LABORATORY MEDICINE FOR THE EVALUATION OF KIDNEY DISEASE: FROM THE FETUS TO THE ADULT

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Abstract: - Spectrum of kidney disease could be roughly divided in chronic kidney disease (CKD) and acute kidney injury (AKI). CKD mainly consists of steady or progressive loss of renal function with a slow but continuous decrease in glomerular filtration rate (GFR); AKI, on the other hand, is characterized by tubular necrosis, oliguria and tissue damage. The widespread availability of enabling technologies such as functional genomics and proteomics has accelerated the rate of novel biomarker discovery and therapeutic targets for kidney diseases. CKD has an insidious onset and is generally detected at a time when it is clinically quite advanced; adverse outcomes of CKD can be prevented through early detection and treatment. A growing body of evidences demonstrated that the concentration of plasma cystatin C correlated better with directly measured values for GFR than did plasma creatinine and subtle decrements in GFR are more readily detected by cystatin C than by creatinine. A number of novel biochemical markers for the early and accurate identification of AKI have been proposed and have been discussed in this review.

Key-Words: - Chronic Kidney Disease (CKD), Acute Kidney Injury (AKI), Glomerular Filtration Rate (GFR), Cystatin C, Neutrophil Gelatinase-Associated Lipocalin (NGAL), Kidney Injury Molecule 1 (KIM-1)

1 Introduction

In the newborn, progressive renal diseases can result from a primary on-going injury which may be multifactorial in origin. To avoid further loss of renal function, the primary insult must be considered. Tests for screening and diagnosis are of critical importance in pediatric nephrology, because patients destined to develop end-stage renal failure often exhibit few signs and symptoms early in their course of disease.

Laboratory management of renal function is usually performed by urinalysis as well as by determining blood urea nitrogen (BUN) and creatinine for the estimation of the glomerular filtration rate (GFR). This traditional strategy, which has existed for more than one hundred years, seems no longer to meet the demands to detect and exclude renal lesions in early and treatable states, especially in the neonatal age [1]. In fact, serum creatinine and BUN increased levels are relatively late manifestations of impaired renal functions. The most important factor appears to be the renal functional reserve that masks renal degeneration, as assessed by GFR, BUN, and creatinine, up to the point where over 75% of the functioning nephrons have been lost. It should be stressed that these factors measure incipient renal failure and in most cases, the finding of normal results does not mean the absence of renal dysfunction. In addition, tubular proteinuria, which reflects tubulotoxic and tubulointerstitial diseases, is likewise not found by the protein test strip. Therefore, there is a growing demand for a clinically convenient and reliable marker of renal function.

2 Biomarkers in pediatric and neonatal nephrology

The widespread availability of enabling technologies such as functional genomics and proteomics has accelerated the rate of novel biomarker discovery and therapeutic targets for kidney diseases [2]. The advent of the microarray, or cDNA chip, allows investigators to search through thousands of genes simultaneously,
making the process very efficient. Such gene expression profiling studies have identified several genes whose protein products have emerged as chronic kidney disease (CKD) and acute kidney injury (AKI) biomarkers [3]. However, microarray-based methods cannot be used for the direct analysis of biological fluids, and usually require downstream confirmation by proteomic techniques prior to clinical use. Advancing technologies have radically improved the speed and precision of identifying and measuring proteins in biological fluids, and proteomic approaches are also beginning to yield novel biomarkers for assessing kidney damage [4]. Urinary proteins include soluble proteins and protein components of solid phase elements of urine. Solid phase elements consist of “sediments” that can be precipitated at low centrifugation speeds and “exosomes” that are of very low density and sediment only with ultracentrifugation. Prefractionation of these components can be useful as a means of enriching for markers of particular types of disease. A study of urine collected from normal human adult subjects indicated that, of the total urinary protein excreted, ~48% was container in sediments, 49% was soluble, and the remaining 3% was in exosomes [5].

