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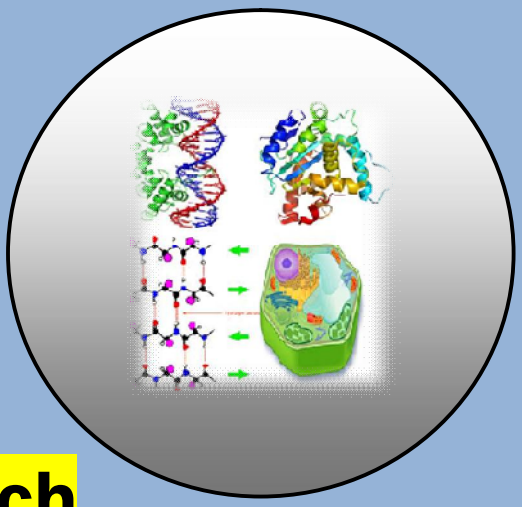

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REVIEW ARTICLE

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Novel Biomarkers in Early Diagnosis of Renal Disease through Proteomics Study of Urine and Serum

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ABSTRACT

Acute kidney injury (AKI) is a common and serious condition, the diagnosis of which depends on serum creatinine (S. Cr.), which is a delayed and unreliable indicator of AKI. The lack of early biomarkers has limited our ability to translate promising experimental therapies to human AKI. Furthermore, kidney injury can be present in the absence of kidney dysfunction. Renal reserve enables normal GFR even when nephrons are damaged. Renal biomarkers, especially those present in urine, may be useful for the study of both acute and chronic nephropathies. In current clinical practice, blood urea nitrogen, serum creatinine level and urine output are the most frequently used indicators of renal dysfunction despite their known limitations such as bias due to non-renal factors. They have limited sensitivity and specificity and creatinine level has a slow rate of change, thus limiting their usefulness in the early detection of AKI. The aim of this article is to review the literature related to AKI biomarker with a focus on the context in which they should serve to add to the clinical challenges facing physicians caring for patients with, or at-risk for, AKI. The optimal and appropriate utilization of AKI biomarkers will only be realized by understanding their characteristics and placing reasonable expectations on their performance in the clinical arena.

Key words: AKI, Clinical proteomics, Urinary proteomics, Biomarker, Kidney diseases and Bio-fluids.

INTRODUCTION

Acute kidney injury (AKI) formerly referred to as acute renal failure (ARF) is a common clinical problem. The term ARF was first coined by Homer W. Smith(Smith 1951) in his textbook in which he referred to kidney dysfunction related to traumatic injuries. Urine has become one of the most attractive bio fluids in clinical proteomics, for its procurement is easy and noninvasive and it contains sufficient proteins and peptides. Urinary proteomics has thus rapidly developed and has been extensively applied to biomarker discovery in clinical diseases, especially kidney diseases. A biomarker should temporally reflect the pathophysiology initiated by a stimulus leading to injury so as to alert the clinician to a potentially reversible stage of the illness.Discharge coding data from a 5% sample of United States Medicare beneficiaries (n = 5.4 million) demonstrated an 11% annual increase in AKI prevalence in hospitalized adults between 1992 and 2001 (the prevalence increased from 14.2 to 34.6 AKI cases per 100 patient discharges)(Xue, Daniels *et al.* 2006). AKI rates likewise increased 20-fold, from 0.5 to 9.9 cases per 1,000 hospitalized children, between 1982 to 2004(Vachvanichsanong, Dissaneewate *et al.* 2006). Acute kidney injury (AKI; or acute renal failure) is an important issue for patients during critical care, with sepsis being the most common trigger for AKI in the intensive care unit (ICU)(Medve, Antek *et al.* ; Uchino, Kellum *et al.* 2005). Because of the lack of sensitive and specific biomarkers for indicating renal cell injury, the mortality rates for septic AKI have remained high(Medve, Antek *et al.* ; Parmar, Langenberg *et al.* 2009; Uchino, Kellum *et al.* 2005). Recently, genomic, transcriptomic, and proteomic techniques have identified neutrophil gelatinaseassociated lipocalin (NGAL) as an early marker of AKI(Matejovic, Chvojka *et al.* ; Mishra, Ma *et al.* 2003). NGAL has been investigated in a range of different clinical settings, such as contrast-induced nephropathy, AKI after cardiac surgery or kidney transplantation and AKI in the critical care setting. Overall, the sensitivity for NGAL to predict AKI is 0.815 (95 % confidence interval, 0.732-0.892) and it is a promising biomarker for AKI, similar to troponin for acute myocardial infarction(Supavekin, Zhang *et al.* 2003). Over the last 10 years several international guideline groups have tried to establish consistent definitions and staging systems for AKI, namely the RIFLE (Risk, Injury, Failure, Loss, End stage kidney disease) system,(Ricci, Cruz *et al.* 2008) which was modified by the AKIN (Acute Kidney Injury Network) group (Bagga, Bakkaloglu *et al.* 2007) and further developed by KDIGO (Kidney Disease: Improving Global Outcomes)(Kellum and Lameire*et al.*,2013).The clinical assessment criteria for AKI are serum creatinine (S. Cr), blood urea nitrogen and urine output levels, where by a rise in measurable quantities of S. Cr and blood urea associated with a decrease in urine levels is deemed indicative of AKI, especially if these occur rapidly within 48 hours.

