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Clinical biomarkers in kidney diseases

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1. ABSTRACT

Biomarkers are “biological parameters that can be objectively measured and evaluated, which act as indicators of normal or pathogenic processes, or of the pharmacological response to a therapeutic intervention”. Renal failure can be broadly divided in acute and chronic renal diseases, two classes of renal pathology that are well distinct each other, not only on the basis of duration and reversibility of loss of kidney function, but also because of their different aetiolopathological processes and their different histopathological characteristics. Unlikely, the conventional measures used for monitoring kidney function are not ideal in the diagnosis of neither acute or chronic kidney diseases and has impaire our ability to institute potentially effective therapies. Therefore, researchers are seeking new early, predictive, non-invasive biomarkers that can aid in the diagnosis for both acute and chronic diseases. These biomarkers will be useful for assessing the duration and severity of kidney disease, and for predicting progression and adverse clinical outcomes. This review article summarized our current understanding of the acute and chronic renal diseases and discussed the most promising biomarkers for facilitating early detection and predicting clinical outcomes.

2. INTRODUCTION

The kidney normally performs a variety of functions essential for the regulation of a constant environment and the maintenance of metabolic homeostasis (1). In order to sustain glomerular filtration and renal metabolism, the renal vascular bed receives a disproportionately large blood flow, averaging 20-25% of resting cardiac output. As a result, cells of the renal vasculature, glomerula, tubules and interstitium are exposed to high volumes of toxicants. The tubular epithelium is especially susceptible to injury, as a result of 1) solute and water reabsorption along the nephron, producing greater concentration of filtered toxicants in the tubular fluid than those seen in the general circulation; 2) transport processes resulting in high intracellular concentration of toxicants and their metabolites; 3) high energy requirements necessary to support epithelial cell metabolism and solute transport. The effects of toxicants on the kidney are protean, with a multitude of different compounds and varying mechanism of injury implicated. Injury may occur secondary to altered renal hemodynamics, direct cellular damage to the tubular epithelium, tubular obstruction of urinary flow due to the precipitation of toxins or their metabolites, interstitial...
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Figure 1. Site and mechanism of injury of toxicants on the kidney. Adapted with permission from (1).

inflammation, and/or thrombotic microangiopathy (Figure 1) (1). Initially, injury is often manifested by subtle changes in tubular function, including altered urine concentration ability and/or electrolyte handling. In the absence of judicious monitoring, injury may evade detection until a significant decline in renal functional capacity occurs.

Renal failure can be broadly divided in acute and chronic renal diseases, two classes of renal pathology that are well distinct each other, not only on the basis of duration and reversibility of loss of kidney function, but also because of their different aetiological processes and their different histopathological characteristics. However, there is ongoing recognition of the important interaction between acute and chronic kidney diseases. Recent studies about the impact of chronic kidney diseases on epidemiology and outcome of acute renal diseases, in fact, showed that the rapid decline in glomerular filtration rate (GFR) occurring over hours to weeks characteristic of acute renal diseases, can occur in the setting of previously normal renal function, or can be superimposed on pre-existing chronic pathology (2).

This review article summarized our current understanding of the acute and chronic renal diseases and discussed the most promising biomarkers for facilitating early detection and predicting clinical outcomes.

3. ACUTE RENAL FAILURE

It was Homer W. Smith who is credited with the introduction of the term Acute Renal Failure (ARF), in a chapter of his textbook “The kidney - Structure and Function in Health and Disease” (3). In most reviews and textbooks the concept of acute kidney dysfunction still emphasized the most severe forms with severe azotemia and often with oliguria or anuria. It is only in the past few years that moderate decreases of kidney function have been recognized as important.
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ARF is the generic term used to indicate a sustained decrease in renal function resulting in retention of nitrogenous (urea and creatinine) and non-nitrogenous waste products. Depending on the severity and duration of the renal dysfunction, this accumulation is accompanied by metabolic disturbances (e.g., acidosis and hyperkalaemia), changes in body fluid balance and effects on many other organ systems (4).

3.1. Causes of ARF in the critically ill patient

Causes of ARF can be broadly divided into three categories: 1) pre-renal ARF; 2) post-renal ARF and 3) intrinsic renal ARF (Figure 2) (4).

1. In the pre-renal form there is a reversible increase in serum creatinine and blood urea concentrations; it results from decreased renal perfusion, which leads to a reduction in glomerular filtration rate (GFR). The reduced GFR means that a greater fraction of salt and water can be absorbed and thus less will enter the tubules. Of course, less tubular filtrate means less urine and less nitrogen excretion. Pre-renal ARF, in which the integrity of the renal tissue is preserved, is a physiological response to renal hypo-perfusion. This can be secondary to congestive heart failure or decompensate liver failure. Other causes are volume depletion, renal artery stenosis and decreased glomerular perfusion pressure secondary to drugs that influence glomerular perfusion by interference with normal auto-regulatory mechanisms (e.g., non-steroidal anti-inflammatory drugs (NSAIDs), angiotensin-II receptor antagonists) (5). Pre-renal azotemia can be corrected if the extrarenal factors causing renal hypo-perfusion are reversed. When not corrected, persistent renal hypo-perfusion will ultimately lead to ischemic acute tubular necrosis.

2. Post-renal ARF is due to obstruction of the urine outflow tract by either intrinsic or extrinsic masses (as for instance hypertrophy of the prostate). An important clinical consequence of post-renal ARF is the post obstructive diuresis (from 4 to 20 L/day) that can occur after the release of the obstruction causing volume depletion: in these cases, patients necessitate careful monitoring and adjustment of the volume and electrolyte status.

3. The remaining patients have the renal form, in which structures of the nephron (glomerula, tubules, vessels or interstitium) are affected. The major cause of intrinsic renal azotemia is acute tubular necrosis. The worldwide range of factors that cause acute tubular necrosis shows great variability among populations, even if it can be often induced by ischemic or nephrotoxic injury (several classes of antibacterial, antifungal, antiviral, anti-neoplastic agents and many radio-contrast molecules are nephrotoxic).
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Pre-renal azotemia and ischemic acute tubular necrosis occur on a continuum of the same pathophysiology process and together account for 75% of the cases of ARF (6).

3.2. Histopathology of ARF

While recent advances have suggested novel therapeutic approaches in animal models, translational efforts in human have yielded disappointing results. The reason for this included an incomplete understanding of the mechanisms of ARF risk factors and the role of the patient’s baseline (7). A vast literature on mechanisms of injury in experimental ARF suggests tubular cell injury leading to tubular obstruction and backleak, leukocyte–endothelial interactions, persistent vasoconstriction secondary to tubulo-glomerular feedback, and inflammation including leukocyte infiltration as major events initiating and extending ARF and loss of glomerular filtration (8). The mechanisms of ARF involve both vascular and tubular factors, with a different relative importance in each patient on the basis of the autopathogenesis of ARF that is considered (9).

Recent evidence has highlighted both structural and functional vascular changes including endothelial dysfunction impairing autoregulation (10) and endothelial injury and obstruction in peritubular capillaries in ARF (11). Moreover, accumulating evidence suggests that progressive vascular obliteration is a significant contributing mechanism to progression after acute injury, which may explain the approximately 30% of survivors of ARF who develop chronic kidney failure and the development of hypertension in many others (12). Thus, while the exact mechanisms remain unclear, impaired renal perfusion probably contributes to both acute and chronic kidney injury. Studies of experimental ARF in animals have developed our understanding of the mechanisms of ischemia-reperfusion and toxic ARF, the two commonest causes.

The typical histological features of human acute tubular necrosis (the major type of intrinsic-renal cause of ARF) include vacuolation, loss of brush border in proximal tubular cells and sloughing of tubular cells into the lumen, leading to cast obstruction. There is “cellular simplification” of both proximal and distal tubules (loss of brush border and basolateral membranes); nevertheless, the major changes appear to be in the distal nephron (13). Interstitial edema with mild to moderate leukocytes infiltration can produce widely spaced tubules (14). Although the disorder is named necrosis, frankly necrotic cells are not a common finding. Specifically, in established ATN the presence of tubular necrosis upon histological examination of the kidney is seen in only occasional tubule cells, and in some cases may not even be detectable (15). What is clear with established ATN, moreover, is that the glomerula are morphologically normal. With advanced injury, tubular epithelial cells detach from the basement membrane and contribute to intraluminal aggregation of cells and proteins resulting in tubular obstruction (16).