The soluble proteins in urine are derived largely from glomerular filtration. The glomerular filter effectively retards passage of high molecular weight proteins. However, even with very low sieving coefficients, proteins that are abundant in the blood plasma such as albumin and various globulins can pass the glomerular filter in substantial amounts to enter the lumen of the nephron. Beyond this, peptides and small proteins (<10 kDa) are freely filtered by the glomerulus. Most of the proteins and peptides that pass the glomerular filter are scavenged and proteolyzed in the proximal tubule by highly specialized apical uptake processes that involves receptor-like recognition of the polypeptide molecules [6]. Thus, a change in the amount of a given soluble protein that reaches the final urine can result from a change in its concentration in the blood plasma, a change in the function of the glomerular filter, or an alteration in the proximal tubule scavenging system. Based on these mechanisms, changes in excretion rate of specific urinary proteins can be indicative of systemic disease, glomerular disease, or diseases affecting the proximal tubule, respectively. Some of the soluble proteins in urine originate as membrane-bound proteins that are proteolytically cleaved from their membrane attachments. One of these is Tamm-Horsfall protein (uromodulin), an abundant soluble urinary protein that is secreted by the thick ascending limb of Henle loop, a nephron segment downstream from the proximal tubule [7]. Tamm-Horsfall protein is generally the most abundant protein in urine, forming similar networks of fiber, which can constrain membrane elements in the urine and interfere with fractionation procedures.

Urine typically contains relatively high density sediments consisting chiefly of sloughed epithelial cells and casts, both of which can be isolated using a low speed centrifugation. In addition to other solid phase components (small fragments of membrane, etc.), urine contains numerous exosomes, derived from virtually every epithelial cell type facing the urine including glomerular podocytes, renal tubule cells from proximal and distal nephron segments, and transitional epithelial cells lining the urinary drainage system [8]. Exosomes are small (~80 nm) vesicles that originate as internal vesicles in multivesicular bodies and are excreted from the cell when the outer membrane of the multivesicular body fuses with the plasma membrane. Exosomes can be isolated from urine by either high speed centrifugation or ultrafiltration.

3 Markers for assessing glomerular filtration rate (GFR)

GFR is widely considered the best overall index to assess kidney function. A recent awareness campaigns in the European Union and in the USA has stressed the issue that the accuracy in assessing GFR is a key issue for the early detection of CKD in adults, children, and newborns (http://www.NKDEP.nih.gov). An early detection of changes in GFR leads to an early appropriate treatment options, such as weight loss, exercise, or blood pressure control, especially with angiotensin-converting enzyme (ACE) inhibitors, slowing or even halting progression of renal injury and/or dysfunction. Serum creatinine concentration and endogenous creatinine clearance have been used for such a long time to assess GFR; nevertheless, serum creatinine has a limited diagnostic value, because of analytical and physiological pitfalls. Muscle mass, diet, renal tubular secretion rate, are “in vivo” interfering factors causing inaccuracy in assessing GFR by serum creatinine; in the first week of life, an additional interference in assessing the true value of creatinine is the maternal rate. Being serum
creatinine concentration and GFR closely related each other, the performance of the creatinine analysis significantly affects GFR estimated on the basis of serum creatinine [9]. In fact, proteins and substances with a ketone group are known to interfere in the Jaffe (alkaline picrate) reaction for the measurement of creatinine in serum and urine; this prevents the measurement of the true concentration of creatinine [10]. Recently, a body of evidences suggest that enzymatic creatinine methods are preferred for evaluation of kidney function in babies and children [11]. The more recent enzymatic creatinine method results in lower determinations compared with the older Jaffe method, even when the latter was improved with a dialysis step and elimination of interfering samples [12]. Finally, there is a lack of availability of pediatric creatinine serum standards referenced to an isotope dilution mass spectrometry method [13].