Risk factors and triggers of AKI induction

There is a considerable amount of clinical information available relating to observed AKI cases over the last 30 years that allows a wide-ranging analysis of AKI predisposition and causative agents. General risk factors are age greater than 65 years, heart failure, liver disease, diabetes, chronic kidney disease with or without diabetes, sepsis, urological obstruction, iodinated contrast agents, nephrotoxic medication and hypovolaemia/ shock(Ftouh and Lewington., *et al.*,2014).

AKI can be induced by many different events such as rapid blood loss to and from the kidney, vasoconstrictive drugs, exposureto harmful substances, hypotension linked to sepsis, and obstruction of the urinary tract. **Table 2** lists the main factors that can lead to AKI,(Hilton 2006)where surgical procedures or medication are often precursors.

Table 1.The Classification of AKI(Medve, Antek *et al.,2011*).

Stage/Category	Serum Creatinine Criteria	Urine Output Criteria
Risk	Increased serum creatinine level ≥0.3 mg/dl or 150% - 200% increase from baseline	Urine production <0.5 ml/kg/hr. for >6 hrs.
Injury	Increased serum creatinine level >200%-300%	Urine production <0.5 ml/kg/hour for > 12 hours
Failure	Increased serum creatinine level from baseline (or ≥4 mg/dl) (acute increase of ≥0.5 mg/dl)	Urine production <0.3 ml/kg/hour x 24 hours or anuria in 12 hours
Loss	Persistent ARF = complete loss of renal function >4 weeks	
ESKD	End Stage Kidney Disease >3 months	

Table 2.Events leading to AKI induction.

Site	Trigger
Pre-renal	-Volume depletion due to hemorrhage, severe vomiting or diarrhea, burns -Edema due to cardiac failure, cirrhosis, nephrotic syndrome -Hypotension due to cardiogenic shock, sepsis, anaphylaxis -Cardiovascular due to severe cardiac failure, arrhythmias -Renal hypoperfusion induced by non-steroidal anti-inflammatory drugs (NSAIDs) or specific enzyme inhibitors or receptor blockers involved in the renin-angiotensin axis, abdominal aortic aneurysm, renal artery stenosis or occlusion, hepatorenal syndrome
Renal	-Glomerular disease due to inflammation (glomerulonephritis), thrombosis, hemolytic uraemic syndrome -Tubular injury due to acute tubular necrosis following prolonged ischaemia, and nephrotoxins such as aminoglycosides, radiocontrast media, cisplatin, heavy metals -Acute interstitial nephritis due to drugs (e.g. NSAIDs), infection or autoimmune diseases -Vascular disease including vasculitis, cryoglobulinaemia, polyarteritisnodosa, thrombotic microangiopathy, cholesterol emboli, renal artery stenosis, renal vein thrombosis, malignant hypertension -Eclampsia
Post-renal	-Urinary tract obstructions due to Calculus formation (i.e. kidney stones), urethral stricture, prostatic hypertrophy or malignancy, blood clot -Papillary necrosis -Bladder tumor -Radiation and retroperitoneal fibrosis -Pelvic malignancy

