URINARY BIOMARKERS FOR FOLLOW-UP OF RENAL TRANSPLANTATION - WHERE WE ARE!

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ABSTRACT

Early diagnosis of renal allograft dysfunction is crucial for the management and long-term survival of the transplanted kidney. Early after transplantation, acute tubular necrosis (ATN) manifesting as delay graft function (DGF) or slow graft function, acute rejection (AR) or drug toxicity (e.g. calcineurin inhibitor) are the leading causes of Acute kidney injury (AKI). Acute rejection, ATN, and calcineurin inhibitor toxicity continue to be major causes of renal allograft dysfunction along with other causes like infections (e.g. BK and CMV viruses, pyelonephritis), obstruction, and recurrence of the original disease. Clinicians have been searching for non-invasive tools that would allow the diagnosis of acute renal allograft dysfunction early and accurately without performing a kidney biopsy. The following comprehensive review will describe the latest findings on urinary biomarkers in acute allograft dysfunction.

Key words: Acute kidney injury (AKI), urinary biomarkers, renal transplantation.

INTRODUCTION

Early diagnosis of renal allograft dysfunction is crucial for the management and long-term survival of the transplanted kidney. Early after transplantation, acute tubular necrosis (ATN) manifesting as delay graft function (DGF) or slow graft function; acute rejection (AR) or drug toxicity (e.g. calcineurin inhibitor) are the leading causes of AKI. Acute rejection, ATN, and calcineurin inhibitor toxicity continue to be major causes of renal allograft dysfunction along with other causes like infections (e.g. BK and CMV viruses, pyelonephritis), obstruction, and recurrence of the original disease [1]. Clinicians have been searching for non-invasive tools that would allow the diagnosis of acute renal allograft dysfunction early and accurately without performing a kidney biopsy. The following comprehensive review will describe the latest findings on urinary biomarkers in acute allograft dysfunction.

Currently, kidney transplantation remains an efficient and major therapeutic strategy for end-stage renal disease. Although surgical techniques and postoperative care have greatly advanced, achievement of long-term allograft survival remains a clinical challenge owing to various confounders, such as viral infection and nephrotoxicity [2].

Therefore, earlier detection and identification of renal graft status may guide clinicians in minimizing the risk of graft rejection events and provide decisive advice regarding withdrawal of immunosup- pression [3]. Subsequent preemptive treatment can be adopted to prevent irreversible damage of graft and rescue allograft. To this end, efficient implementation of biomarker identification can realize personalized therapy for renal transplant patients. Indeed, biomarkers for monitoring graft function have been studied for decades [3, 4]. As molecular biological techniques are developing, various types of biomarkers have been successfully identified. However, no suitable biomarkers are widely used for renal transplant patients since clinical cases are normally complicated. Much of the data from experimental findings failed to connect bedside clinical applications. Therefore, standardization of identified biomarkers is required on a large scale in prospective multicenter trials.

In practice, the ideal biomarkers for renal transplantation should be independently validated and standardized by multiple international centers, which can prove their reliability and reproducibility in parallel with corresponding histological analysis [5, 6]. It is encouraging that the European Union Reprogramming the Immune System for the Establishment of Tolerance Consortium undertook such collaborative studies in different European laboratories [7]. Bestard et al presented their very recent data in which a cross-validation of the interferon gamma enzyme-linked immunosorbent spot assay was used to assess the circulating alloreactive memory/effector T-cells for renal transplant patients [6]. Early identification and prognosis of graft dysfunction are also required so that a preemptive regimen can be promptly administered to prevent irreversible damage to the allograft. In addition, optimal biomarkers are expected to precisely reflect alteration of allograft function at an early stage [3]. Herein, our present review analyzes laboratory techniques for identifying biomarkers to better understand the develop-ment of diagnostic tools. Current studies on biomarkers for living and nonliving donors are discussed to acknowledge their predictive values and practical considerations. The develop-ment of laboratory techniques has facilitated the production of novel biomarkers. Firstly, the real-time polymerase chain reaction (RT-PCR) is a sensitive and accurate technique, well-accepted to identify and validate potential biomarkers at the gene level for decades