Because serum creatinine concentration is influenced by both the production rate and the excretion rate, the nephrology community concluded that results should be interpreted in light of the expected rate of production of creatinine. Thus, equations based on serum creatinine are more accurate and precise than serum creatinine alone for estimating GFR (eGFR), as recommended by the clinical practice guidelines for Chronic Kidney Disease (CKD) in children [14]. Estimating equations include variables such as sex, age, body size, and ethnicity in addition to serum creatinine, as surrogates for muscle mass. Equations have the advantage of providing an estimate of GFR which empirically combines all of these average effects while allowing for the marked differences in creatinine production between individuals [15]. Clinical laboratories should report an estimate of GFR using a prediction equation, in addition to reporting the serum creatinine measurement [16]. The most widely used estimate of GFR is the original Schwartz equation [17]: based on serum creatinine (Scr), height, and an empirical age-related constant k, this equation was firstly devised in the mid-1970s. It has been successful because it relates GFR to (patient’s height)/(Scr) rather than to 1/(Scr) [18]. Although simple to use, the Schwartz formula may give biased and imprecise eGFR, unless the values of k are derived from local estimates of mean height, GFR, and Scr. In 2009, Schwartz proposed a new formula for calculating eGFR in children with CKD [19]. The new equation includes serum creatinine, measured by an enzymatic analytical method, cystatin C, and BUN together with an accurate height measurement; the results seem to provide a more accurate, noninvasive method of estimating the GFR in the pediatric and neonatal age. This formula yielded 87.7% of eGFR within 30% of the GFR assessed by iohexol plasma disappearance and 45.6% within 10%. This new equation permits to improve the adjustment of drug dosing and can be used as a research tool. But it can also be used clinically as a confirmatory screening tool for children with impaired renal function and to determine whether CKD in children is stable or progressing. Unfortunately, it cannot yet be used as a general screening tool, since it has not yet been verified in a cohort of children with normal renal function.

Taking into account the extreme importance of the accuracy in the measurement of serum creatinine when creatinine-based equation are used to estimate GFR [20], the National Disease Education Program (NKDEP) has developed several recommendations for the improvement and development of creatinine assays [9]. These recommendations include optimizing creatinine assays to provide accurate (traceable to MS-IDMS) and precise measurements (imprecision goal of approximately 8% to meet the maximum 10% impact on eGFR) particularly at a concentration of 1.00 mg/dL, revising GFR-estimating equations based on more accurate methods, and introducing proficiency testing programs that use commutable serum materials with target values traceable to MS-IDMS procedure [21]. The National Institute for Standards and Technology has developed a reference material at concentrations of approximately 0.80 and 4.00 mg/dL to help manufacturers in the standardization of creatinine assays [22]. Finally, pediatricians need to recognize that the formula requires updating when analytically specific methods of measuring serum creatinine, such as enzymatic assays, are used in their institutions [23].

3.1 Low-molecular mass (Low-$M_t$) proteins

Since 1968, it was postulated a close relationship between blood amount of low- molecular mass proteins (low-$M_t$ proteins) and kidney function [24]; these proteins are freely filtered through the capillary wall and then almost completely reabsorbed and catabolized in the proximal tubular cells. A reduction in GFR corresponds to an increase in their blood plasma concentration [25]. On the other hand, if the tubular reabsorption
capacity is reduced, or if tubular cells have been damaged by nephrotoxic drugs or agents, the urinary excretion of these proteins increases and may be taken as suitable index of renal tubular impairment and dysfunction [26]. Multiple low-\(M_r\) proteins have been investigated in several clinical studies in order to obtain a candidate suitable endogenous markers better reflecting changes in GFR than creatinine; among these proteins, lysozyma, ribonuclease, retinol binding protein (RBP), factor D, \(\beta_2\)-microglobulin, \(\alpha_1\)-microglobulin (protein HC), \(\beta\)-trace protein, cystatin C, etc. However, only \(\alpha_1\)-microglobulin and cystatin C were found to be predictive of severe kidney failure [27]; in particular, cystatin C is released into the blood at a relatively constant rate by all nucleated cells, being not influenced by muscle mass, gender, body composition, and age after 12 months of life, while \(\alpha_1\)-microglobulin is stable in urine samples, being not degraded at acidic pH [28]. In addition, both these proteins can be easily and fast measured by immunometric methods in most standard clinical chemistry laboratories.