Proteomics

Proteins are the main actors in biological processes, and their diversity is much greater than that of genes. Proteomics is a large-scale study of proteins and their function and structure. Proteome composition is constantly changing and varies with physiological changes. Although proteins are products of genes, multiple distinct protein isoforms can be created from the same gene. Proteins are further susceptible to posttranslational modifications (PTMs). Isoforms and PTMs are detectable only by studying the proteins directly and can be indicative of diverse protein functions. Furthermore, there is evidence that only one third of proteins with altered expression display a concomitant change in mRNA expression(Cagney, Park *et al.* 2005). Thus proteomics seems to reflect the actual cellular processes more accurately than do genomics or transcriptomics.Proteomic studies can be discovery based or targeted.Discovery approaches, which predominate in renal literature, can be either broad, when 2 types of samples are compared in a qualitative or quantitative sense, or focused, such as with investigations of protein– protein interactions. Broad approaches can involve taking on a global examination of the entire proteome, or evaluation of a specific subproteome, such as all proteins with a particular PTM.In a targeted approach the investigator has previous knowledge of the protein candidates of interest and evaluates them in different types of samples using single-reaction or multiple-reaction monitoring (MRM). In this approach, MS analyzers with high sensitivity and high dynamic range are required. As an example, Quintana and colleagues searched for markers of interstitial fibrosis and tubular atrophy not otherwise specified (IF/TA-NOS)(Quintana, Campistol *et al.* 2009). Raw data generated by MS are processed by sophisticated computer algorithms leading to peptide and protein identification and quantification. Owing to the nature of peptide and protein assignment by database searching, additional methodologies are used to verify the candidates of interest. Verified candidates should be subjected to testing in a new cohort of patients. Large-scale validations involving prospective studies are the ultimate tests of biomarker adequacy.Despite its promise, the translation of proteomic biomarkers to the clinic from a plethora of studies has been slow. Why? First, there is a lack of a unifying method for sample collection or analysis. This is true of all biosamples including urine, for which composition is influenced by diet,(Thongboonkerd*et al.,* 2007) timing of collection,(Lafitte, Dussol *et al.* 2002; Schaub, Rush *et al.* 2004) exercise,(Hortin and Sviridov 2007) sex,(Schaub, Rush *et al.* 2004) and age(Zurbig, Decramer *et al.* 2009). Generation of a universal protocol for urine collection is the focus of international organizations(Vlahou, Schanstra *et al.* 2008; Yamamoto). Urine is a dilute bio-fluid that requires protein concentration, particularly in gel-based proteomics studies(Thongboonkerd, Klein *et al.* 2004). Different methods for urine concentration and protein isolation will yield distinct proteins. Numerous methods have been used for urine concentration, including precipitation with organic solvent, centrifugal filtration, ultracentrifugation, and lyophilization (Thongboonkerd 2007; Thongboonkerd, Chutipongtanate *et al.* 2006). Furthermore, it remains unclear which approaches are most effective: addition of protease inhibitors, depletion of high-abundance proteins, and collection of spot urine v/s collection of urine for a longer time period. Urine spot collections can be dilute or concentrated depending on their water content, thus requiring adjustment of biomarker concentrations.

The most common adjustment factor is urine creatinine, although specific gravity also has been used. Urine creatinine has been validated as a normalization factor for urine albumin and total protein. Specific gravity is a measure of the weight of the solution compared to that of an equalvolume of distilled water and is determined by both thenumber and size of particles in the solution. Specificgravity can be problematic, particularly when largermolecules are present in urine(Zwelling and Balow*et al.,* 1978). Only a few studieshave compared biomarker normalization with eitherurine creatinine or specific gravity,(Andersson, Haraldsson *et al.* 2008; Gaines, Fent *et al.* ; Heavner, Morgan *et al.* 2006; Moriguchi, Inoue *et al.* 2009) and theirconclusions have varied. Depending on the biomarkertested either normalization method may be adequate.If protein or peptide ratios are evaluated as biomarkers,normalization may not be necessary. In acute kidneyinjury (AKI), in which the steady state is lost, longercollections may be preferred (Waikar, Sabbisetti *et al.,2010*) overspot samples.