3.3. Epidemiology of ARF

Nowadays, ARF is a common clinical problem encountered in critically ill patients and characteristically portends an increase in morbidity and mortality (ARF is often associated with anemia, neuromuscular diseases, infections, immune suppression and leads to cardiovascular and pulmonary consequences in critically ill patients) (17). Clearly, trials of prevention and therapy are not comparable because widely disparate definitions have to be used. The spectrum of patients presenting with ARF has changed over the last few decades. As instance, we have seen a decline in the number of cases due to trauma and obstetrical causes, but at the same time, the number of patients with multi-organ dysfunction has increased (18). Two recent papers describe the epidemiologic and prognostic evolution of ARF over the last decade. In the first paper, Xue and colleagues report the incidence of ARF as 23.8 per 1000 discharges with rates increasing by approximately 11% per year from 1992 to 2001 (19). The other analysis by Waikar and colleagues reported, instead, an increased incidence of ARF from 61 to 288 per 100.0.00 population over a 15-year period from 1988 to 2002 (20). These studies indicated that, despite technical advances in renal replacement therapy and supportive care over the past few decades, the mortality rate with patients with ARF remains high, especially in ICUs. In contrast, Ympa and colleagues performed a systematic review of literature and found that over the last 50 years the mortality rates have remains unchanged at round 50% (21). The clinical condition of ARF is said to occur in anywhere from 15 to 25% of critically ill patients (22), depending on the population being studied and the criteria used to define its presence. Furthermore, mortality in these populations ranges from 28% to 90% (23). The lack of a uniform definition of ARF may be partly responsible for these discrepancies. Prospective studies utilizing a common definition of ARF will provide a significant advance in this field.

3.4. Definition and classification systems for ARF

Despite several advances in treatment and in the understanding of the pathogenesis of ARF, many aspects in this field remain subject to controversy, confusion and lack of consensus. These problems include the definition of ARF (24), the choice, validity and relevance of animal models of ARF (13), and the choice regarding appropriate physiological and clinical end-points for trials of new treatments (25). They also include principles that should govern fluid management in patients with ARF (26) and use of information technology to optimize all areas of patients care in this field.

About the problem of a universally accepted definition of ARF, indeed, a recent survey revealed the use of more than 35 different definitions in medical literature (27). These definitions describe the whole spectrum of severity grades of ARF, from mild (an increase of 25% in serum creatinine) to severe (with need for renal replacement therapy (RRT)). This is probably one of the main reasons of the wide variation in the reported incidence and outcome of ARF in different studies (incidence ranges between 1 and 31% and mortality between 28 and 82%) (28). Bellomo and colleagues (29) suggested that it is important to consider the following features in any definition of ARF: it should consider changes from baseline; it should include classifications for acute and...
3.4.1. RIFLE classification

Recognizing the need for uniform standards, the Acute Dialysis Quality Initiative (ADQI) group, a group of experts in acute kidney dysfunction consisting of nephrologists and intensivist from around the world, in 2002 proposed the RIFLE criteria for the definition and staging of ARF. The RIFLE criteria classified ARF into three increasing severity categories (risk, injury and failure) defined on the basis of the changes in serum creatinine or urine output, and two clinical outcome categories (loss and end-stage renal disease) defined by the duration of loss of kidney function (Figure 3) (34) (29). In the successive years the RIFLE classification has been evaluated in a number of clinical studies of critically ill patients with ARF (30); (31); (32); (33). These criteria have now been applied to more than 70,000 patients with varying acute problems and chronic comorbidities. All studies showed an increase in mortality with worsening RIFLE class. In a systematic review of 13 studies, Ricci and colleagues concluded that there was a clear correlation between the RIFLE classification and outcome (34). So, in general, these criteria have been found to have clinical relevance for monitoring the progression of ARF, as well as having modest predictive ability for mortality.

3.4.2. Modifications of the RIFLE criteria

In September 2005, nevertheless, a somewhat larger, multi-disciplinary, international group, the Acute Kidney Injury Network (AKIN) proposed some small modifications to the RIFLE criteria and suggested the term acute kidney injury (AKI) to represent the entire spectrum of acute renal dysfunction ranging from mild elevation in serum creatinine to severe forms requiring renal replacement therapy (RRT) (35). In particular, the AKIN group proposed that patients meeting the definition of AKI be classified from stage 1 to 3 (Figure 4) (36). The proposed staging is based on the following principles:

- with the purpose of increasing the sensitivity of the RIFLE criteria, they used a smaller change in serum creatinine (≥ 26.2 µmol/L) as a threshold to define the presence of AKI and identify patients with Stage 1 AKI (analogous of RIFLE-
Figure 4. AKIN criteria for classification of AKI. Adapted with permission from (36).

Risk. As a consequence, those patients who are classified as having “Injury” or “Failure” match respectively to Stage 2 and 3; “Loss” and “ESRD” categories were removed from the staging system, since they represent outcomes;

- a 48 hours time window for the diagnosis of AKI was introduced to ensure that the process was acute;

- finally, any patients receiving renal replacement therapy were be classified as Stage 3 AKI (RIFLE-Failure).

It is currently unknown whether discernible advantages exist with one approach to definition and classification versus the other. However, in the current clinical practice AKI is considered to be present when there is an abrupt (within 48 hours) reduction in kidney function, defined as an absolute increase in serum creatinine of either ≥ 0.3 mg/dl or a percentage increase of ≥ 50% or reduction in urine output (documented oliguria of < 0.5 ml/kg/hr for >6 hours). It is, however, necessary that the diagnosis is made following estimation of at least two creatinine values within 48 h. If the diagnosis of AKI is based on the urine output criterion alone, urinary tract obstructions and other reversible causes of oliguria (e.g. hydration status and diuretic use), should be excluded.

3.5. Diagnostic approach

As previously described, most definitions of AKI have common elements including the use of serum creatinine and, often, urine output. Although the kidney has numerous functions, these are the only functions that are routinely and easily measured and that are unique in the kidney. Unlike the conventional measures used for monitoring kidney functions (e.g. serum creatinine, blood urea nitrogen (BUN) and other urinary tests) are not ideal in the diagnosis of AKI.

In particular, creatinine, an amino acid compound derived from creatine metabolism in skeletal muscle and from meat dietary intake, is released into the plasma at a relatively constant rate, is freely filtered by the glomerulus and is not reabsorbed by the kidney. The clearance of creatinine is widely used for estimating glomerular filtration rate (GFR), because of the non-linear inverse relationship between GFR and serum creatinine levels: so, a decrease in GFR corresponds to a rise in serum creatinine and indicates a reduced kidney function. Nevertheless, there are some limitations in the use of creatinine clearance for estimation of GFR: first of all, it must be considered that from 10% to 40% of clearance of creatinine depends on tubular secretion of serum creatinine into the urine (37). Therefore, the accuracy of a creatinine clearance measurement is limited because, when GFR falls, creatinine tubular secretion is increased and thus the rise in
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Figure 5. Three hypothetical receiver-operating characteristic (ROC) curves representing a biomarker that is no better than expected by random chance (line in red; AUC of 0.5), a good biomarker (line in purple; AUC of 0.75) and an excellent biomarker (line in green; AUC of 0.9). Adapted with permission from (41).

Thus, these traditional markers suffered from lack of specificity and dearth of standardized assays for the diagnosis of AKI. Fortunately, the application of innovative technologies such as functional genomics and proteomics to human and animal models of AKI has uncovered several novel gene products that are emerging as prominent biomarkers.

3.6. Emerging biomarkers for AKI

Biomarkers are “biological parameters that can be objectively measured and evaluated, which act as indicators of normal biologic or pathogenic processes, or of the pharmacologic response to a therapeutic intervention” (definition by NIH, 2001). The sensitivity, specificity and time course of a biomarker are critical factors in determining the utility of a particular biomarker in a disease process.

An ideal biomarker for detecting AKI for use in clinical practice could have some desirable performance characteristics.

First, it should be non invasive and easy to perform at the bedside, using easily accessible samples such as blood or urine. Regarding the sample source, the majority of AKI biomarkers have been measured in urine. Urinary diagnostic have several advantages, like the reduced number of interfering proteins and the potential for the development of patient self-testing kits. However, several disadvantages also exist, including the lack of sample from patients with severe oliguria and potential influence on urinary biomarker concentrations induced by diuretic therapy. Thus, in case of AKI is important to develop both urinary and plasma biomarkers.

Second, it should be rapid, reliable and not expensive to be measured with standardized assay methods.

Third, it should be highly sensitive to facilitate early detection of AKI and it would enable monitoring of the course of disease over time and have some ability to predict the severity of AKI. Moreover, a good biomarker should have a satisfactory discrimination level, defined as the ability to distinguish “case” from “non-case”, and a high level of calibration, defined as the capacity of a biomarker to predict risk in sub-groups of the population relative to actual observed risk (Figure 5) (41).

Finally, it would be specific in order to allow the classification of different subtypes and pathogenesis of AKI. In the diagnosis of AKI, in fact, a perfect biomarker could be useful for identifying the primary location of injury and understanding the pathogenesis of AKI, for defining the course of the disease and making a prognosis of the duration of kidney failure and, finally, for monitoring the response to therapeutic interventions.

Unfortunately, at this moment, no single biomarker exist which satisfy all these ideal conditions. As AKI is a complex disease with multiple causes, it is possible that one biomarker will not be sufficient to make an early diagnosis. So it is likely that a “AKI panel of biomarkers” will be required to differentiate subtypes of
AKI and to define the phase and the severity of injury. Nowadays, there is a discrete number of serum and urine AKI biomarkers that are under investigation, in different phases of development. In developing new biomarkers for diagnostic purposes, different phases have been identified (42): a preclinical phase in animal models (first phase); two translational phases in which the potential biomarker is tested in a limited clinical setting (second and third phases); the validation phase in which the biomarker is tested in a large scale clinical study (fourth phase) and can be made available for clinical use.