3.2 Cystatin C

Cystatin C is a non-glycosylated 13 kDa basic protein that acts as a cysteine proteinase inhibitor; cystatin C is produced at a relatively constant rate and its blood plasma level is approximately 1 \(\text{mg/L}\) in healthy individuals of age >1y, while in the neonatal age and in the early infancy is significantly higher [29, 30]. Cystatin C does not cross the placental barrier, being neonatal cystatin C blood plasma levels not influenced by maternal cystatin C concentration [31]. Cystatin C is catabolized and almost completely reabsorbed by renal proximal tubular cells, so that a very small amount is excreted in the urine; because it is metabolized and not excreted, cystatin C cannot be used to measure GFR by standard urinary clearance techniques [32]. From a number of clinical studies on cystatin C, two key findings are evident: first, the concentration of serum cystatin C correlated better with directly measured values for GFR than did serum creatinine; second, subtle decrements in GFR are more readily detected by the determination of serum cystatin C than by creatinine concentration [33]. Thus, while cystatin C is not a conventional marker of GFR, reciprocal values of serum cystatin C levels are reasonably well correlated with GFR [34]. Most of clinical studies reported in the literature have clearly demonstrated that blood plasma cystatin C increase already with mild reduced GFR of 70 to 90 \(\text{mL/min}\)/1.43m\(^2\) in the “creatinine-blind range”. Cystatin C-based equations for estimating GFR have been extensively proposed in the literature [35]; however, these equations have been generated and validated in small data set of patients, in single center settings, and by using different gold standard measurements for GFR. This partially explains the variation between individual equations. Additional equations using both cystatin C and creatinine to estimate GFR have also been reported [36], but their application in the routine seems to be poor reliable, because they summarize analytical imprecision of different lab tests as well as use different constants for different categories of patients. Factors other than renal function could limit the diagnostic use of cystatin C [37]; in particular, corticosteroids [38], thyroid dysfunction [39], chronic inflammatory state associated with atherosclerosis [40], obesity, and smoking [41] could significantly change plasma cystatin C concentration, leading to an underestimation of GFR. Recently, cystatin C has emerged as a strong independent predictor of cardiac mortality in hospitalized patients for worsening chronic heart failure (CHF) with normal to moderately impaired kidney function [42]; in addition, Cystatin C may substantially improve the admission risk stratification in patients with non-ST elevation acute coronary syndrome [43]. Cystatin C has been measured in fetal urine [44] and in amniotic fluid [45, 46] as a marker for assessing fetal obstructive uropathies and kidney malformations.

4. Markers for assessing kidney injury

A number of clinical conditions affecting newborns and children admitted to the intensive care unit (ICU), like asphyxia, ischemia-reperfusion, anoxia, nephrotoxic agents and drugs, sepsis, surgery, fluid imbalance, etc., could cause tissue injury and damage and, ultimately, could lead to a rapid loss of kidney function characterized by oliguria/anuria. This clinical condition, previously identified as “acute renal failure”, has recently redefine and named acute kidney injury (AKI) [47]. Critically ill newborns are at risk of having AKI, as they are commonly exposed to nephrotoxic medications and have frequent infections that lead to multi-organ failure. Published studies estimate that the incidence of AKI in critically ill newborns is between 8% and 24% and that mortality rates are between 10% and 61% [48]. In a retrospective analysis, it was found a 25% hospital mortality.
rate in newborns with AKI; 47% of those newborns had non-oliguric renal failure and premature infants constituted 31% of the cases [49]. In current clinical practice, AKI is typically diagnosed by measuring serum creatinine. In 2007, the Acute Kidney Injury Network (AKIN), a collaborative group of investigators from all major critical care and nephrology societies, proposed a staging system based 3 categories (mild, moderate, and severe) in a way similar to those (risk, injury, and failure) used by the RIFLE staging system [50]. In children, it was proposed a modified pediatric RIFLE (pRIFLE) classification in which similar criteria were used for pediatrics [51]. Despite these working classification systems, the diagnosis of AKI is problematic, as current diagnoses rely on two functional abnormalities: functional changes in serum creatinine and oliguria. Both these are late consequences of injury and not markers of the injury itself. Partially following a very recent suggestion [52], an ideal biomarker for AKI should: (a) distinguish pre-renal AKI from apoptotic and necrotic damage; (b) be specific for kidney tissue lesion; (c) be up-regulated shortly after an injury in the organ tissue; (d) predict outcome; (e) act as surrogate end point useful for clinical interventional approaches; (f) be independent of GFR level.