[3]. However, the data from RT-PCR is insufficient for high throughput screening and cannot meet researchers' needs. As a replacement, microarray profiling arose to generate a large amount of potential biomarkers for comprehensive analysis on various renal transplant patients in different transplant scenarios[8-10]. During the past years, mass spectrometry analysis was employed to identify potential biomarkers at the protein level in patients' serum samples[9]. Apart from specimens from peripheral blood, urine was utilized to identify qualified biomarkers for early detection of glomerular injury [6]. Research findings exhibited that urinary cystatinC, clusterin, and beta-2 microglobulin could significantly monitor kidney injury better than serum creatinine or blood urea nitrogen, as supported by in situ hybridization, gene and protein expression analysis, and immunohistochemistry [6]. Other multiple platforms are also used in empirical studies, such as enzyme-linked immunosorbent assay, flow cytometry, and immune cell functional assays, although their efficiency is unsatisfactory [11,12]. In addition, perfusate was utilized to detect applicable non invasive biomarkers for renal transplant patients .

What are Biomarkers?

Biomarker is a very broad term that can be used to describe any indicator of a biological state. The term biomarker, or biological marker, was introduced in 1989 as a Medical Subject Heading (MeSH) term and it was defined as "measurable and quantifiable biological parameters (eg, specific enzyme concentration, specific hormone concentration, specific gene phenotype distribution in a population, presence of biological substances) which serve as indices for health- and physiology-related assessments." More recently, in 2001, the definition was standardized by the Biomarker Definitions Working Group as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention". In practice, biomarkers can improve understanding about a disease and provide new knowledge of pathological mechanisms, making possible the earlier diagnosis and the delivery of more efficacious and safer therapies. Presently, it is not well established how biomarkers are categorized. Within the field of health care, biological markers are commonly classified based on the sequence of events from exposure to disease: biomarkers of exposure, which are used in risk prediction, and biomarkers of disease, which are used in screening, diagnosis and prognosis. To be clinically useful and prevent false-positive screening tests, a biological marker should be highly sensitive and specific in detecting disease or any other outcome. Regardless of the purpose for its use, it should be accurate, reproducible and standardized across different clinical units. Furthermore, it should be easily measured in a standard biological source (as blood or urine), obtained from a person (as blood pressure or electrocardiogram) or image based (echocardiogram or computerized tomography scan), so that the information would be readily available and easy to interpret by clinicians [5,6].

In summary and according to the Food and Drug Administration, an ideal biomarker should be specific, sensitive, predictive, robust, simple, accurate, and inexpensive. In organ transplantation, initial graft dysfunction is one of the most important early post-operative problems, mainly due to the unavoidable ischemia-reperfusion injury that occurs in the transplanted organ. In kidney transplantation, ischemic injury of the renal allograft is a critical early insult that augments the risk of acute tubular necrosis and long-term graft loss [7, 8]. The development of effective interventions is constricted by the limited ability of early detection of graft dysfunction. Current clinical indicators of kidney injury, like serum creatinine, are inadequate for timely diagnosis and prognosis. Thus, application of biomarkers in the field of kidney transplantation will allow to detect incipient graft dysfunction or rejection, will refine diagnoses and enable more effective post-transplant management, and thereby potentially improve short-term (e.g., delayed graft function, acute rejection) and long-term (e.g., allograft failure) outcomes. Discovery of biomarkers is expanding at an unprecedented rate. Numerous biomarkers in kidney transplantation have been evaluated in the past decade, but, so far, evidence to support their use in routine practice is limited. In this article, we review the promising role of three biomarkers of delayed graft dysfunction, namely, neutrophil gelatinase-associated lipocalin, oxidative stress, and cystatinC.

An ideal biomarker of AKI would fulfill the following:

· Increase in the urine or blood within minutes or hours after a renal insult;

· Remains elevated as long as the renal injury persists

· Correlates quantitatively with the extent of renal injury;

· Decreases proportionally with the renal recovery status.

As molecular biological techniques are developing, various types of biomarkers have been successfully identified, however, no suitable biomarkers are widely used for renal transplant patients since clinical cases are normally complicated. Therefore, standardization of identified biomarkers is required on a large scale in prospective multicenter trials.

Depending on the time of appearance after acute injury and rejection, the urinary biomarkers may be classified into biomarkers of structural injury, and those of functional injury. Structural injury biomarkers are those appearing in the urine immediately after tubular cell apoptosis, and include KIM-1, NGAL, NAG, IL-18, and clusterin. Functional injury biomarkers are delayed markers for injury which may indicate global renal dysfunction, and constitute cystatin C, total protein, albumin and â-2 microglobulin (Table1).