There are two ways for defining renal damage; one of these is linked to biochemical markers and the other to morphological changes. So, the most promising AKI biomarkers are: interleukine-18 (IL-18); neutrophil gelatibase-associated lipocalin (NGAL); kidney injury molecule-1 (KIM-1); cystatin C; fatty acid binding protein (FABP); other biomarkers and tubular enzymes and markers of tubular dysfunction.

### 3.6.1. Interleukin-18

Numerous cytokines have been detected in the urine of critically ill patients with AKI (e.g. IL-1, IL-6, IL-8, IL-18, tumour necrosis factor-α) (43); the increased production of these cytokines in AKI may be both a consequence of and predispose to AKI.

The best characterized cytokine in AKI is urinary IL-18: it is a pro-inflammatory cytokine (known as interferon-γ-inducing factor), produced as a 24kDa inactive precursor that is cleaved by caspase-1 to generate its mature, biologically active form (44) (45). This active form exits the cell and may enter the urine after being activated in the proximal tubules after AKI. IL-18 is involved in many functions; it plays a critical role in innate and in T-cells mediated immunity, in the activation of macrophages and natural killer cells (46) and it is a mediator of ischemic tissue injury in the heart (47) and in brain (48). IL-18 was found to potentiate ischemic AKI and it can be detectable in the urine of mice subjected to ischemic kidney injury (49). To prove that IL-18 plays an injurious role in ischemic AKI, wild-type mice were injected with rabbit antimurine IL-18 neutralizing antiserum before the ischemic insult. These mice were protected against AKI to a similar degree as caspase-1-deficient mice. Immunohistochemistry of mouse kidneys showed an increased staining for IL-18 protein in injured tubular epithelial cells in AKI mice compared with normal control mice (49). Moreover, urine IL-18 was increased in mice with ischemic AKI compared with sham-operated mice. The discovered that IL-18 could be released from the injured tubular epithelial cells into the urine both in mice and in human (also immunohistochemistry of human kidneys with AKI showed an increased IL-18 immunoreactivity in the tubules) let Edelstein to hypothesize that IL-18 may serve as a urinary biomarker of AKI in humans (50).

The first AKI study of urinary IL-18 in humans was a cross-sectional comparison of patients with different renal dysfunctions: acute tubular necrosis (ATN), pre-renal failure, urinary tract infection (UTI), chronic kidney disease (CKD), transplant recipients and healthy controls (51). The highest levels of urinary IL-18 were observed in patients with established ATN and within 24 h after kidney transplantation in patients with delayed allograft dysfunction (52). Urinary IL-18 levels displayed sensitivity and specificity of >90%, with an area under the receiver-operating characteristic curve (AUC-ROC) of 95% for the diagnosis of established AKI: thus, IL-18 seems to be an excellent tool to differentiate ATN from other types of acute renal diseases and raised urinary IL-18 concentrations early after kidney transplantation have been shown as predictive of delayed graft failure (51); (52).

In 2005, a study of critically ill adult patients with acute respiratory distress syndrome (ARDS) first investigated the potential of urinary IL-18 as an early marker of AKI. Urine samples from 52 AKI cases (50% increase in serum creatinine) and 86 control patients were tested for urinary IL-18 using samples previously collected as part of a National Institutes of Health-sponsored Acute Respiratory Distress Syndrome network trial (53). Urine IL-18 levels above 100 pg/mg predicted the development of AKI 24 h before the serum creatinine, with an adjusted odd ratio of 6.5 and AUC of 73% to predict AKI in the next 24 hours. Importantly, urine IL-18 on day of initiation of mechanical ventilation was also a strong predictor of mortality, independently of severity of illness scores, serum creatinine or urine output. Similarly, in a paediatric cohort of 137 critically ill patients (average age of 6.5 years; 53% male), urine IL-18 increased as much as 48 h before AKI and is a predictor of mortality: the peak levels of IL-18 correlated with severity of AKI by the RIFLE classification.

AKI is a frequent complication of cardiopulmonary bypass (CPB). Urinary IL-18 (together with urinary NGAL) was recently shown to represent an early and predictive AKI biomarker in children undergoing cardiac surgery (54). Using serum creatinine, AKI was detected only 48-72 h after CPB; urinary IL-18 levels, in contrast, increased at 4 to 6 h after CPB, peaked at over 25-fold at 12h after surgery (AUC 75%) and remained markedly elevated up to 48 h after CPB. Moreover, urine IL-18 was associated with the duration of AKI and renal recovery, suggesting that it may be a marker of AKI severity.

Delayed graft function (DGF) because of tubule cells injury, frequently complicates kidney transplants from cadavers. In a single-centre study and in a prospective multicentre study of children and adults, urine IL-18 levels in samples collected at the day of transplant clearly identified cadaveric kidney recipients who subsequently developed DGF and dialysis recruitment. In patients with DGF, in fact, while peak postoperative serum creatinine typically occurred 2 to 4 days after transplant, urine IL-18 values were maximally elevated in the first 24 h after transplant: in this study Parikh and colleagues showed that urine IL-18 represents an excellent early and predictive biomarker of DGF with an AUC of 90% (52).
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In conclusion IL-18 represent a promising candidate for inclusion in the urinary “AKI biomarkers panel”. This proinflammatory cytokine is a differential marker for ischemic AKI and other forms of acute tubular necrosis, distinguishing them from prerenal azotemia, chronic kidney disease and urinary tract infections. IL-18 also offers prognostic information about severity, duration and mortality at the time of diagnosis of AKI.

3.6.2. Neutrophil gelatinase-associated lipocalin

Neutrophil Gelatinase-Associated Lipocalin (NGAL) is one of the best studied urinary biomarkers of AKI to date. Human NGAL, also known as lipocalin-2 or siderocalin, belongs to the lipocalin superfamily of over 20 structurally related secreted proteins and was originally identified as a 25 kDa protein synthesized during granulocyte maturation in bone marrow (55) and covalently bound to gelatinase in specific granules of the neutrophil (56). NGAL is a critical component of innate immunity and is normally expressed at very low levels in several human tissues, including kidney, trachea, lung, stomach and colon. NGAL expression is markedly induced in epithelium in the setting of inflammation or malignancy or in other disease states (57): for example, elevated concentrations of NGAL have been seen in serum of patients with acute bacterial infections, in the sputum of subjects with asthma or chronic obstructive pulmonary disease and in the bronchial fluid from patients with a pulmonary emphysema.

Recently, using cDNA microarray screening techniques, Devarajan and colleagues identified NGAL as one of the seven genes whose expression was upregulated >10 fold within the first few hours after ischemia-reperfusion injury (58) or after cisplatin-induced nephrotoxicity (59) in kidney epithelium in animal models. Immunohistochemistry studies demonstrated minimal NGAL expression in control mouse kidney, but marked upregulation in proximal tubules within 3 hours from ischemia (59). NGAL is a small secreted protein that is protease resistant and thus may be easily detected in the urine. NGAL protein increases massively (100 fold) in the first urine output in early ischemic AKI in rats and mice, preceding the appearance of other urinary markers. So, these studies show that NGAL may represent an early, sensitive and non invasive urinary biomarker for ischemic and nephrotoxic kidney injury.

Comparable results were obtained in a cross-sectional study, in which human ICU adults with established AKI (defined as a doubling of the serum creatinine in <5 days) displayed a greater than 10-fold increase in plasma NGAL an more than 100-fold increase in urine NGAL revealed by Western Blot, when compared with controls. Moreover, kidney biopsies in these patients show intense accumulation of immunoreactive NGAL in 50 % of cortical tubules. These results identified NGAL as a widespread and sensitive response to established AKI in humans (60).

A prospective study in a cohort of 71 paediatric patients undergoing cardiopulmonary bypass (CPB) for surgical correction of congenital heart disease, found urinary NGAL to be a powerful early marker of AKI (defined as a 50% increase in serum creatinine), preceding any increase in serum creatinine by 1-3 days (61). In this study, serial urine and blood samples were analyzed by Western Blot and by enzyme-linked immunosorbent assay for NGAL expression: these measurements revealed a >10 fold increase of NGAL in the urine and plasma within 2-6 hours from the surgery in patients who subsequently developed AKI. Even if serum NGAL was inferior to urinary NGAL for the identification of AKI, both 2-hours urine and plasma NGAL measurements were powerful independent predictor of AKI with an AUC respectively of 98% and 91%. In addiction, a larger follow-up study of 120 children by Dent and colleagues, showed, on multivariate analysis, that the concentration of NGAL 2 hours after surgery was a strong independent predictor of clinical outcomes such as postoperative change in serum creatinine levels, duration of AKI and length of hospital stay and mortality after CPB among cases (62).