Because the incidence of AKI continues to rise, while the outcomes remain poor, nephrologists and intensivists continue to devote numerous resources for the better improvement of outcomes. Recent advances in the field of early AKI biomarkers have provided great optimism. Novel urine and serum biomarkers may significantly improve outcome and reduce mortality if they are able to indicate AKI hours after an insult, in comparison with the days it may take serum creatinine to rise substantially. As opposed to our current functional markers of AKI (GFR and urine output), these biomarkers promise to signal injury early in the disease process, and, hopefully, they will allow us to intervene in the disease process at the onset of acute kidney ‘injury’ as opposed to attempting to fix acute kidney ‘failure’ [53]. Currently, the most promising early non-invasive biomarkers of AKI are serum and urinary neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), urinary interleukin-18 (IL-18), and serum cystatin C. More focused proteomic approaches have recently yielded additional biomarkers for AKI. Other markers continue to be investigated, but they need clinical and analytical validation.

4.1 Neutrophil Gelatinase-associated Lipocalin (NGAL)

Neutrophil Gelatinase-associated Lipocalin (NGAL), also known as Lipocain-2 (lcn2) as well as Siderocalin, is a ubiquitous 25 kDa protein covalently bound to gelatinase from human neutrophils. NGAL is a member of the lipocalin super family with various biological functions such as the induction of apoptosis, the suppression of bacterial growth, and modulation of inflammatory response [54]. NGAL is physiologically expressed at very low concentrations in various human tissues, including the kidney, lungs, stomach, trachea, and colon [55]. NGAL expression increases greatly in the presence of inflammation and injured epithelia and therefore NGAL is one of the earliest proteins induced in the kidney after ischemic or nephrotoxic insult. Consequently, NGAL significantly rises in blood and urine soon after AKI [56]. These findings have spawned a number of translational proteomic studies to evaluate NGAL as a novel biomarker in human AKI.

In a cross-sectional study, subjects in the intensive care unit with established ARF displayed a greater than 10-fold increase in plasma NGAL and more than a 100-fold increase in urine NGAL by Western blotting when compared to normal controls [57]. Both plasma and urine NGAL correlated highly with serum creatinine levels. Kidney biopsies in these patients showed intense accumulation of immuno-reactive NGAL in 50% of the cortical tubules. These results identified NGAL as a widespread and sensitive response to AKI [58]. In a prospective study of children undergoing cardiopulmonary bypass, AKI (defined as a 50% increase in serum creatinine) occurred in 28% of the subjects, but the diagnosis using serum creatinine was only possible 1-3 days after surgery. In marked contrast, NGAL measurements revealed a robust 10-fold or more increase in the urine and plasma, within 2-6 hours of the surgery in patients who subsequently developed AKI. Both urine and plasma NGAL were powerful independent predictors of AKI, with an AUC of 0.998 for the 2 hour urine NGAL and 0.91 for the 2 hour plasma NGAL measurement [58]. The 2 hour NGAL level represented a strong independent predictor of clinical outcomes such as duration of AKI among cases. Thus, plasma and urine NGAL have emerged as sensitive, specific, and highly predictive early biomarkers of AKI after cardiac surgery in children. In a prospective multicenter
study of children and adults, urine NGAL levels in samples collected on the day of transplant clearly identified cadaveric kidney recipients who subsequently developed delayed graft function and dialysis requirement (which typically occurred 2-4 days later). The ROC curve for prediction of delayed graft function based on urine NGAL at day 0 showed an AUC of 0.9, indicative of an excellent predictive biomarker [59]. Urine NGAL has also been shown to predict the severity of AKI and dialysis requirement in a multicenter study of children with diarrhea-associated hemolytic uremic syndrome [60]. Preliminary results also suggest that plasma and urine NGAL measurements represent predictive biomarkers of AKI following contrast administration [61] and in the intensive care setting. In summary, NGAL is emerging as a center-stage player in the AKI field, as a novel predictive biomarker [62]. However, it is acknowledged that the studies published thus far are small, in which NGAL appears to be most sensitive and specific in relatively uncomplicated patient populations with AKI. NGAL measurements may be influenced by a number of coexisting variables such as pre-existing renal disease and systemic or urinary tract infections [63]. Large multicenter studies to further define the predictive role of plasma and urine NGAL as a member of the putative “AKI panel” have been initiated; simultaneously, a new chemiluminescent microparticle method, optimized on a fully-automated analytical platform (ARCHITECT, Abbott Diagnostics Inc, Abbott Park, IL, USA) has been developed for the measurement of urine NGAL in the clinical practice and, specifically, in the emergency setting [64]. This new simple method permits to measure urine NGAL in all the clinical laboratories in a time closely comparable with that occurring for measuring creatinine.