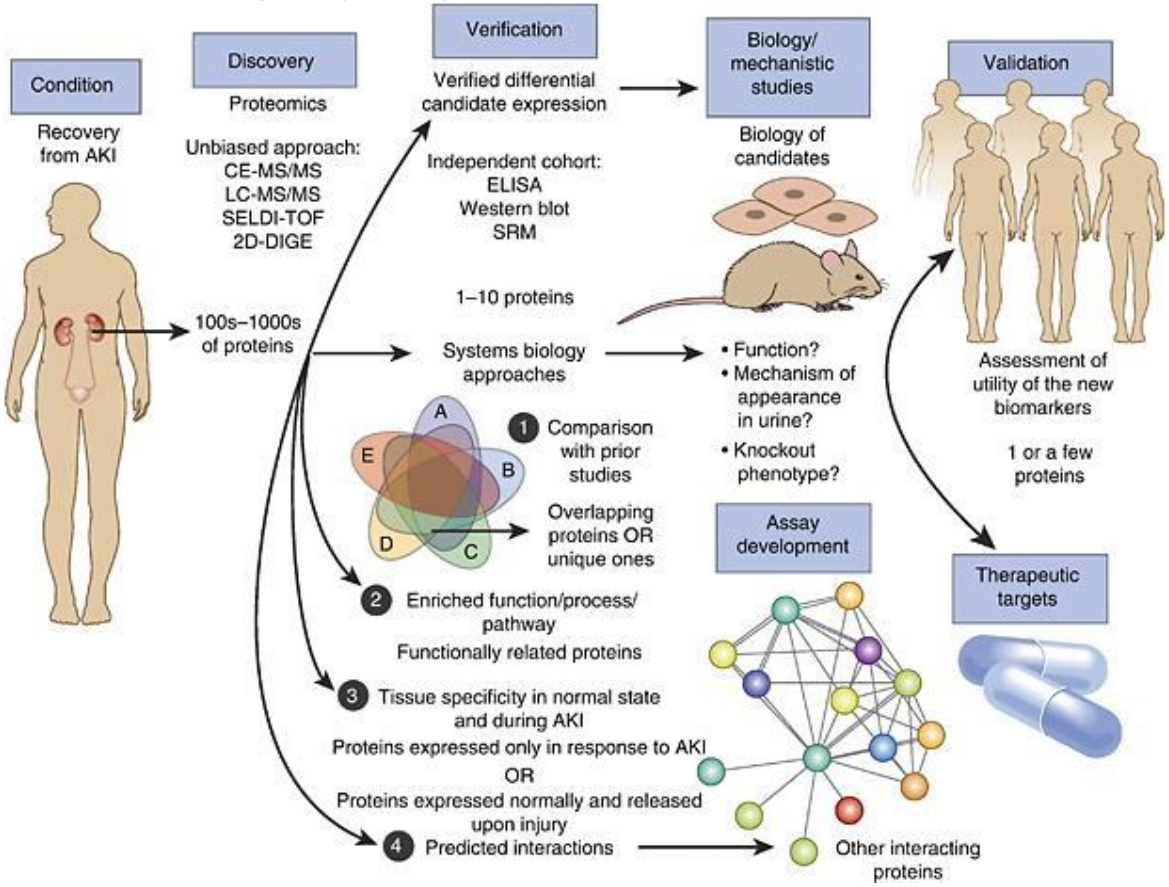

Figure 1.A proposed model for studying protein biomarkers discovered through unbiased mass spectrometry-based approaches: (Konvalinka*et al.,2014*)

The initial step involves a careful selection of the patient population. Subsequent discovery is made by using one of the fractionation approaches coupled to mass spectrometry analysis to identify proteins including those differentially regulated between conditions of interest.

Discovery is followed by verification in a similar but independent cohort of patients. Another potential approach involves using systems biology to narrow down or expand the candidate list. This approach can include comparison with prior studies, the search for enriched functional processes among candidate proteins, evaluation of tissue specificity of the candidate(s), and the search for predicted interaction, which may also be of potential interest. The candidates of interest should then undergo mechanistic studies to inform their role in acute kidney injury. In addition, robust and sensitive assays would need to be developed if they are not already available. Finally, if proven to be of biological utility, a few potential markers should be validated in a large-scale study. Lastly, development of novel therapies based on the processes involved in the pathogenesis and progression of disease may be of tremendous potential. AKI, acute kidney injury; CE, capillary electrophoresis; 2D-DIGE, two-dimensional fluorescence-difference gel electrophoresis; ELISA, enzyme-linked immunosorbent assay; LC, liquid chromatography; MS/MS, tandem mass spectrometry; SELDI-TOF, surface-enhanced laser desorption/ionization–time of flight; SRM, selected reaction monitoring.

Urine Proteome Composition

Under normal physiologic conditions, urine contains <20 mg of albumin and <150 mg of total protein per day. Approximately 30% of the protein content derives from plasma, whereas 70% is produced by the kidney and lower urinary tract(Pieper, Gatlin *et al.* 2004). Normal urine contains at least 1500 proteins, most of which are extracellular and membrane bound(Adachi, Kumar *et al.* 2006). To appear in urine, proteins or their fragments must be filtered at the glomerulus and bypass or otherwise avoid tubular reabsorption. Alternatively, they must be secreted by the kidney or lower urinary tract directly into urine. During plasma filtration in the glomeruli, the glomerular capillary wall discriminates among molecules of different size, charge, and configuration. The filtration barrier consists of the fenestrated endothelium, glomerular basement membrane, and slit diaphragm. Typically, proteins <20 kDa are freely filtered, whereas those<60 kDa are almost completely restricted. Despite this filtering, the most abundant urine protein is albumin, a negatively charged molecule with a molecular weight slightly more than 66.4 kDa. It is thought that the relative abundance of albumin in urine is a result of the presence of large pores able to filter albumin, immunoglobulin's, and other macromolecules(Deen, Bridges *et al.* 1985). The actual amount of albumin filtration is unknown, but is thought to be in the order of 2–4 g/day. Renal hemodynamics and serum concentration of a particular protein also determine the extent of filtration. For example, hyperfiltration states such as pregnancy result in increased filtration of most proteins. Overproduction diseases such as multiple myeloma result in filtration of large amounts of immunoglobulin light chains(Konvalinka, Scholey *et al.,2012*). In addition to being an important source of proteins, urine appears to be enriched with peptides of <750 Da. A study of the normal urine peptidome evaluated polypeptides in multiple fractions segregated on the basis of size(Norden, Sharratt *et al.* 2004). Tubules reabsorb most of the filtered proteins. Proximal tubules also catabolize proteins and excrete their peptides in urine. Albumin is reabsorbed via endocytosis mediated by megalin and cubilin receptors in proximal tubules.