These findings have now been confirmed in a prospective study of 81 adults who developed AKI after cardiac surgery. In this study, urine samples were collected immediately preoperatively and at various time intervals (1, 3, 18 and 24 hours after surgery) to measure NGAL levels. AKI, defined as 50% of increase in serum creatinine concentration, did not occur until the third postoperative day. Urinary NGAL concentrations in patients who went on to develop clinically significant AKI resulted to be significantly higher compared with patients who did not developed AKI (the AUC for NGAL ranged from 0.6-7 (immediately after surgery) to 0.8-0 (18 hours after surgery)), even if a substantial overlap between patients who did and did not developed AKI was noted (63). A somewhat inferior performance perhaps could be reflective of confounding variables such as old age, pre existing kidney disease, prolonged bypass times, or comorbid conditions and chronic illness (e.g. diabetes, hypertension, atherosclerosis).

NGAL was also been evaluated as a biomarker of AKI in kidney transplantation. Parikh and colleagues studied, in a prospective multicentre study, plasma and urinary NGAL levels in 53 consecutive patients (adults and children) undergoing living or deceased donor kidney transplantation (52). Plasma and urine samples collected on the day of transplantation identified cadaveric kidney recipients who subsequently developed delayed graft function (which typically occurred 2 to 4 days later). NGAL levels (normalized to urine creatinine concentration) were significantly higher in deceased donor recipients with delayed graft function (DGF) than prompt graft function with an AUC of 0.9, for urine NGAL, indicative of an excellent predictive biomarker. Moreover, biopsies of kidney obtained 1 hour after vascular anastomosis revealed a significant correlation between NGAL immunohistochemistry staining intensity and the subsequent development of delayed graft function (64).

Several researchers have investigated the role of NGAL as an early sensitive biomarker of AKI following...
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contrast administration. In a prospective study of patients undergoing percutaneous coronary interventions (PCIs), Bachorzewa-Gajewska and colleagues measured NGAL in urine and plasma before and 2,4,12,24, and 48 hours after PCI. This study showed that both urine and plasma NGAL predicted contrast-induced AKI (defined as a 50% increase of serum creatinine from the baseline concentration): there was a significant rise in serum NGAL 2 and 4 hours after PCI and a rise in urinary NGAL 4 and 12 hours after PCI (65). A similar study of 91 children (age 0-18 years) undergoing elective cardiac catheterization and angiography with contrast administration, revealed that NGAL (both in serum and in urine) is an early predictive biomarker of contrast-induced nephropathy (CIN) within 2 hours from the administration of contrast, with an AUC of 0.9,2 and 0.9.1 respectively. On the contrary, detection of CIN by an increase of serum creatinine was only possible 6 to 24 hours after cardiac catheterization (66).

In a study of 140 ICU children (age 0-21 years) requiring mechanical ventilation, urine was collected daily for 4 days. The authors of this study showed that mean and peak urinary NGAL levels were higher in patients with worsening degrees of AKI. Urine NGAL was a good diagnostic marker for AKI development (48 hours before the development of AKI) in the urine had an AUC of 0.7,8, and for the persistence of AKI for 48 hours or longer (AUC=0.79), but not for AKI severity (AUC=0.63) (67).

Recently, NGAL is also emerging as an early biomarker used in the evaluation of some interventional trials (at least 10 ongoing clinical trials listed in the ClinicalTrials.gov registry). For example, urine NGAL was attenuated in adult cardiac surgery patients who experienced a lower incidence of AKI after sodium bicarbonate therapy when compared with sodium chloride (68). Moreover, urinary NGAL has also been studied as a marker of acute kidney injury after aprotinin (a nephrotoxic fibrinolytic) use during cardiac surgery. Wagener and colleagues found that postoperative levels of urinary NGAL dramatically raised (almost 20 times) in patients who received aprotinin (69).

Ultimately, NGAL is also increased in other clinical conditions besides ischemic AKI, like polycystic kidney disease, diarrohea associated haemolytic uremic syndrome (70) or lupus nephritis and where is able to predict the severity of AKI and dialysis requirement. In summary, NGAL is emerging as an important biomarker of AKI, with a tremendous potential for early diagnosis. Nevertheless, the studies published until now often involved small numbers of participants and NGAL measurements may be influenced by a great number of coexisting variables, such as preexisting renal disease and systemic or urinary tract infections.

3.6.3. Kidney injury molecule-1

Kidney Injury Molecule 1 (KIM-1 in humans, or Kim-1 in rodents) is a type 1 cell membrane glycoprotein containing, in its extracellular portion, a unique six-cysteine immunoglobulin-like domain and a threonine-serine and proline-rich domain characteristic of mucin-like O-glycosylated proteins, suggesting its potential involvement in cell-cell and/or cell-matrix interactions (71). KIM-1 is normally minimally expressed in kidney tissue even if it has been demonstrated a marked upregulation of KIM-1 in adult proximal tubular epithelial cells of the kidney in response to ischemic or nephrotoxic AKI (e.g. after nephrotoxicity following cisplatin) (72); (73). KIM-1 appears to be a marker of injury associated with renal tubular cells dedifferentiation that occurs when urinary concentrations rise to very high levels. Following upregulation, KIM-1 is proteolytically clipped and the extracellular domain is shed from the cells into the urine in rodents and in human and it is easily detected in urine by immunoassay (74).

Rat cDNA encoding Kim-1 was initially identified by Ichimura and colleagues by a polymerase chain reaction-based cDNA subtraction analysis designed to identify genes with differential expression between normal and regenerating kidneys following ischemia/reperfusion (I/R) injury in rats (75). This same group found that Kim-1 is expressed at undetectable levels in the normal adult kidney, but is dramatically upregulated both in ischemia/reperfusion and in cisplatin-induced nephrotoxicity models in rat (75). In this study, a sandwich KIM-1 enzyme-linked immunosorbent assay (ELISA sandwich) test showed that there was a 3 to 5 fold increase in urinary Kim-1 at 1 day after cisplatin administration compared with no increases of the conventional urinary biomarkers at the same time. Comparably, at 24 h of post ischemic reperfusion, KIM-1 levels were 10 fold higher than control levels. So, urinary KIM-1 is a non invasive, rapid, sensitive and reproducible biomarker for the early detection of both cisplatin-induced AKI and ischemic AKI in rats (76). KIM-1 is also a tissue and urinary biomarker for nephrotoxicant-induced kidney injury: in recently studies with different mechanistically proximal tubule nephrotoxicant in rats, Kim-1 had an AUC of 99% for proximal tubules toxicity (using histopathological analysis as gold standard) and among 21 different urinary markers, Kim-1 was the most sensitive and specific (76).

KIM-1 is also a biomarker of AKI in humans. Han and colleagues, demonstrated an extensive expression (greater than 12-fold) of KIM-1 in proximal tubules in kidney biopsy specimens from patients with established AKI (primarily ischemic) due to ATN and found that urinary KIM-1 distinguished ischemic AKI from other forms of acute renal failure, prerenal azotemia and chronic renal disease (74). Concentrations of other urinary brush border enzymes like gamma-glutamyltransferase and alkaline phosphatase did not correlated with clinical diagnostic groupings.

Recent preliminary studies showed a role for KIM-1 as a potential predictive AKI biomarker. In a cohort of 103 adults undergoing CPB surgery, AKI (defined as an increased in serum creatinine of 0.3 mg/dL) developed in 31% of patients. In patients developing AKI, KIM-1 levels increased by 40% 2 hours after surgery and over 100% at 24 hours. In an analogue study of 40 children undergoing
cardiac surgery, in patients with AKI (AKI defined as a 50% increase in serum creatinine), urinary KIM-1 levels resulted marked enhanced 12 hours after surgery (AUC of 83%) (77).

Moreover, Han and colleagues showed that KIM-1 is also detectable at high levels in the urine of patients with renal cell carcinoma (RCC): this discover suggested that KIM-1 could have a potential role for early non invasive diagnosis of RCC (78).

Urinary KIM-1 can also be used as a non invasive biomarker of multiple kidney diseases that present tubulointerstitial fibrosis and inflammation besides AKI (e.g. focal glomerulosclerosis, immunoglobulin A nephropathy, acute rejection, chronic allograft nephropathy, systemic lupus erythematosus, hypertension, diabetic nephropathy, etc.): KIM-1, in fact, is highly increased at the luminal side of dedifferentiated proximal tubules in areas with fibrosis and in areas of inflammation in macrophages and this increase correlated positively with kidney damage and negatively with kidney function (50).

The diagnostic and prognostic role of KIM-1 in kidney transplant recipients was evaluated by Zhang in 25 protocol biopsies of patients with active tubular injury: KIM-1 staining identified proximal tubular injury and correlated with the degree of renal dysfunction (79). Focal KIM-1 expression was found in 28% of protocol biopsies despite the absence of conventional histological evidences of tubular cell injury: therefore, KIM-1 should be more sensitive than histology for detecting early tubular injury. Another study of 145 stable kidney-transplant recipients by van Timmeren and colleagues, showed that high KIM-1 urinary excretion at 24 hours from graft, was associated with significantly higher risk of loss of graft over a follow up period of 4 years. This study concluded that urinary KIM-1 was a predictor of graft loss independent of creatinine clearance, proteinuria and donor age (80).

