients for interventional trials.

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Figure 1. Common Nephrotoxicants : Site and Mechanism of Injury

ACE-I: angiotensin-converting enzyme inhibitors; ARB: angiotensin II receptor blockers; NSAID: nonsteroidal anti-inflammatory drugs

Table 1

Biomarkers of Nephrotoxic Acute Kidney Injury

Biomarker	Comments	References
<i>Urinary Proteins with Enzymatic Activity</i>		
Alanine aminopeptidase	1 Proximal tubule brush border enzyme	(Mueller <i>et al.</i> , 1986; Rybak <i>et al.</i> , 1987; Holdt-Lehmann <i>et al.</i> , 2000; Mukhopadhyay <i>et al.</i> , 2004)
	2 Instability may limit clinical utility	
Alkaline phosphatase	1 Proximal tubule brush border enzyme. Human intestinal alkaline phosphatase is specific for proximal tubular S3 segment; human tissue non-specific alkaline phosphatase is specific for S1 and S2 segments.	(Jung <i>et al.</i> , 1982; Nuyts <i>et al.</i> , 1994; Han <i>et al.</i> , 2002)
	2 Levels may not correlate with extent of functional injury	
	3 Instability may limit clinical utility	
α -Glutathione-S-transferase	1 Proximal tubule cytosolic enzyme	(Eger <i>et al.</i> , 1997; Kharasch <i>et al.</i> , 1997; Cressey <i>et al.</i> , 2002; Van Kreel <i>et al.</i> , 2002; Westhuyzen <i>et al.</i> , 2003; Pai <i>et al.</i> , 2005)
	2 Requires stabilization buffer for specimen storage and processing	
γ -Glutamyl transpeptidase	1 Proximal tubule brush border enzyme	(Mueller <i>et al.</i> , 1986; Jung and Grutzmann, 1988; Emeigh Hart, 2005)
	2 Instability requires samples to be analyzed quickly after collection, limiting clinical utility	
π -Glutathione-S-transferase	1 Distal tubule cytosolic enzyme	(Eger <i>et al.</i> , 1997; Cressey <i>et al.</i> , 2002; Westhuyzen <i>et al.</i> , 2003; Pai <i>et al.</i> , 2005)
	2 Requires stabilization buffer for specimen storage and processing	
N-acetyl- β -D-glucosaminidase	1 Proximal tubule lysosomal enzyme	(Bondiou <i>et al.</i> , 1985; D'Amico and Bazzi, 2003; Westhuyzen <i>et al.</i> , 2003; Mukhopadhyay <i>et al.</i> , 2004; Emeigh Hart, 2005; Vaidya and Bonventre, 2006)
	2 More stable than other urinary enzymes	
	3 Extensive clinical data in a variety of conditions (nephrotoxicant exposure, cardiopulmonary bypass, delayed renal allograft function, etc.)	
	4 Endogenous urea may inhibit activity	
<i>Filtered low-molecular weight proteins</i>		
α_1 -microglobulin	1 Synthesized by the liver	(Yu <i>et al.</i> , 1983; Herget-Rosenthal <i>et al.</i> , 2004; Emeigh Hart, 2005; Wolf and Boldt, 2007)
	2 Filtered by the glomerulus and reabsorbed by proximal tubule cells	
	3 Early marker of tubular dysfunction; high levels may predict poorer outcome	
	4 Stable across physiologic urinary pH	