Tubules secrete proteins directly into urine as a result of the normal maintenance process or in response to injury. Tubular injury could result in decreased reabsorption or catabolism of the filtered proteins and in secretion of tubular proteins in response to the injury.

Emerging Novel Early Biomarkers

AKI biomarkers can be components of serum or urine. Urine biomarkers are quite promising to detect early AKI, hence it can be anticipated earlier; therefore, it could be useful for early diagnosis, identification of mechanism disorders and determination of location and severity of dysfunction(Vaidya, Ferguson *et al.* 2008). The term biomarker (acronym for biological marker) was first described in 1989, which means measurable indicator for a specific biologic condition and for specific disease process. In 2001, biomarker definition were standardized to be a characteristic that can be measured and evaluated as normal biological process, pathological process or pharmacological response to therapeutic intervention. Moreover, the Food Drug and Administration (FDA) use biomarker term to describe any diagnostic indicator that can be measured and used to assess any risk or disease. The ideal biomarker for AKI should be affordable, fast and easy to measure, precise and accurate, and able to determine the severity of dysfunction, specific for kidney, increase in the early stage of dysfunction, with high sensitivity and specificity(Molitoris, Melnikov *et al.* 2008). An ideal AKI biomarker would be one that could be easily measured without interference from other biological variables, able to detect early kidney damage and stratify its risk(Cullen, Murray *et al., 2012*). There are still a lot of opportunities to develop a biomarker that can actually detect earlier stage of tubular cell disturbances before it disrupts renal filtration capacity. Several biomarkers have been found, some seem to be promising, such as netrophilgelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), IL-18,Cystatin-C, N-acetyl-β-D-glucosaminidase (NAG), L-FABP, netrin-1 and vanin-1.

NeutrophilGelatinase-Associated Lipocalin (NGAL)

Human NGAL was originally identified as a 25 kDa protein covalently bound to matrix metalloproteinase-9 (MMP-9) from neutrophils(Mishra, Mori *et al.* 2004b). Like other lipocalins, NGAL forms a barrel-shaped tertiary structure with a hydrophobic calyx that binds small lipophilic molecules(Devarajan*et al.*, 2007). The major ligands for NGAL are siderophores, small iron-binding molecules. On the one hand, siderophores are synthesized by bacteria to acquire iron from the surroundings, and NGAL exerts a bacteriostatic effect by depleting siderophores. Although NGAL is expressed only at very low levels in several human tissues, it is markedly induced in injured epithelial cells, including the kidney, colon, liver and lung. These findings provide a potential molecular mechanism for the documented role of NGAL in enhancing the epithelial phenotype, both during kidney development and following AKI(Mishra, Mori *et al.* 2004b). In the normal kidney, only the distal tubules and collecting ducts stain for NGAL expression. NGAL's composite molecule binds ferric siderophores, and furthermore, it is a potent epithelial growth inducer, has protective effects in ischaemia,(Mishra, Mori *et al.* 2004b; Yang, Goetz *et al.* 2002) and is up-regulated by systemic bacterial infections(Fjaertoft, Foucard *et al.* 2005; Nielsen, Borregaard *et al.* 1996; Shapiro, Trzeciak *et al.* 2009; Xu, Pauksen *et al.* 1995).

In the case of AKI, proximal tubule cells also stain for NGAL proteins, which is explained by megalin–cubilin-mediated re-uptake of NGAL present in the glomerular filtrate(Hvidberg, Jacobsen *et al.* 2005; Mori, Lee *et al.* 2005). Urinary NGAL originates from local production in the distal tubules and collecting ducts.