Different types of biomarkers for renal allograft monitoring in Kidney transplantation

Diverse confounding factors such as viral infection, malignancy, surgical complication, and nephrotoxicity from immunosuppressive drugs can affect graft function, and

even survival. Therefore, specific biomarkers were investigated for such varying clinical scenarios of kidney transplantation[15-17]. First, transplant organs can be categorized into those received from living donors, and those from deceased donors. To some extent, the quality of donor organs determines transplant outcome[18]. It is well known that living donor organs function better than those from deceased donors. Accordingly, the biomarkers for kidney transplantation can be divided into two types: biomarkers for living donors, and biomarkers for deceased donors[16].

Urinary biomarkers are regarded more non-invasive, easy to measure, easily obtainable, and clinically earlier detection than blood biomarkers[19,20]. Injury to dif- ferent segment regions of nephrons may excrete different specific urinary biomarkers. Detection of high-molecular weight protein, like albumin, immunoglobulin, and trans- ferrin in the urine may be associated with glomerular injury. Low-molecular weight proteinuria (e.g. $á$-1 mi-croglobulin, $â$-1 microglobulin and retinol binding pro- teins)[21,22], brush border antigens [23,24,25], urinary enzymes [21,25] and other urinary proteins [26-29] are associated with damage to renal tubules. However, sufficient validation is needed to use these markers for the screening and differentiating the site of injury clinically.

Table 1. Biomarkers for specific conditions of donor kidney grafts

TYPE OF BIOMARKERS	TARGET INJURY TISHU
Biomarkers in functional injury	
Cystatin C	Glomerular injury, in urine indicates proximal tubular injury
Beta 2 microglobulin	Glomerular and tubular disfunction
Brush border antigens	
Adenosine desaminase protein	Proximal tubular disfunction
Carbonic anhydrase	Proximal tubular injury
Urinary enzymes	
N-acetyl-b-d-glucosaminidase	Proximal tubular injury
Alanin aminopeptidase	Proximal tubular injury
Cathepsin B	Proximal tubular injury
Alfa-gluthatione S-transferase	Proximal tubular injury
Beta-Glukosidase	Proximal tubular injury
Biomarkers of structural injury	
Kidney injury molecule-1(KIM-1)	Proximal tubular injury(Ischemia, acute insuficience, nephrotoxines)
N-acetyl-b-d-glucosaminidase	Proximal tubular injury
NGAL	Tubule and collecting duct injury
Interleukin 18	Tubule injury
Metabolomics in monitoring in Kidney transplantation	
*Urine Proteomics	Analysis relies of the exctraction and separation of proteins(MALDI,SELDI)
*Urine Genomics	Function of Genes and proteins
*CD103 mRNA	Roles in T-cell activation(effectors function)
*FOXP3 mRNA	X-linked fork head /winged helix transcription factor
*Cytokines and Binding Receptors	Control of leukocyte migration during inflammatory processes
Others	
Type IV collagen	Glomerular injury
Gama-Gluthamyl trasnferase	Tubular epithelium injury
Retinol binding protein	Proximal tubular injury
α- Gluthatione S- transferase	Proximal tubular injury
π- Gluthatione S transferase	Distal proximal injury
Tamm Horsfall Glycoprotein	Proximal tubular injury