4.2 Kidney Injury Molecule 1 (KIM-1)

Kidney injury molecule 1 (KIM-1) is a type-1 transmembrane protein with glycosylated mucin and IgG-like domains in the ectodomain of the protein and a relatively short intracellular domain that is tyrosine phosphorylated. The ectodomain is cleaved by metalloproteinases. The intracellular domain has a tyrosine phosphorylation site that may be critical for the regulation of KIM-1 function. Downstream proteomic studies have also shown KIM-1 to be one of the most highly induced proteins in the kidney after AKI in animal models, and a proteolytically processed domain of KIM-1 is easily detected in the urine soon after AKI [65]. In a small human cross-sectional study, KIM-1 was found to be markedly induced in proximal tubules in kidney biopsies from patients with established AKI (primarily ischemic), and urinary KIM-1 distinguished ischemic AKI from prerenal azotemia and chronic renal disease [66]. Patients with AKI induced by contrast did not have increased urinary KIM-1.

Recent preliminary studies have expanded the potential clinical utility of KIM-1 as a predictive AKI biomarker [67]. In a small case-control study of 40 children undergoing cardiac surgery, 20 with AKI (defined as a 50% increase in serum creatinine) and 20 without AKI, urinary KIM-1 levels were markedly enhanced, with an AUC of 0.83 at the 12 hour time point [68]. In a larger prospective cohort study of 201 hospitalized patients with established AKI, both urinary KIM-1 as well as urinary N-Acetyl-β-(D)-Glucosaminidase (NAG) were found to be associated with adverse clinical outcomes, including dialysis requirement and death [69]. KIM-1 represents a promising candidate for inclusion in the urinary “AKI panel”. An advantage of KIM-1 over NGAL is that it appears to be more specific to ischemic or nephrotoxic AKI, and not significantly affected by prerenal azotemia, urinary tract infections, or chronic kidney disease. On the other hand, analytical methods for measuring KIM-1 should be improved and optimized on automated platforms in order to introduce this test in clinical practice. It is likely that NGAL and KIM-1 will emerge as tandem biomarkers of AKI, with NGAL being most sensitive at the earliest time points and KIM-1 adding significant specificity at slightly later time points.

4.3 Additional protein markers for assessing AKI

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 [70]. In a cross-sectional study, urine IL-18 levels were markedly increased in patients with established AKI, but not in subjects with urinary tract infection, chronic kidney disease, nephritic syndrome, or prerenal failure [71]. Urinary IL-18 was significantly upregulated up to 48 hours prior to the increase in serum creatinine in patients with acute respiratory distress syndrome who develop AKI, with an AUC of 0.73, and represented an independent predictor of mortality in this cohort [72]. Both urinary IL-18 and NGAL were recently
shown to represent early, predictive, sequential AKI biomarkers in children undergoing cardiac surgery [73]. In patients developing AKI 2-3 days after surgery, urinary NGAL was induced within 2 hours and peaked at 6 hours whereas urine IL-18 levels increased around 6 hours and peaked at over 25-fold at 12 hours post surgery (AUC 0.75). Both IL-18 and NGAL were independently associated with duration of AKI among cases. In a prospective multicenter study of children and adults, both NGAL and IL-18 in urine samples collected on the day of transplantation predicted delayed graft function and dialysis requirement with AUC of 0.9 [74]. IL-18 is more specific to ischemic AKI, and not affected by nephrotoxins, chronic kidney disease or urinary tract infections. The application of innovative technologies such as functional genomics and proteomics to human and animal models of kidney disease has uncovered several novel candidates that are emerging as biomarkers and therapeutic targets [78, 79]. Gene expression studies have provided several additional clues regarding the AKI proteome, but human data are hitherto lacking. For example, Muramatsu et al., have utilized a subtractive hybridization approach to identify Cyr61 (also known as CCN1) as a markedly upregulated gene in the rat kidney very early after ischemic injury [80]. Cyr61 protein was induced in the kidney within one hour and detectable in the urine at 3-6 hours after ischemic injury, but not after volume depletion. However, this detection required a complex bioaffinity purification step with heparin-Sepharose beads, and even after such purification, several crossreacting peptides were apparent.