The genesis and sources of plasma and urinary NGAL following AKI require further clarification. Although plasma NGAL is freely filtered by the glomerulus, it is largely reabsorbed in the proximal tubules by efficient megalin-dependent endocytosis. However, uNGAL excretion is proportional to albumin excretion in mouse models of diabetic nephropathy and is thus augmented when the proximal transport maximum is exceeded(Mori, Lee *et al.* 2005; Schmidt-Ott, Mori *et al.* 2006; Schmidt-Ott, Mori *et al.* 2007). Preclinical transcriptome profiling studies identified *NGAL*(also known as lipocalin 2 or *lcn2*) to be one of the most upregulated genes in the kidney very early after acute injury in animal models(Supavekin, Zhang *et al.* 2003). Downstream proteomic analyses also revealed NGAL to be one of the most highly induced proteins in the kidney after ischemic or nephrotoxic AKI in animal models(Mishra, Ma *et al.* 2003; Mishra, Mori *et al.* 2004a; Mori, Lee *et al.* 2005). The serendipitous finding that NGAL protein was easily detected in the urine soon after AKI in animal studies has initiated a number of translational studies to evaluate NGAL as a noninvasive biomarker in human AKI. In a cross-sectional study of adults with established AKI (doubling of serum creatinine) from varying etiologies, a marked increase in urine and serum NGAL was documented by western blotting when compared with normal controls(Mori, Lee *et al.* 2005). Urine and serum NGAL levels correlated with serum creatinine and kidney biopsies in subjects with AKI who demonstrated intense accumulation of immune-reactive NGAL in cortical tubules, confirming NGAL as a sensitive index of established AKI in humans.

KidneyInjury Molecule-1 (KIM-1)

Human KIM is a type 1 transmembrane glycoprotein with an immunoglobulin and mucin domain that is not detectable in normal kidney tissue or urine, but is expressed at very high levels in dedifferentiated proximal tubule epithelial cells in human and rodent kidneys after ischemic or toxic injury. The KIM-1 (designated as Kim-1 in rodents, KIM-1 in humans) was found to be markedly up-regulated after 24–48 h in the proximal tubule of the post-ischemic rat kidney(Ichimura, Bonventre *et al.* 1998). Although KIM-1 gene or protein expression is undetectable in the normal kidney, following injury KIM-1 mRNA is rapidly synthetized and protein is generated and localized at very high levels in the apical membrane of proximal tubule. In human ischemic and toxic AKI, it is found in all three segments of the proximal tubule. KIM-1 is a transmembrane protein that is specifically upregulated in dedifferentiated proximal tubule cells after ischemic or nephrotoxic AKI. An extracellular domain of KIM-1 is detectable in the urine soon after AKI(Vaidya, Ramirez *et al.* 2006). KIM-1 represents a promising biomarker for the early diagnosis of AKI and its clinical outcomes(Han, Waikar *et al.* 2008; Liangos, Perianayagam *et al.* 2007; Liangos, Tighiouart *et al.* 2009). In hospitalized patients with established AKI, urinary KIM-1 levels predicted adverse clinical outcomes such as dialysis requirement and mortality(Liangos, Perianayagam *et al.* 2007). KIM-1 is also an excellent marker of nephrotoxicity in preclinical studies(Vaidya, Ozer *et al.,2010*).

The recent availability of a rapid urine dipstick test for KIM-1 will facilitate its further evaluation in preclinical and clinical studies(Vaidya, Ford *et al.* 2009). Urinary KIM-1 is also increased in a number of chronic kidney diseases(van Timmeren, Vaidya *et al.* 2007; van Timmeren, van den Heuvel *et al.* 2007; Waanders, Vaidya *et al.* 2009).

Interleukin-18 (IL-18)

Kidney injury molecule-1 (KIM-1) is renal tubular protein that has been demonstrated to be elevated in experimental animals with AKI. Clinical studies reveal that KIM-1 is highly effective at distinguishing true acute tubular necrosis from other types of renal injury (including CKD) or controls(Han, Bailly *et al.* 2002; Han, Waikar *et al.* 2008). In the first published report, a 1U increase in KIM-1 was associated with 12-fold higher odds for ischemic acute tubular necrosis(Han, Bailly *et al.* 2002). In the second report, the AUC (area under the curve) of KIM-1 for the diagnosis of AKI was 0.90.37 Urine IL-18 measurements also represent early biomarkers of AKI in the pediatric intensive care setting, being able to predict this complication about 2 days prior to the rise in serum creatinine(Han, Bailly *et al.* 2002). Early urine IL-18 measurements correlated with the severity of AKI as well as mortality. Overall, IL-18 appears to be more specific to ischemic AKI, and largely unaffected by chronic kidney disease or urinary tract infections. However, IL-18 measurements may also be influenced by a number of coexisting variables, such as endotoxemia, immunologic injury and cisplatin toxicity. Furthermore, plasma IL-18 levels are known to be increased in various pathophysiologic states, such as inflammatory arthritis, inflammatory bowel disease, and systemic lupus erythematosus. Standardized platforms for the clinical measurement of urinary IL-18 remain to be developed and validated.