A. Urine biomarkers in acute allograft rejection
Tubular Enzymes and Markers of Tubular Dysfunction

The apical surface of proximal tubular epithelial cells contains numerous microvilli that form the brush border and contain proteins with enzymatic functions to carry out the specialized tasks of the proximal tubule. Intracellular enzymes can be released into the urine with injury either by exocytosis or leakage. The proteins can exist in the free form or may be membrane- encased as exosomes. Several different classes of enzymes can be found: lysosomal proteins, such as N-acetyl- β - D -glucosaminidase (NAG), brush border enzymes including gamma-glutamyl transferase (GGT) and alkaline phosphatase, as well as cytosolic proteins such as α-glutathione S-transferase (α -GST). Furthermore,when proximal tubular epithelial cells are injured, they may not metabolize cystatin C properly, and filtered intact cystatin C may appear in the urine. Similarly, injured cells may not completely reabsorb low-molecular weight proteins that are freely filtered into the urinary space, such as α1 - and β 2 -microglobulin. Westhuyzen et al. compared the predictive value of a number of tubular enzymes for the subsequent development of AKI, defined as a 50% rise in serum creatinine to at least 1.7 mg/dl [26]. Four of 26 subjects developed AKI; baseline levels of GGT, AP, NAG, β -GST and π -GST were higher in those who developed AKI, compared to those who did not. α-GGT and π - GST had the best predictive value on their own, with areas under the receiver-operating characteristic curve (AUC-ROC) of 0.95 (95% CI 0.79–1.0) and 0.93 (95% CI 0.74–1.0), respectively.Several investigators have examined the ability of tubular enzymes to predict adverse clinical outcomes. Herget-Rosenthal et al. risk-stratified patients with nonoliguric AKI (defined as a doubling in creatinine from a baseline concentration of <106 µmol/l to at least 115 µmol/l) using tubular enzymes as biomarkers[27]. Of the tubular enzymes studied,NAG had the best predictive value, with an AUC-ROC of 0.81. In another study, Chew et al. found that levels of NAG and tissue non-specific alkaline phosphatase were higher in AKI patients with poor outcomes (defined as need for dialysis or death) [28]. Liangos et al.recently performed a study of NAG and kidney injury molecule-1(KIM-1) in 201 patients with established AKI [29]. They found that elevated NAG levels portended poor clinical outcomes, with the odds of death or dialysis requirement increased over fivefold in patients with the highest versus lowest quartiles or urinary NAG levels, even after careful multivariable adjustment for disease severity and comorbidity. Tubular enzymes present in the urine have long been studied as markers of AKI, but they have not been adopted in widespread clinical use either as early diagnostic tests, prognostic indicators, or surrogate endpoints for interventional studies. Some authors have suggested that tubular enzymes are overly sensitive, because they tend to rise after injuries such as cardiopulmonary bypass without an associated rise in SCr [30, 31]. Investigators should exercise caution, however, in interpreting performance characteristics of new biomarkers against a gold standard like SCr that has poor specificity and sensitivity: cardiac troponin would appear to be nonspecific against earlier (and now discredited) cardiac biomarkers like lactate dehydrogenase.

Kidney injury molecule-1(KIM-1) was identified as the single most upregulated gene in postischemic rat kidney using a PCR-based technique [32]. KIM-1encodes a type I cell membrane glycoprotein containing, in its extracellular portion, a novel sixcysteine immunoglobulin-like domain and a threonine/ serine and proline-rich domain characteristic of mucin like O-glycoslyated proteins, suggesting its potential involvement in cell-cell and/or cell-matrix interactions [33]. After proximal tubular kidney injury, the ectodomain of KIM-1 protein is shed from cells into the urine in rodents and in humans. In both ischemia-reperfusion and cisplatin-induced nephrotoxicity models in the rat, urinary Kim-1 is a sensitive and specific indicator of proximal tubular kidney injury and is increased earlier than any of the conventional biomarkers, e.g. plasma creatinine, blood urea nitrogen, glycosuria, proteinuria, and urinary NAG [34]. In recently completed studies of 8 mechanistically different proximal tubule nephrotoxicants and 2 different hepatotoxicants in rats, Kim-1 had an AUC-ROC of 0.99 for proximal tubular toxicity, using histopathology as the gold standard; of 21 urinary markers studied, Kim-1 was found to have the highest sensitivity and specificity [35]. Human studies have begun to confirm the promise of urinary KIM-1 for the diagnosis of AKI. Han et al. demonstrated marked expression of KIM-1 in kidney biopsy specimens from 6 patients with acute tubular necrosis (ATN), and found elevated urinary levels of KIM-1 in 7 patients with ischemic ATN; urinary levels of KIM-1 were significantly lower in contrast nephropathy (n = 7), although the levels did correlate with the severity of contrast-induced injury(36). Levels of urinary KIM-1 were lower in AKI not due to ATN (n = 9), CKD (n = 9), and were below limits of detection in normal subjects (n = 8)[36]. KIM-1 is also highly expressed in the setting of renal cell carcinoma (RCC), as shown by Han et al. [37]. KIM-1 was detectable in the urine of patients with RCC, suggesting a potential role for early non-invasive diagnosis. In all other disease conditions, KIM-1 protein was identified in dedifferentiated proximal tubular cells and correlated with tubulointerstitial fibrosis and inflammation. In the subset of patients who underwent urine collection near the time of biopsy, urinary KIM-1 levels correlated with tissue expression of KIM-1. Urinary KIM-1 may therefore hold promise as a non-invasive assessment of the activity and prognosis of a variety of acute and chronic kidney diseases. Proximal tubule KIM-1 expression was found in all patients with histologic evidence of tubular cell injury, and higher KIM-1 staining correlated with improved renal outcomes at 18 months. Higher KIM-1 excretion was associated with significantly higher risk of graft loss over the follow-up period. High KIM-1 excretion was also associated with proteinuria, low creatinine clearance, and high donor age, but was independently associated with graft loss after multivariate adjustment for these variables. How KIM-1 and other markers will compare to other predictive markers and clinical scoring systems is the subject of extensive ongoing studies.