Biomarker	Comments	References
β_2 -microglobulin	1 Light chain of the MHC I molecule expressed on the cell surface of all nucleated cells	(Tolkoff-Rubin <i>et al.</i> , 1988; Chapelsky <i>et al.</i> , 1992; Dehne <i>et al.</i> , 1995; Miyata <i>et al.</i> , 1998; Schaub <i>et al.</i> , 2005)
	2 Monomeric form is filtered by the glomerulus and reabsorbed by the proximal tubule cells	
	3 Early marker of tubular dysfunction in a variety of conditions	
	4 Instability in acidic urine limits clinical utility	
Cystatin-C	1 Important extracellular inhibitor of cysteine proteases	(Uchida and Gotoh, 2002; Herget-Rosenthal <i>et al.</i> , 2004; Shlipak <i>et al.</i> , 2005; Confi <i>et al.</i> , 2006)
	2 Filtered by the glomerulus and reabsorbed by proximal tubule cells	
	3 Elevated urinary levels reflect tubular dysfunction; high levels may predict poorer outcome	
Retinol binding protein	1 Synthesized by liver, involved in vitamin A transport	(Bernard <i>et al.</i> , 1987; Tolkoff-Rubin <i>et al.</i> , 1988; Roberts <i>et al.</i> , 1990)
	2 Filtered by glomerulus and reabsorbed by proximal tubule cells	
	3 Early marker of tubular dysfunction	
	4 Increased stability in acidic urine when compared to p2-microglobulin	
<i>Heart-type fatty acid binding protein</i>		
	1 Expressed in distal tubule epithelial cells	(Maatman <i>et al.</i> , 1992; Gok <i>et al.</i> , 2003a; Gok <i>et al.</i> , 2003b; Pelsers <i>et al.</i> , 2005)
	2 Functions in intracellular fatty acid transport to sites of p-oxidation	
<i>Interleukin-18</i>		
	1 Cytokine with broad immunomodulatory properties, particularly in setting of ischemic injury	(Melnikov <i>et al.</i> , 2001; Pomerantz <i>et al.</i> , 2001; Melnikov <i>et al.</i> , 2002; Gracie <i>et al.</i> , 2003; Parikh <i>et al.</i> , 2004; Parikh <i>et al.</i> , 2005; Striz <i>et al.</i> , 2005)
	2 Constitutively expressed in distal tubules; strong immunoreactivity in proximal tubules with transplant rejection	
	3 Elevated urinary levels found to be early marker of AKI and independent predictor of mortality in critically ill patients	
<i>Kidney Injury Molecule-1</i>		
	1 Type-1 cell membrane glycoprotein upregulated in dedifferentiated proximal tubule epithelial cells	(Ichimura <i>et al.</i> , 1998; Han <i>et al.</i> , 2002; Ichimura <i>et al.</i> , 2004; Vaidya <i>et al.</i> , 2005; Vaidya <i>et al.</i> , 2006)
	2 Protein ectodomain found to be shed in urine following acute injury	
	3 Elevated urinary levels found to be highly sensitive and specific for AKI	
<i>Liver-type fatty acid binding protein</i>		
	1 Expressed in proximal tubule epithelial cells	(Maatman <i>et al.</i> , 1991; Ek-Von Mentzer <i>et al.</i> , 2001; Kamijo <i>et al.</i> , 2004; Nakamura <i>et al.</i> , 2005; Pelsers <i>et al.</i> , 2005; Kamijo <i>et al.</i> , 2006; Nakamura <i>et al.</i> , 2007; Yamamoto <i>et al.</i> , 2007; Ferguson <i>et al.</i> , unpublished data)
	2 Important functions include intracellular fatty acid transport and possible sequestration of reactive fatty acid oxidation products	
	3 Increased levels reported in ischemic and nephrotoxic AKI; CKD	

Biomarker	Comments	References
<i>Microalbumin</i>	1 Established marker for monitoring progression of chronic kidney disease	(Koch Nogueira et al., 1998; Kern et al., 2000; Tugay et al., 2006; Russo et al., 2007)
	2 Elevated urinary levels may be indicative of proximal tubule dysfunction	
	3 Lack of specificity for AKI may limit utility	
<i>Neutrophil gelatinase-associated lipocalin</i>	1 Initially identified bound to gelatinase in specific granules of the neutrophil, but also may be induced in epithelial cells in the setting of inflammation or malignancy	(Borregaard et al., 1995; Cowland and Borregaard, 1997; Cowland et al., 2003; Mishra et al., 2003; Mishra et al., 2005; Mishra et al., 2006; Wagener et al., 2006)
	2 Expression upregulated in proximal tubule cells following ischemic renal injury	
	3 Found to be an early indicator of AKI following cisplatin administration	