Cystatin-C

IL-18 is a pro-inflammatory cytokine that is known to be induced and cleaved in the proximal tubule, and subsequently easily detected in the urine following ischemic AKI in animal models(Melnikov, Ecder *et al.* 2001). In a cross-sectional study, urine IL-18 levels measured by a sensitive commercially available ELISA (Medical and Biological laboratories, Nagoya, Japan) were markedly elevated in patients with established AKI but not in subjects with urinary tract infection, chronic kidney disease, nephrotic syndrome, or prerenal azotemia(Parikh, Jani *et al.* 2004). Cystatin-C is a 13-kDa non-glycosylated cysteine protease inhibitor produced by all nucleated cells at a constant rate. In healthy subjects, Cystatin-C is excreted through glomerular filtration and metabolized completely by the proximal tubules. Furthermore, there is no evident tubular secretion. Several studies claim the superiority of Cystatin-C against S.Cr. to detect minor reductions in glomerular filtration rate (GFR)(Dharnidharka, Kwon *et al.* 2002). However, the interpretation of Cystatin-C levels is biased by older age, gender, weight, height, cigarette smoking and high levels of C-reactive protein (CRP)(Knight, Verhave *et al.* 2004; Okura, Jotoku *et al.*). It has been found useful both as serum or urinary biomarker. In 85 critically ill patients at high risk for developing AKI, serum cystatin-C was found to detect AKI almost 2 days earlier compared to detecting AKI by the RIFLE Classification using S. Cr.(Herget-Rosenthal, Marggraf *et al.* 2004). Many other studies have confirmed utility of serum cystatin-C as a useful early biomarker(Delanaye, Lambermont *et al.* 2004; Villa, Jimenez *et al.* 2005).

Koyner et al (Koyner, Bennett *et al.* 2008) found urinary cystatin-C a very promising early (within 6 h after surgery) biomarker of AKI in adult cardiac surgery patients.

N-acetyl-β-D-glucosaminidase (NAG)

NAG is a lysosomal enzyme, found predominantly in proximal tubules and the increased activity of this enzyme in the urine suggests injury to tubular cells, therefore can serve as a specific urinary marker for the tubular cells. In the course of active kidney disease, urinary NAG levels remain persistently elevated. The increase in urinary NAG activity indicates damage to tubular cells, although it can also reflect increased lysosomal activity without cellular damage(Liangos, Perianayagam *et al.* 2007). However, the use of NAG remains limited by the fact, that urinary excretion of the enzyme is also elevated in glomerular diseases such as diabetic nephropathy(Marchewka, Kuzniar *et al.* 2001).

Liver-type fatty acid binding protein (L-FABP)

Liver-type fatty acid binding protein is a protein expressed in the proximal tubule of the kidney(Noiri, Doi *et al.* 2009). Liver fatty acid binding protein is a 14-kDa protein normally expressed in the kidney proximal convoluted and straight tubules(Maatman, van de Westerlo *et al.* 1992). Fatty acids binding proteins (FABPs) are a family of small cytosolic proteins that facilitate beta-oxidation by binding and transporting long chain fatty acids. There are currently nine specific FABPs found in each specific tissue. The liver type, or L-FABP (or FABP-1) is a 14–kDa protein synthesized by the liver and localized in the liver, in the intestine and in the proximal renal tubule epithelium, a fatty-acid-dependent cell type in primary metabolism(Sirota, Klawitter *et al.* ; Slocum, Heung *et al.,2012*). One pilot study examined the role of urinary L-FABP in contrast-induced AKI, defined as an increase in serum creatinine of > 0.5 mg/dl or a relative increase of > 25% at 2 – 5 days after the procedure(Yamamoto, Noiri *et al.* 2007). In a recent prospective study of 40 children undergoing cardiac surgery, 21 developed AKI (defined as a 50% increase in serum creatinine from baseline, which typically occurred 2–3 days post-surgery). In this group, ELISA analysis showed increased urine L-FABP from levels before surgery of 20 ± 4 to 1885 ± 500 ng/mg creatinineat 4 h(Portilla, Dent *et al.* 2008). Western blot analysis confirmed the presence of urinary L-FABP. Urinary L-FABP levels at 4 h post cardiac surgery were a powerful independent risk indicator for AKI. For concentration of urinary L-FABP at 4 h, the AUC was 0.810 for a cutoff value of 486 ng/mg creatinine.