Neutrophil Gelatinase-Associated Lipocalin (NGAL) NGAL is one of the best-studied urinary biomarker of acute injury and rejection to date. Also known as lipocalin-2 or siderocalin, NGAL was first discovered as a protein in granules of human neutrophils; animal studies showed its promise as an early marker of ischemic and nephrotoxic kidney injury [38]. Mishra and others serum NGAL was inferior to urinary NGAL for the identification of AKI(39). As encouraging as these results were, it should be noted that 29% of eligible patients were excluded due to perioperative use of ibuprofen, angiotensin-converting enzyme inhibitors, gentamicin, or vancomycin. A larger follow-up study of 120 children (using similar exclusion criteria) by Dent et al**.** (40) showed that 2-hours postoperative serum NGAL was predictive of AKI (AUC-ROC 0.96) and correlated with postoperative change in SCr, duration of AKI, and length of stay Parikh and others(41)studied urinary NGAL in 53 consecutive patients undergoing living or deceased donor kidney transplantation. NGAL levels (normalized to urine creatinine concentration) were significantly higher in deceased donor recipients with delayed graft function (DGF) (n = 10, median 3,306 ng/mg creatinine) than prompt graft function (n = 20, median 756 ng/mg creatinine). A cutoff value of 1,000 ng/mg creatinine had 90% sensitivity and 83% specificity for the identification of DGF; the AUC ROC was 0.90. Urinary NGAL has also been studied as a surrogate marker of kidney injury from aprotinin, a nephrotoxic fibrinolytic used in cardiac surgery. Wagener et al. found that postoperative levels of urinary NGAL were almost 20 times higher in patients who received aprotinin compared to epsilon amino-caproic acid, lending support to the suggestion of aprotinin's nephrotoxicty. Urinary NGAL was also found to be higher after coronary angiography in 13 patients with AKI than in 27 controls without AKI [42].

Vascular Endothelial Growth Factor. Vascular endothelial growth factor (VEGF), mRNA and protein are detected in glomerular podocytes, distal tubules, and in some proximal tubules [43]. In AR, these cells express VEGF ligand and VEGF receptors are upregulated [44]. VEGF expression is increased in pathological conditions associated with macrophage infiltration [45]. When VEGF increases, macrophage and leukocyte infiltration is facilitated by the increasing of capillary permeability [46] and leukocyte migration is augmented to the allograft, which may lead to rejection [47]. Fifteen different sequence polymorphisms have been identified within the VEGF gene. The correlation among VEGF gene polymorphisms, VEGF production and AR has been investigated. Homozygotes with – 1154G/G genotype and – 2578C/C genotype showed the greatest risk of rejection and had the highest production of VEGF, as compared with – 1154A/A and – 2578A/A, heterozygotes with – 1154G/A and – 2578C/A [48]. In a recent study, urinary VEGF was determined by ELISA in 215 allograft recipients and 80 healthy controls. Patients with AR (n=67) excreted urinary VEGF at a significantly higher level [49].