A recent study examined the relationship between KIM-1 level and the adverse clinical outcomes (dialysis or death) in 201 hospitalized patients with AKI. Urinary KIM-1 level possessed an AUC of 61% for the prediction of the end point: patients with the highest levels in urinary KIM-1 had the higher odds for dialysis requirement or hospital death (81).

In conclusion, KIM-1 clearly represent a promising candidate for inclusion in the urinary AKI panel. An advantage of KIM-1 is that it seems very specific for differentiating among various subtypes of AKI. Its utility, however, seems limited because the increase in urinary KIM-1 is delayed by 12-24 h after the insult.

3.6.4. Cystatin C

Butler and Flynn in 1961 studied the urine proteins of 223 individuals by starch gel electrophoresis and found a new urine protein fraction in the post γ-globulin fraction: they found cystatin c (82). Cystatin C is a non-glycosylated low molecular weight (13.4 kDa) endogenous cysteine protease inhibitor which is produced by all nucleated cells at a relative constant rate and released into the blood (83). More than 99% of cystatin C is freely filtered by the glomerulus, completely reabsorbed by the proximal tubules and not secreted by the renal tubules (84): as a consequence, in physiological conditions, there is little to no detectable cystatin C present in the urine. Moreover, cystatin C appears to be relatively easily measured (with a specific immunonephelometric assay), is not affected by routine clinical storage conditions or by common interfering substances (85): thus, some of the limitations of serum creatinine (e.g. effect of muscle mass, diet, sex and tubular secretion) may not be a problem with cystatin C. These characteristics explain cystatin C superiority in the detection of acute changes of GFR compared to serum creatinine, especially with minor GFR reductions. Uzun and colleagues studied the diagnostic significance of cystatin C, serum creatinine and creatinine clearance in a group of patients with GFRs of 10 to 60 mL/min/1.73 m² and healthy controls, using serum technetium-diethylene-triamine-penta-acetic-acid (Tc-DTPA) as the reference standard clearance. This study showed that reference clearance was best correlated with creatinine clearance and cystatin C (respectively r = 0.957 and r = 0.828) compared to creatinine (r = 0.682) and indicated that serum cystatin C levels can be used as a marker of GFR in patients with kidney failure (86). Another similar study of 127 patients undergoing cardiac catheterization by Artunc and colleagues, compared serum creatinine and serum cystatin C with the clearance of the iodinated contrast dye iopromide as reference standard. The results of this study revealed that serum cystatin C showed a higher correlation (r = 0.805) with the reference standard clearance than creatinine (r = 0.652). A serum cystatin C value > 1.3 mg/L showed an 88% sensitivity and a 96% specificity for the detection of kidney failure (87).

After a multinational meeting held in Germany in 2002 (88), in which it was established that cystatin C is at least equal if not superior to serum creatinine as a marker of GFR, many studies have pointed their attention to demonstrate that changes in cystatin C occur sooner than changes in serum creatinine after impairment of kidney function. For example, Herget-Rosenthal studied patients after uninephrectomy before living kidney donation. This research showed that serum cystatin C increased 1 day after nephrectomy, while the increase in serum creatinine was observed only 2 days after nephrectomy (85). In another comparable study, in the intensive care setting, 85 patients at high risk to develop AKI manifested an increase by more than 50% in serum cystatin C, that predicts AKI 14 hours before the rise in serum creatinine, with an AUC of 0.97 (89).

Urinary excretion of cystatin C has been shown to predict the requirement for renal replacement therapy (RRT), in patients with established AKI, about 1 day earlier with an AUC of 0.75 (89): so it appears to be a marker of adverse outcome, even if the association between cystatin C and outcome in AKI and critically ill patients remains to be demonstrated.
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A recent prospective study of a cohort of children patients undergoing cardiac surgery for cardiopulmonary bypass, compared the ability of serum cystatin C and NGAL in the prediction of AKI.Of 129 patients in the study, 41 developed AKI (defined as a 50% increase in serum creatinine) 1 to 3 days after cardiopulmonary bypass. In AKI cases, serum NGAL levels were elevated at 2 hours after surgery, whereas serum cystatin C levels raised only after 12 hours. This study showed that, at 12 hours after surgery, both NGAL and cystatin C levels were strong independent predictors of AKI, but NGAL outperformed cystatin C at earlier time points (90).

Nevertheless, there are limitations in the use of cystatin C as a marker of GFR. In fact, even if it is reportedly not significantly affected by patient age, sex, muscle mass or changes in diet, in a large cross-sectional study of 8058 patients, several factors were found to be associated with an elevated cystatin C, including older age, male sex, greater height, greater weight, current smoking status and elevated C-reactive protein levels (suggesting that cystatin C is a marker of inflammation) (91). Cystatin C levels have also been found to be influenced by abnormal thyroid function, use of immunosuppressive therapy (e.g. glucocorticoid therapy) and by the presence of systemic inflammation (92), independently of kidney function.

In conclusion, cystatin C may represent a promising biomarker candidate for inclusion in the blood “AKI panel”. It is primarily a sensitive marker of reduction in GFR, superior to serum creatinine because it can detect acute renal failure earlier than creatinine. However, it is not a marker of kidney injury: thus, it is an early marker of injury when filtration is affected but it cannot differentiate between different types of AKI. Early detection may provide time to prevent the progression of AKI and may improve its negative impact on outcome.

3.6.5. Fatty acid-binding protein

Mammalian fatty acid binding proteins (FABPs) are expressed from a large multigene family that encode 14 kDa proteins, members of the superfamily of lipid-binding proteins (LBP). There are nine different FABPs with different tissue specific distribution. The FABPs are small cytoplasmic proteins abundantly expressed in tissue with an active fatty acid binding metabolism. The primary function of the FABPs is the facilitation of long-chain free fatty acid transport from the plasma membrane to sites for oxidation (mitochondria and peroxisomes) (93). Two types of FABP have been identified in the human kidney: heart-type FABP (H-FABP) in the distal tubules and liver-type FABP (L-FABP) in the proximal convoluted and straight tubules (94); (95).

H-FABP levels have been found to be a sensitive marker for aminoglycoside induced kidney injury in rats (96). In contrast, clinical studies into the utility of H-FABP as urinary biomarker in human models of AKI are lacking. Urinary L-FABP has been studied extensively in preclinical and clinical models and has been found to be a potential biomarker in a number of pathological renal conditions. Nowadays, a two-step sandwich ELISA method using monoclonal antibodies is routinely used for quantification of L-FABP in urine, and it is commercially available (CMIC Co.Ltd, Tokyo, Japan).

One pilot study examined the role of urinary L-FABP in contrast-induced AKI (defined as an increase in serum creatinine > 25%). Urinary L-FABP levels were significantly increased at 2–5 days after the procedure before the increase in serum creatinine only in those patients that developed AKI post contrast dye (97). In a model of cisplatin-induced AKI, there was a rise in urinary L-FABP within the first 24 h, whereas a rise in serum creatinine was detectable only after 72 h of cisplatin treatment (98). In addition, more recently, Ferguson and colleagues have performed preliminary studies that suggest that L-FABP is an early indicator of AKI in the setting of aminoglycoside administration, preceding changes in serum creatinine (99).

Moreover, a recent study involving 12 living-related kidney transplant recipients immediately after reperfusion of their transplanted organs, showed that urinary L-FABP levels are highly correlated with peritubular capillary blood flow and ischemic time of the transplanted kidney, as well as hospital stay (100).

However, urinary L-FABP measurements may also be influenced by several confounding variables. For example, several studies have documented that L-FABP is also abundantly expressed in the liver, and as a consequence, urinary L-FABP may be influenced by serum L-FABP levels (101). The hypothesis is that urinary L-FABP may become not specific for AKI in the setting of acute liver injury: this will be important to investigate as AKI and acute liver injury commonly co-occur in the critically ill population. More exhaustive studies seem to indicate that serum L-FABP levels do not influence urinary L-FABP levels: evidences for this notion has recently been provided in patients who developed AKI post cardiac surgery, who also developed significant early acute liver injury. In this subgroup, of 40 children, 21 developed AKI (defined as a 50% increase in serum creatinine from baseline) 2–3 days post surgery. This study revealed that there was a significant increase in serum L-FABP levels at 12 h post cardiac surgery, but not at 4 h. By contrast, urinary L-FABP levels were dramatically increased within the first 4 h post surgery, while urinary levels at 12 h had actually begun to decline. The findings that the increase in urinary L-FABP levels at 4 h post cardiac surgery was a powerful independent risk indicator for AKI (AUC = 0.8.10) confirm the dissociation between plasma and urine L-FABP levels in AKI (102).

Thus, L-FABP also appears to be a promising candidate for inclusion in the urinary “AKI Biomarker Panel”.

3.6.6. Other biomarkers

A number of other biomarkers of AKI have been proposed, but required further characterizations. The most promising for its potentially useful, is the sodium-hydrogen
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exchanger isoform 3 (NHE3) (103). In an intensive care population, NHE3 was able to distinguish among patients with AKI and patients with pre-renal failure. This biomarker may therefore have a role in differential diagnosis rather than in the prediction of AKI.