Netrin-1

Netrin-1, a laminin-related neuronal guidance molecule, is not or barely expressed in tubular epithelial cells of normal kidneys andit is highly expressed and excreted in the urine after AKI in animals(Urbschat, Obermuller *et al.*,2011). Netrin-1 is a 50-75 kDalaminin-like protein, previously known as chemotropic and cell survival factor in the development of nervous system and possibly has a role in neovascularization, cell adhesion and tumorigenesis(Basnakian *et al.,* 2008). In a recent study, Netrin-1 levels rose 2 h after cardiopulmonary bypass operation (CPB) and peaked at 6 h, remaining elevated up to 48 h. Furthermore, a correlation with duration and severity of AKI and hospital stay was found. Therefore, the authors postulated Netrin-1 being a new early predictive biomarker of AKI after CPB(Ramesh, Krawczeski *et al.,2010*).

In a mouse model, urinary Netrin-1 levels increased markedly within 3 h of ischemia–reperfusion, reaching a peak level at 6 h, with a decrease thereafter, returning to near baseline values by 72 hinterestingly, serum creatinine did not increase significantly until 24 h of reperfusion, but in cisplatin, folic acid, and lipopolysaccharide- treated mice, urine Netrin-1 excretion increased as early as 1 h and reached a peak level at 6 h after injection. Here too, serum creatinine only rose significantly after 6, 24, and 72 h, respectively. The authors concluded therefore that urinary Netrin-1 is a promising early up-regulated biomarker for detection of renal injury(Reeves, Kwon *et al.* 2008).

Vanin-1

Vanin-1 an epithelial glycosylphos-phatidylinositol-anchored pantetheinase(Aurrand-Lions, Galland *et al.* 1996; Pitari, Malergue *et al.* 2000).Participates in the response to oxidative stress in vivo(Berruyer, Martin *et al.* 2004).A recent study showed that the protein levels of renal vanin-1 increased in rats with streptozotocin-induced diabetic nephropathy, and urinary vanin-1 was detected in patients with diabetic nephropathy(Fugmann, Borgia *et al.,2011*). Hosohata et al. also reported that urinary concentration of vanin-1 elevated before the conventional markers changed in rats with nephron-toxicant-induced renal tubular injury(Hosohata, Ando *et al.,2011*). Therefore, it is anticipated that urinary vanin-1 is a potential biomarker for the early detection of AKI.

CONCLUSION

This review has focused on a number of potential and early biomarkers of acute kidney injury that finally are moving from a laboratory setting to the bedside. AKI is a common clinical scenario. Urine and serum are two important bio fluids, used routinely for diagnosis of the AKI and both have great future prospects for novel proteomic biomarkers. The properties of an ideal biomarker have been described above, but as the pathophysiology behind AKI is multifaceted, it is unlikely that we will find a single marker that fulfills all criteria. Many researchers have shown promising and satisfactory results and new biomarkers are being discovered every year.The search for new AKI biomarkers has been evolving rapidly with advancements in modern technologies and in the recent year several protein biomarkers, including KIM-1, NGAL, IL-18, cystatin-C, and L-fatty acid binding protein, have emerged through the application of functional genomics and proteomics to human and animal AKI models. For identification and severity of the AKI, theAKI biomarkers must ideally allow for the early detection of kidney injury, which reflect the improvement and worsening of the kidney injury, and be amenable to quick and reliable measurement at the bedside or in the clinical laboratory.But there practical running cast is too high. Further research is going on and is expected in near future, to search and use few novel biomarkers whose cast would be similar or lower than currently used laboratory parameters for the diagnosis of AKI for the resource limited countries like India and African countries.Available studies have several limitations of new AKI biomarkers in the early detection and prediction of AKI. These limitations preclude the mainstream acceptance of AKI biomarkers in the clinical setting.In our view, these studies advanced the field in 2015 and collectively provide important information on the current state of medicine pertaining to AKI as well as suggesting future directions.

We believe that some existing biomarkers such as NGAL, Cystatin-C, IL-18 and KIM-1 will begin to shape clinical practice while new biomarkers will continue to be discovered. This ongoing process of new discovery and reinvention of existing tools, will advance the field further and we will eventually emerge with a set of tools that will not only help us diagnose AKI, but will also help us determine its cause, monitor its course and predict response to therapy. We eagerly await this bright future.

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