Cytokines and Binding Receptors. Chemokines play a role in the control of leukocyte migration during inflammatory processes by interaction with their respective G-protein-coupled receptors. The chemokine receptors comprise two groups, the CC receptors 1-10 (CCR1 to CCR10), which bind CC chemokines, and the CXC receptors 1–5 (CXCR1 to CXCR5), which bind CXC chemokines [50,51]. The following are examples of cytokines studied in kidney transplantation: Using a cytometric bead array technique, the concentrations of six human cytokines (IL-2, IL-4, IL-5, IL-10, tumor necrosis factor-α (TNF-α) and interferon (IFN)-λ) were measured in the urine. Results showed that patients who developed kidney allograft rejection had high levels of IL-10 and IFN-λ cytokines in urine compared to patients without rejection [52]. In a study conducted by Smith et al. [42], soluble ILs, cyclic GMP (cGMP), nitrate, and nitrite were measured in 192 urine samples collected from 13 patients during the first 3 months of renal transplantation. For follow-up analysis, 67 urine samples were collected randomly from 24 patients. TNF-α, IL-6, and IL-8 were found to increase at the time of rejection (p ≤0.01). A study investigated the kidney gene, protein expression and the urinary excretion rate of IL-6 and epidermal growth factor (EGF) in 29 renal transplant recipients: 16 with AR and 13 with acute tubular damage or cyclosporine toxicity. Urinary IL-6/EGF ratio was markedly increased in AR, and only slightly increased in the other group [53]. Urine IL-6 levels were also measured daily in another study in 101 recipients during post-transplant hospital stay. DGF was associated with very high urine IL-6 levels. Steroid-sensitive acute rejection was associated with significantly increasing urine IL-6 [54]. Levels of soluble IL-2-receptor (sIL-2R), IL-6 and IL-8 were measured in serum and urine of 79 patients with kidney transplantation; AR was associated with an increase in the urine levels of IL-6 and sIL-2R [55]. The concentration of monocyte chemoattractant protein-1 (MCP-1) in urine and serum of 19 renal transplant patients was investigated by Prodjosudjadi et al. who found that urinary excretion of MCP-1 was increased in patients with acute rejection [56]. Urine samples were collected in another study from 35 patients with acute rejection and 65 with a stable graft function within the first 6 months after transplantation. MCP-1 level was tenfold higher inacute rejection than in patients with a stable graft function [57]. Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine and a potent activator of macrophages and T cells. Brown et al. have used ELISA to detect MIF in 9 allograft patients who had serial urine MIF concentrations measured in the first 14 days after transplantation [58]. Levels increased on day 1 post-transplantation and subsequently fell in parallel with the serum creatinine, also increased before episodes of acute rejection. Chemokine monokine induced by IFN-γ (MIG) (CXCL9) and IFN-γ-inducible protein 10 (IP-10) (CXCL10) are studied as early markers of acute rejection in renal transplantation. In a prospective study of 69 patients, urine samples were collected for a median of 29 days after transplant. Urine MIG and IP-10 were analyzed by ELISA and were elevated in 14 patients who had biopsy-proven acute rejection. Urinary MIG predicted acute rejection with a sensitivity of 93% and a specificity of 89% [59].

Urine Genomics. Genomics refers to the analysis of the genomes; on the other hand, functional genomics is the field that uses global approaches to understand the functions

of genes and proteins. Most human sequenced genes and genomes' biological functions remain unknown. Human genomes are encoded in the DNA, which is copied into RNA; the RNA molecules are used to make proteins. The functions of unknown genes can be determined by repeated measurements of their RNA transcripts.Quantifying mRNAs can be done by Northern blotting or quantitative polymerase chain reaction (PCR), which measures a few genes at a time. DNA microarray allows a rapid analysis of gene expression; it is based on the principle that complementary sequences of DNA can be used to probe and hybridize to the immobilized DNA molecules [60]. Microarray has been utilized in kidney biopsies; thus far, data is still limited on the utilization of microarray methods in urine samples of transplant recipients. In the following we present mRNAs measured by PCR in urine samples of kidney transplant recipients: CD103 mRNA (CD103) is a member of integrin family heterodimers that plays diverse roles in T-cell activation, homing, and delivery of effectors function [61]; Ding et al. found that CD103 mRNA levels were higher in urinary cells from 30 patients with acute rejection compared with the levels in 12 patients with other findings on renal allograft biopsy, and 25 patients with stable graft function (p=0.001) [62].