Other novel biomarkers discovered through genome wide arrays of renal tissue or proteomic analysis of plasma or urine in experimental ischemia-reperfusion induced AKI have not yet been validated in humans. Examples include the human analogue of the mouse keratinocyte chemokine (Gro-alpha) (104), Exosomal Fetuin-A (105) and metalloproteinase Meprin A (106), and as yet undefined proteins (e.g. protein biomarkers with 6.4, 28.5, 43 and 66 kDa (107)).

3.6.7 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 functions of the proximal tubules. Urinary enzymes have been extensively studied in a number of pathological conditions predisposing to acute and chronic renal injury (e.g. hypertension, renal ischemia, renal transplantation, etc.). In presence of kidney injury, enzymes that normally are present in tubular epithelial cells may be released into the urine as a consequence of damage or secondary to intensified enzyme induction during the repair and regeneration process (43). The detection of enzymes released from proximal and/or distal tubular cells can also be used as a biomarker of AKI and there is often a correlation between the dose-dependent increase in urinary enzymatic activity and the degree of tissue damage present (108).

Several different classes of enzymes can be found in the urine: lysosomal proteins (N-acetyl-β-D-glucosaminidase (NAG)), brush border enzymes (γ-GT and alkaline phosphatase (AP)), or cytosolic proteins (α-glutathione S-transferase (α-GST)). Furthermore, when proximal tubular epithelial cells are injured, they not completely reabsorbed low-molecular weight proteins that are freely filtered into the urinary space (α1- and β2-microglobulins).

A pilot study prospectively evaluated the potential for urinary γ-GT, AP, α-GST and NAG to predict the subsequent development of AKI (defined as an increase of 50% in serum creatinine) in a small cohort of 26 patients admitted to an ICU. 4 of the 26 subjects developed AKI; baseline levels of all the enzymes analyzed were higher in the patients who developed AKI, compared with those who did not. In particular, γ-GT and α-GST had high sensitivity (100%) and high specificity (90%) in predicting subsequent AKI, with an AUC of respectively 0.9.5 and 0.9.3. Anyway, the ROC curves for the other enzymes were all >0.8. Changes in enzyme levels preceded the rise of serum creatinine (from 12 hour to 4 days before) (109). However, when the authors test the generalizability of their results in a test population of 19 patients (four developed AKI), the sensitivity and the specificity of these enzymatic tubular biomarkers were significantly reduced.

Several investigators have also examined the ability of tubular enzymes to predict adverse clinical outcomes. Herget-Rosenthal and colleagues, for example, proposed with their study to prospectively investigated the diagnostic accuracy of the urinary excretion of tubular enzymes as predictor of a need for renal replacement therapy in non-oliguric AKI (110). In 73 consecutive patients with initially non-oliguric AKI, they measured urinary excretion of γ-GT, α-GST and NAG early in course of AKI. 26 patients (36%) required RRT a median of 4 days after detection of proteinuria and enzymuria. Of the tubular enzymes studied, NAG had the best predictive value, with an AUC-ROC of 0.8.1.

A recent study of urinary NAG and KIM-1 in a cohort of 201 hospitalized patients with established AKI also demonstrated that these biomarkers were associated with poor clinical outcomes, with the odds of dialysis requirement or hospital death increased five fold in patients with the high urinary levels of these biomarkers, highlighting that these urinary markers could be of prognostic value (81).

However, a number of potential pitfalls have been noted with respect to the use of enzymuria in the setting of AKI. For example, very important in terms of practicability is the instability of many urinary enzymes. For example the brush border enzymes AP and γ-GT are stable for only 4 hours after urine collection and samples require gel filtration to eliminate interfering substances (111). As a consequence, the utility of urinary enzyme excretion as diagnostic or predictive biomarkers for AKI remains an area that needs further investigations.

Biomarkers for the identification of acute kidney disease are summarized in the following table (Table 1).

4. BIOMARKERS FOR MONITORING PROGRESSION FROM ACUTE TO CHRONIC KIDNEY DISEASES

Relatively little is currently known regarding the potential for transition from AKI to CKD. There is emerging evidence that an AKI episode can lead to chronic kidney disease and can accelerate the progression to end stage renal disease. Patients that survive after AKI present a higher long-term mortality risk, especially those with partial renal recovery (112). In both acute and chronic renal diseases, early intervention can significantly improve the dismal prognosis. Recently, the application of innovative technologies has identified some candidate biomarkers that are emerging as diagnostic tools in both AKI and CKD, since they hold tremendous promise as methods for monitoring the progression from acute to chronic forms of renal disease (113). The most promising of these biomarkers include neutrophil gelatinase-associated lipocalin (NGAL), liver-type fatty acid binding protein (L-FABP), and kidney injury molecule-1 (KIM-1). It is likely that they will be useful for timing the initial insult and assessing the duration and the severity of the disease (114). Actually, Studies to
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Table 1. Biomarkers for the identification of acute kidney injury

<table>
<thead>
<tr>
<th>Biomarker name</th>
<th>Sample</th>
<th>Detection method</th>
<th>REFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL1B</td>
<td>Urine</td>
<td>ELISA</td>
<td>(51); (52); (53)</td>
</tr>
<tr>
<td>KIM-1</td>
<td>Urine</td>
<td>ELISA</td>
<td>(77)</td>
</tr>
<tr>
<td>KIM-1</td>
<td>Tissue</td>
<td>Immunohistochemistry, Western Blot analysis</td>
<td>(74)</td>
</tr>
<tr>
<td>NGAL</td>
<td>Tissue</td>
<td>Immunohistochemistry, Western Blot analysis</td>
<td>(59) *in animal models</td>
</tr>
<tr>
<td>NGAL</td>
<td>Plasma</td>
<td>ELISA, Biosite</td>
<td>(61)</td>
</tr>
<tr>
<td>NGAL</td>
<td>Urine</td>
<td>ELISA, Abbott</td>
<td>(63); (64)</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>Plasma</td>
<td>Nephelometry, Dade-Behring</td>
<td>(85); (89)</td>
</tr>
<tr>
<td>L-FABP</td>
<td>Urine</td>
<td>2-step sandwich ELISA</td>
<td>(97); (102)</td>
</tr>
<tr>
<td>Tubular enzymes</td>
<td>Urine</td>
<td>Enzymatic dosage assay</td>
<td>(81); (109)</td>
</tr>
</tbody>
</table>

validate the sensitivity and specificity of these biomarkers in clinical samples from large cohorts and from multiple clinical situations are in progress.

5. CHRONIC KIDNEY DISEASE

CKD is characterized by a progressive decline in kidney function that is associated with excess of morbidity and mortality (115). During the past decade, the patient population with end-stage renal disease (ESRD) more than doubled. Arresting its progression mandates recognition of risk factors and the earlier stages of chronic kidney disease (CKD), a worldwide public health problem that affects 11% of individuals >65 years of age (116). CKD is a devastating illness that has reached epidemic proportions worldwide.

The major outcomes of chronic kidney disease, regardless of cause, include progression to kidney failure, complications of decreased kidney function, and cardiovascular disease (CVD). Increasing evidence indicates that some of these adverse outcomes can be prevented or delayed by early detection and treatment (117). Unfortunately, chronic kidney disease is underdiagnosed and undertreated, resulting in lost opportunities for prevention (118); (119); (120), in part because of a lack of agreement on a definition and classification of stages in the progression of chronic kidney disease (121) and a lack of uniform application of simple tests for detection and evaluation.

In February 2002, the Kidney Disease Outcomes Quality Initiative (K/DOQI) of the National Kidney Foundation (NKF) published 15 clinical practice guidelines on chronic kidney disease. The goals of the guidelines are to 1) define chronic kidney disease and classify its stages, regardless of underlying cause; 2) evaluate laboratory measurements for the clinical assessment of kidney disease; 3) associate the level of kidney function with complications of chronic kidney disease; and 4) stratify the risk for loss of kidney function and development of CVD (122).

Kidney disease can be diagnosed without knowledge of its cause. Kidney damage is usually ascertained by markers rather than by kidney biopsy. According to the Work Group, persistent proteinuria is the principal marker of kidney damage (122). An albumin–creatinine ratio greater than 30 mg/g in untimed (spot) urine samples is usually considered abnormal; proposed sex-specific cut points are greater than 17 mg/g in men and greater than 25 mg/g in women (123); (124). Other markers of damage include abnormalities in urine sediment, abnormalities in blood and urine chemistry measurements, and abnormal findings on imaging studies. Persons with normal GFR but with markers of kidney damage are at increased risk for adverse outcomes of chronic kidney disease.

Glomerular filtration rate is the best measure of overall kidney function in health and disease. The normal level of GFR varies according to age, sex, and body size. Normal GFR in young adults is approximately 120 to 130 mL/min per 1.73 m² and declines with age (125). A GFR level less than 60 mL/min per 1.73 m² represents loss of half or more of the adult level of normal kidney function. Below this level, the prevalence of complications of chronic kidney disease increases.