Supavekin et al., performed detailed mouse kidney microarray analyses at early time points after ischemia-reperfusion injury to identify consistent patterns of altered gene expression, including transcription factors, growth and regenerative genes, and apoptotic molecules [81]. Prominent among the last category included FADD, DAXX, BAD, BAK, and p53, all of which were confirmed by immunohistochemistry. Mounting evidence now indicates that apoptosis is a major mechanism of early tubule cell death in contemporary clinical AKI [82, 83]. Several human models of AKI have consistently demonstrated the presence of apoptotic changes in tubule cells [84]. Importantly, proteomic studies have now identified a multitude of apoptotic pathways, including the intrinsic (Bcl-2 family, cytochrome c, caspase 9), extrinsic (Fas, FADD, caspase 8), and regulatory (p53) factors, that are activated in tubule cells following human AKI [85]. As a consequence of these studies, inhibition of apoptosis has emerged as a promising approach in human AKI [86]. Cell-permeant caspase inhibitors have provided particularly attractive targets for study. In this regard, an orally active small molecule pan-caspase inhibitor (IDN-6556, Pfizer) has been shown to be effective in preventing injury after lung and liver transplantation in animals [87]. While many of them have now been confirmed by downstream proteomic analysis, the majority of these studies remain in the pre-clinical research realm, and convincing data attesting to their utility in human AKI are currently unavailable.

5 Molecular biology for assessing AKI

Spermidine/spermine N1-acetyltransferase (SSAT), the rate-limiting enzyme in polyamine catabolism, is a novel early biomarker of tubular cell damage after ischemic injury in rats. SSAT protein appears to play a role in the initiation of oxidant-mediated injury to tubules, raising the possibility of inhibition of polyamine catabolism as a future therapeutic approach [75]. It was found that another maximally induced gene identified very early after ischemic injury in animal models is Zf9, a Kruppel-like transcription factor involved in the regulation of a number of downstream targets [76]. Zf9 protein is markedly upregulated in the postischemic tubule cells, along with its major trans-activating factor, TGF-β1. Gene silencing of Zf9 abrogated TGF-β1 protein expression and mitigated the apoptotic response to ischemic injury in vitro. These studies have thus identified a novel pathway that may play a critical role in the early tubule cell death that accompanies ischemic renal injury. Thakar et al., have employed transcriptome profiling in rat models to identify thrombospondin 1 (TSP-1), a previously known p53-dependent pro-apoptotic and anti-angiogenic molecule, as another maximally induced gene early after ischemic AKI [77]. The TSP-1 protein product is upregulated in the postischemic proximal tubule cells, where it colocalizes with activated caspase-3. TSP-1 null mice were partially protected from ischemic injury, with striking structural preservation of kidney tissue. These results have thus identified yet another previously unknown apoptotic protein that is activated in proximal tubule cells early after ischemic AKI in animals.
6 Conclusions

There is a growing demand for a clinically convenient and reliable marker of renal fetal and neonatal function both to assess GFR and kidney injury [88]. From a clinical point of view, the introduction of such biochemical markers should represent a significant advantage for the early identification and treatment of kidney diseases. To be clinically reliable, markers should be very high sensitive and specific, easy to measure in short time for emergency, and with a very high positive ratio cost/effectiveness. From an analytical point of view, results should be accurate, precise, and standardized in order to minimize inter-laboratory variability. It is likely that cystatin C will be introduced in the clinical practice for the assessment of GFR; similarly, it is likely that NGAL, IL-18 and KIM-1 will emerge as sequential urinary biomarkers of AKI [89].

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