CystatinC. CystatinC is a 13kD cysteine protease inhibitor protein that is produced by all nucleated cells into plasma, and freely filtered from glomerulus, completely reabsorbed and not secreted in the tubules. It is less influenced by factors other than glomerular filtration rate (e.g. age, gender, race, or muscle mass) [63]. Several studies have demonstrated that a change in serum and urine CystatinC is more sensitive than a change in Serum creatinine in predicting a change in glomerular filtration [64-67]. In a prospective study of 85 critically ill patients at high risk to develop AKI, a 50% increase in serum cystatinC was noted one to two days before serum creatinine with an AUC of 0.97 and 0.82 [68]. Furthermore, serum cystatinC also predicts the risk of AKI-associated cardiovascular morbidity and mortality in critically ill patients [69]. Urine cystatinC has also been studied and some studies found that urine CystatinC performed better for AKI prediction than did serum CystatinC [70,71]. In addition, increased urinary cystati C and á1-microglobulin may be early predictors of an unfavorable clinical outcome in ATN, reflected by the requirement for Renal Replecement Therapy. Severity prediction with these markers could assist in improving the outcome of ATN [71]. CystatinC also is extensively studied in chronic kidney disease patients. Since serum cystatin C has higher sensitivity and higher negative predictive value in determination of reduced GFR than serum creatinine, many studies examine the serum cystatinC to use clinically in GFR determination [72]. A recent cross-sectional study found that the combined creatinine-cystatinC equation to estimate GFR better than either of these markers alone in chronic kidney disease patients [73]. Although these findings are encouraging, additional studies are further needed for clinical use of serum and urine cystatinC in acute and chronic renal disease conditions.

B. Urine biomarkers for Acute Allograft Ischemic Injury.

Interleukin 18. IL-18 was found to potentiate ischemic AKI and to be detectable in the urine of mice subjected to ischemic kidney injury [75]. Urinary IL-18 has been studied by Parikh and coworkers in a variety of clinical settings, including delayed graft function [74], cardiac surgery [76], acute respiratory distress syndrome [77] and cross-sectionally in patients with and without kidney disease [79]. The first AKI study of urinary IL-18 in humans was a cross-sectional comparison of patients with ATN (n =14), pre-renal azotemia (n = 8), UTI (n = 5), CKD (n = 12), transplant recipients (n = 22), and healthy controls (n = 11) [22]. The highest levels of urinary IL-18 were observed in patients with ATN and delayed graft function, with relatively little overlap from patients with pre-renal azotemia, urinary tract infections, and CKD. The AUC-ROC from this cross-sectional cohort (for the identification of ATN, including delayed graft function) was 0.95, with a sensitivity of 85% and specificity of 88% at a cutoff of 500 pg IL-18/mg creatinine. Parikh and colleagues performed a prospective, nested, case-control study in 138 of the 861 patients enrolled; exclusion criteria included a baseline SCr 11.2 mg/dl. They found that urinary IL-18 levels were higher in those patients who developed AKI (defined as a 50% increase in SCr within 6 days of enrollment), and that higher levels were predict live of mortality. The AUC-ROC for IL-18 (not normalized to urine creatinine) was 0.73 at 24 h prior to AKI diagnosis; this value does not compare favorably with the AUC-ROC of 0.95 from the cross-sectional study of urinary IL-18. Parikh et al. also measured IL-18 in urine samples collected in the pediatric cardiac surgery cohort used to study NGAL [77]. They measured IL-18 in all 20 cases of AKI and in 35 of the 51 non-AKI cases (matched according to race, gender, and age to AKI cases). Not surprisingly for a pro-inflammatory cytokine that plays an important role in sepsis, urinary IL-18 was significantly higher in patients with sepsis than in those without, and limited its diagnostic ability for the early identification of AKI in this cohort. Urinary IL-18 has also been studied as a biomarker of contrast nephropathy with mixed results.

C. Urine Biomarkers Associated with Infections

Some urine biomarkers are also correlated with infections in kidney transplant recipients. Perforin (P), granzyme B (GB) and Fas-ligand (FAS-L) gene expression in urine were upregulated in urinary tract infections (UTI) and CMV infections [80]. Fischer et al. [81] found that IL-6 and IL-8 levels did not change in CMV infections or systemic extrarenal bacterial infections; however, acute pyelonephritis resulted in a higher serum IL 6 level. In another study, the level of IL-8 in urine was elevated in patients with UTI. Patients with higher concentrations of serum creatinine during UTI had high urine levels of IL-8 [82]. Smith et al. [83] found that IL-2 was decreased in UTI 1–5 days prior to diagnosis but increased 1–5 days after diagnosis; the same study showed that cGMP increased and nitrate decreased with UTI.