Although the age-related decline in GFR has been considered part of normal aging, decreased GFR in the elderly is an independent predictor of adverse outcomes, such as death and CV. In addition, decreased GFR in the elderly requires adjustment in drug dosages, as in other patients with chronic kidney disease. Therefore, the definition of chronic kidney disease is the same, regardless of age. Because GFR declines with age, the prevalence of chronic kidney disease increases with age; approximately 17% of persons older than 60 years of age have an estimated GFR less than 60 mL/min per 1.73 m² (126).

The guidelines define kidney failure as either 1) GFR less than 15 mL/min per 1.73 m², which is accompanied in most cases by signs and symptoms of uremia, or 2) a need to start kidney replacement therapy (dialysis or transplantation). Approximately 98% of patients with kidney failure in the United States begin dialysis when their GFR is less than 15 mL/min per 1.73 m² (121). Kidney failure is not synonymous with end-stage renal disease (ESRD). "End-stage renal disease" is an administrative term in the United States. It indicates that a patient is treated with dialysis or transplantation, which is the condition for payment for health care by the Medicare ESRD Program. The classification of ESRD does not include patients with kidney failure who are not treated with dialysis and transplantation. Thus, although the term ESRD provides a simple operational classification of patients according to treatment, it does not precisely define a specific level of kidney function (120).

Data from the Third National Health and Nutrition Examination Survey (NHANES III) show the increasing prevalence of complications of chronic kidney disease at lower levels of GFR. These data and other studies...
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Table 2. NKF Classification of Chronic Kidney Disease and Action Plan

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>GFR (ml/min/1.73 m²)</th>
<th>Action plan</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kidney damage with normal or elevated GFR</td>
<td>≥ 90</td>
<td>Diagnosis and treatment; treat co-morbid conditions; slow progression of CKD; reduction of modifiable cardiovascular disease risk factors</td>
</tr>
<tr>
<td>2</td>
<td>Kidney damage with mildly decreased GFR</td>
<td>60-89</td>
<td>Estimation of disease progression</td>
</tr>
<tr>
<td>3</td>
<td>Moderately decreased GFR</td>
<td>30-59</td>
<td>Evaluation and treatment of complications of CKD</td>
</tr>
<tr>
<td>4</td>
<td>Severely decreased GFR</td>
<td>15-29</td>
<td>Preparation for kidney replacement therapy</td>
</tr>
<tr>
<td>5</td>
<td>Kidney failure</td>
<td>&lt;15 or dialysis</td>
<td>Kidney replacement therapy</td>
</tr>
</tbody>
</table>

provide a strong basis for using GFR to classify the stage of severity of chronic kidney disease (Table 2). shows the classification of stages of chronic kidney disease and the prevalence of each stage, estimated by using data from NHANES III (126). Approximately 11% of the U.S. adult population (20 million persons from 1988 to 1994) have chronic kidney disease. The prevalence of early stages of disease (stages 1 to 4; 10.8%) is more than 100 times greater than the prevalence of kidney failure (stage 5; 0.1%). The burden of illness associated with earlier stages of chronic kidney disease has not been systematically studied (127); (128). The National Institute of Diabetes and Digestive and Kidney Disease has initiated a prospective cohort study, the Chronic Renal Insufficiency Cohort (CRIC) study, for this purpose (120).

5.1. Histopathology of CKD

CKD presents a common pathology of glomerulosclerosis and tubulointerstitial fibrosis (TIF); however, the degree of tubulointerstitial involvement provides the best correlation with renal impairment. Tubulointerstitial fibrosis is characterized by an inflammatory cell infiltrate, an increase in interstitial cell number with the appearance of myofibroblasts expressing the cytoskeletal protein a-smooth muscle actin, tubular atrophy, obliteration of the peritubular capillaries and accumulation of extracellular matrix (ECM) (129). The inflammatory infiltrate results from both activation of resident inflammatory cells and recruitment of circulating inflammatory cells. The increase in interstitial cell number is due to increased proliferation and decreased apoptosis of resident interstitial cells, as well as migration of cells into the tubulointerstitium. Myofibroblasts arise by differentiation of resident interstitial fibroblasts and infiltrating cells, including bone marrow-derived progenitor cells (130) and inflammatory cells. An increasing literature now also points to the transdifferentiation of tubular epithelial cells as another source of myofibroblasts (130); (131). Tubular cell apoptosis is a prominent feature of TIF, although epithelial–mesenchymal transdifferentiation (EMT) also likely contributes to tubular atrophy and loss. The hallmark of TIF is the expansion of the ECM. Matrix accumulates as a consequence of both increased production and decreased turnover of ECM proteins. Matrix turnover is regulated primarily by the matrix metalloproteinases (MMPs) and their endogenous inhibitors, the tissue inhibitors of metalloprotease (TIMPs) and the plasmin–plasminogen activator–plasminogen activator inhibitor (PAI) cascade. TIF is characterized by increased levels of TIMP-1, and PAI-I, likely acting to suppress ECM turnover and promote accumulation although MMP-independent effects of TIMPs on cell proliferation, survival and differentiation, may also be relevant (129); (132).

5.2. Emerging biomarkers for CKD

Fortunately, deterioration of kidney function can be delayed and patient outcomes favorably affected if kidney disease is recognized and treated in a timely manner. Monitoring CKD activity requires biomarkers that provides clinicians with quick, non invasive, and specific measurements that correlate with pathophysiologic processes occurring within the kidney (113).

Current biomarkers of CKD and its progression that are in widespread clinical use, namely serum creatinine and urine protein (proteinuria), have limitations in serving these goals. The measured serum creatinine level is used to calculate an estimated glomerular filtration rate (GFR). Screening of proteinuria often alerts physician to the presence of chronic kidney disease before changes in the GFR become apparent (113). Marked day-to-day variations in serum creatinine levels complicates identifications of trends. It is important to note that serum creatinine can remain within the normal range even when renal function is seriously impaired (133); (134). For this reason calculated or measured creatinine clearance should always be used to assess renal function. Accurate 24-hour urine collection can be difficult but it is possible to predict creatinine clearance or GFR from serum creatinine and demographic, anthropometric and other data (135). Proteinuria is an important marker of kidney function decline. Multiple studies have demonstrated that proteinuria is a marker of kidney damage and that higher degrees of proteinuria result in more rapid progression of renal disease (136). Unfortunately proteinuria may occur long after renal insult has occurred and it may not be present in many types of renal injury such as hypertensive renal disease and tubulointerstitial disease.

Novel biomarkers that reflect tissue pathology are currently being evaluated in order to identify those that will predict disease progression before the development of changes in traditional markers for monitoring kidney function.

The most promising biomarkers of CKD are NGAL, cystatin C, ADMA, and L-FABP.

5.2.1. Neutrophil gelatinase-associated lipocalin

The search for early, specific substances able to reveal the onset of AKI has uncovered NGAL as one of the most promising biomarkers in the future of clinical nephrology (137). As discussed above, NGAL is a superb marker of AKI. In CKD there is growing body of literature suggesting that NGAL is also a marker of kidney disease and severity.
NGAL is massively released in blood and urine from injured tubular cells after various conditions potentially detrimental to the kidney in experimental and human clinical models. No less important NGAL release from renal tubule occurs soon after damage, notably preceding the rise in serum creatinine and thus allowing the initiation of preventive therapeutic measure in a timely manner.

Recently Bolignano and colleagues showed that, in a cohort of patients affected by nonadvanced CKD with stable renal function, there was a strict, independent and inverse correlation between NGAL and estimated GFR, suggesting that under these particular conditions this protein may also represent a surrogate index of residual renal function, similar to what has previously described elsewhere (138).

In the 2007 Mori and Nakao proposed an interesting theory which might explain the relationship between NGAL and GFR, suggesting that the increase in NGAL is not just the passive consequence of a reduced renal clearance (139). This hypothesis, called the “forest fire theory”, assumes that the increase in NGAL in chronic kidney disease is the consequence of a sustained production by inflamed but vital tubular cells, whereas the rise in serum creatinine and the contraction of GFR are the mere passive result of a general loss of functional cells or nephrons. From this point of view, NGAL would represent a real-time indicator of how much active kidney damage exists within the overall condition of chronic renal impairment.

It was demonstrated that in subject affected by nonterminal CKD, NGAL represent a novel, independent renal predictor of CKD progression which also provides a good reflection of the severity of renal disease.

However further studies are required to better determine the pathophysiologic role that NGAL plays in models of CKD and to define its association with CKD progression.

5.2.2. Cystatin C

Data from several studies suggests that cystatin C is more sensitive to changes in GFR and is less subject to extrarenal factors than creatinine concentration (140); (141). These data led to hypothesis that cystatin C may be a better marker of kidney function in elderly persons and in persons with mild reduction in kidney function.