Limitations of Biomarker

The heterogeneity of acute injury and rejection especially in intensive care unit needs more than one biomarker to obtain sufficient sensitivity and specificity for AKI screening. An analysis of multiple biomarkers may need in additional studies before biomarkers may be used in routine clinical practice. Recently, none of the promising biomarkers have been systematically evaluated in the various clinical settings of AKI. Additionally, no cutoff value that is predictive of AKI has been available nowadays. Although the above discussed urinary biomarkers data is promising, there have been some limitations to expand the application of these markers from clinical research to clinical practice. In general, these clinical studies included small numbers of participants, making the statistical analysis underpowered and not significantly robust. On the other hand, in spite of the advances in the proteomic and genomic techniques, data has not been sufficient to yield using these expensive methods in clinical practice. Also, Bioplex multiple ELISA and Luminex techniques have been extensively used in cytokines and other substances analysis resulting in the detection of few biomarkers; most of these data could not provide a strong evidence on the causality and correlations between the biomarkers and the clinical outcomes, resulting in suboptimal clinical benefits of these markers. However, despite these limitations, the discovery of biomarkers in renal transplantation is an evolving field of significant clinical implication. Larger and more comprehensive multi-center studies are urgently needed.

CONCLUSION:

New biomarkers under clinical investigation will likely perform differently with respect to disease specificity (e.g. sepsis vs. nephrotoxic after transplantation), time course (early vs. late markers), and prognostic characteristics (markers of incipient AKI vs. markers of prognosis in established acute rejection). Establishing the optimal test(s) for a given clinical scenario will require prospective validation in large numbers of patients with a variety of causes of acute rejection, preferably with measurement of numerous candidate biomarkers for the purpose of efficiency. The possibility that new biomarkers may be superior to SCr for the identification of AKI will require investigators to test the creatinine independent associations between biomarker levels and exposures (e.g. cardiopulmonary bypass time, dose of nephrotoxin administration) and outcomes (e.g. not only acute rejection as defined by creatinine but also length of stay, need or dialysis, and mortality). Early and accurate diagnosis of acute allograft rejection will allow interventional studies to be performed in a timely fashion, which is a prerequisite for the future development of effective prevention and therapeutic strategies that have eluded nephrology for years. Because manipulation of the immune system is key to transplantation, monitoring of the immunological response is crucial in understanding the environment in which the allograft functions in any given individual. Currently, there is no best immunological monitoring method, but promising advancements have been achieved over the past few years. With the development of these technologies, understanding the strengths and weaknesses of each test will allow clinicians to integrate these monitoring methods with clinical assessment to achieve the best long-term outcomes in transplant recipients. Biomarkers of AR such as NGAL, KIM-1, IL-18 and CystatinC are now becoming greatest interest among different acute clinical settings. Early measurements of acute rejection biomarkers predict subsequent development of intrinsic AR, dialysis requirement, ICU stay, days of hospital stay and finally affects mortality. Future studies should evaluate biomarker outcomes independent of serum creatinine, and should consider biomarkers as entry criteria for AR therapeutic protocols. Such an advance would finally find out the gold standard biomarker for AR, as in case of troponin I for potential myocardial ischemia.

FUTURE DIRECTIONS:

As experimental transplant models are improving, a considerable number of potential biomarkers are being identified,including more biomarkers for living or nonliving donors. Suitable biomarkers available for both patient populations are required. It is well-accepted that the great advances in translational medicine can potentiate validation of these identified biomarkers from bench to bed in a transplant center. Nevertheless, international standardization of biomarkers is still required to test their predictive power through prospective multicenter studies and their reliability and reproducibility will be accordingly validated. The sensitivity and specificity of these biomarkers for monitoring allograft function is an important issue, particularly in the settings of various confounders [3]. Combined use of biomarker patterns might provide reliable and significant indication on allograft function, which might shed light on the appropriate preemptive therapeutic strategies [3].

Furthermore, systematic analysis using bioinformatics tools can be utilized to avoid repetitive scientific research. Taken together, our goal is to achieve long-term allograft survival using personalized biomarkers.

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