If cystatin C is in fact a stronger risk factor among patients with advanced CKD, 2 reasons may explain these findings. Cystatin C may be a better measure of kidney function than is actual GFR because of considerable variability in iohalamate clearance due to short-term changes in true GFR and to measurement error (142). As reported by Levey and colleagues, the median intratest variability for iohalamate GFR was 9,4% in the modification of diet in renal disease (MDRD) study and the intertest coefficient of variation for 2 measurements of GFR performed 3 months apart was 6,3% (143). The reported intratest coefficient of variation for the cystatin C assay ranged from 2,1 to 4,8 and reported intertest coefficient for the cystatin C assay were 2,3% to 3,1% (144) and 3,8% (145). Thus cystatin C may be measured more precisely than GFR. On the other hand, the average of 2 measured GFRs provided risk estimates similar to models that used 1 measure of GFR. This suggests that the strength of the relationship between measured GFR and outcomes was not weakened by the imprecision of GFR measurements, although measurements errors cannot be excluded.

An alternate explanation is that cystatin C provides prognostic information beyond its role as an index of kidney function and is a better overall measure of the spectrum of pathophysiologic abnormalities that accompany kidney disease (146).

Unfortunately cystatin C levels are known to be affected by HIV and glucocorticoid use (113). Therefore, further studies are needed in order to address whether cystatin C is truly a better biomarker than serum creatinine and in which populations its use is most appropriate.

5.2.3. Asymmetric dimethylarginine

Asymmetric dimethylarginine (ADMA) is an endogenous methylated amino acid that inhibits the endothelial and neuronal isoforms of nitric oxide synthase (NOS). It is a less potent inhibitor of the inducible isoform (147). There are three types of methylated arginines ADMA, N²-monomethyl-L-arginine (L-NMMA) and symmetric dimethylarginine (SDMA). Because only minor amounts of L-NMMA are found in human plasma and SDMA has no effects on NOS activity, ADMA is now thought to be a major type of endogenously generated methylated arginines that possess the inhibitory activity of NOS (148). ADMA is synthesized in endothelial cells and it is known that more than 90% of the circulating ADMA is metabolized by the action of dimethylarginine dimethylaminohydrolase (DDAH) in rats. This substance raises arterial pressure and it is strongly associated with insulin resistance (149), the basic abnormality of metabolic syndrome. ADMA has profound renal hemodynamic effects (150). Furthermore it is associated with intima hyperplasia (151); (152), a widespread alteration encompassing medium and small renal vessels and large elastic arteries as well (153). ADMA increases as renal function deteriorates and two recent prospective cohort studies showed that ADMA is a strong predictor of renal disease progression in patients with CKD (154); (155).

In vitro studies has shown that physiologically relevant levels of ADMA significantly inhibit NOS and subsequently reduce NO generation in cultured endothelial cells (EC) and in isolated human blood vessels (156). Administration of ADMA to normal rats causes an increase in renal vascular resistance and blood pressure (BP) (157); (158).

Recently Zoccali and colleagues studied the potential role of ADMA in CKD. According to their theory, there are at least four possible mechanisms that may...
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Table 3. Biomarkers for the identification of chronic kidney disease

<table>
<thead>
<tr>
<th>Biomarker name</th>
<th>Sample</th>
<th>Detection method</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGAL</td>
<td>Plasma</td>
<td>ELISA</td>
<td>(137); (138)</td>
</tr>
<tr>
<td>NGAL</td>
<td>Urine</td>
<td>ELISA</td>
<td>(137); (138)</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>Plasma</td>
<td>Dade-Behring</td>
<td>(138); (139); (146)</td>
</tr>
<tr>
<td>ADMA</td>
<td>Plasma</td>
<td>ELISA</td>
<td>(154); (155)</td>
</tr>
<tr>
<td>L-FABP</td>
<td>Urine</td>
<td>ELISA</td>
<td>(161); (162)</td>
</tr>
</tbody>
</table>

explain the accumulation of ADMA in CKD: 1) increased methylation of proteins; 2) increased protein turnover; 3) decreased metabolism by DDAH; and 4) impaired renal excretion. As dimethylarginines are excreted in urine, impaired renal clearance may, at least in part, account for the elevation of ADMA levels in patients with CKD (159).

In fact, the plasma level of ADMA is a strong predictor for the progression of renal dysfunction in patients with CKD. NOS inhibition may accelerate the progression of renal injury by impairing the angiogenic response and subsequent loss of peritubular capillaries via suppression of NO.

In addition, Matsumoto and colleagues (160) have recently found that plasma levels of ADMA are associated with decreased number of peritubular capillaries, enhanced tubulointerstitial fibrosis and progressive loss of renal function in the remnant kidney model, all of which were prevented by DDAH overexpression-elicted ADMA reduction.

Large longitudinal studies are needed to demonstrate the ability of ADMA to identify and predict CKD and its progression in cohorts with CKD of multiple aetiologies.

5.2.4. Liver type-fatty acid binding protein

Liver-type fatty acid-binding protein (L-FABP) is a protein expressed in the proximal tubule of the kidney. Its expression and urinary excretion are increased in the setting of CKD.

The sensitivity and specificity of predicting the progression of chronic renal disease, were established by using the cutoff values determined on the basis of a receiver operating characteristic (ROC) curve (161). By setting the cut off values for urinary L-FABP and urinary protein at 17.4. g/g Cr. and 1.0. g/g Cr., respectively, urinary L-FABP was found to be more sensitive than urinary protein which was generally known to be a predictor for the progression of chronic renal disease (93.8% and 68.8%, respectively). However, urinary protein was more specific than urinary L-FABP (93.8% and 62.5%, respectively).

In an experimental study of protein overload–induced nephropathy, L-FABP gene expression in the kidney was up-regulated and urinary excretion of L-FABP was increased by the stress which causes the tubulointerstitial damage. In the clinical study, urinary excretion of L-FABP was correlated with the severity of the tubulointerstitial damage. Furthermore, the level of urinary L-FABP was significantly higher in patients whose kidney function deteriorated than in those whose kidney function was stable, and therefore urinary L-FABP may be a new and unique clinical marker for predicting the progression of CKD (162). Urinary L-FABP may be a useful marker for the screening of kidney function to identify patients who are likely to experience deterioration of renal function in the future. The combined use of urinary L-FABP and urinary protein may allow to precisely determine the state of chronic renal disease and therefore administer more appropriate treatments to patients affected with chronic renal disease.

In conclusion, L-FABP may be a useful clinical biomarker for monitoring CKD. We thus assume that urinary L-FABP may be utilized in the management of CKD.

Biomarkers for the identification of chronic kidney disease are summarized in the following table (Table 3).

6. SUMMARY AND PERSPECTIVE

The incidence of both AKI and CKD is rising and reaching epidemic proportions. In both situations, early intervention can significantly improve the prognosis.

Recently, studies on critically ill patients, in the last years provided us with new biomarkers for the clinical investigation of AKI in humans, with potentially high sensitivity and specificity. Potential biomarkers of AKI have been detected in preclinical studies in animal models. In clinical studies only a few of these biomarkers have been validated in established AKI, and because of their ability in detect AKI before the increase in serum creatinine they are potentially able to give us an early and accurate diagnosis of AKI.

The most promising of these biomarkers are NGAL, IL-18, KIM-1, CYSTATIN C and L-FABP, as well as the urinary presence of some tubular enzymes or proteins that are not usually found in the urine: all together they form the “AKI biomarkers panel”.

As they represent sequential biomarkers, it is likely that the AKI panels will be useful for timing the initial insult and assessing the duration and severity of AKI. Based on the differential expression of the biomarkers, it is also likely that the AKI panels will help distinguish between the various types and aetiologies of AKI, and predict clinical outcomes.

Fortunately, the application of innovative technologies has identified candidates that are emerging as early biomarkers of both AKI and CKD. The most promising of this include N-GAL, L-FABP and KIM-1: it is likely that they will be useful for timing the initial insult and assessing the duration and the severity of disease.

Moreover, the list of prognostic biomarkers is in continuous growth also in patients with CKD. However,
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Inconsistencies across different studies are frequent. These may depend on differences in study design, study population, study power, analytical reliability of the biomarker being assessed and other factors. No biomarker per se may offer an easy and all purpose solution to the diverse problems posed by clinical practice. When used for prognostic purposes, biomarkers should be properly validated in the specific setting where their use is recommended, such as in CKD patients without pre-existing cardiovascular complications or in high risk dialysis patients.

However, until now these biomarkers have been tested only in small studies and in a limited number of clinical situations. Prospective screening studies to validate the sensitivity and specificity of these biomarkers in larger populations are underway, facilitated by the development of commercial tools for the reproducible measurements across different laboratories that also promised to practice multicentre studies with the participation of various specialties (intensivists, cardiologist, surgeons).

In conclusion, combined together, panel(s) of biomarkers will be developed to facilitate early detection, identification of disease subtypes and aetiologies (differential diagnosis), prediction of clinical outcomes, monitoring the response to interventions. The widespread availability of such information promises to revolutionize renal care in both children and adults, and allow for the practice of personalized and predictive medicine at an unprecedented level.

7. ACKNOWLEDGEMENTS

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impact on renal function measurement.


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**Key Words:** Clinical Biomarkers, Kidney Diseases, Review
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