Biomarkers of Acute Kidney Injury in Different Clinical Settings: A Time to Change the Paradigm?

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Key Words

Acute kidney injury • End-stage renal disease • Biomarker

Abstract

Acute kidney injury (AKI) is diagnosed in 5% of all hospitalized patients and in up to 50% of all ICU patients. In the last years a dramatic rise in the prevalence of AKI has been observed with virtually no change in mortality, reaching up to 50–80% in all dialyzed ICU patients. AKI may progress to end-stage renal disease, and even subclinical episodes of AKI, which are common, may also progress to end-stage renal disease. The early detection of AKI may enable timely intervention and prevention of progression; however, in animal models and in human studies the ‘window of therapeutic intervention’ is narrow. Different urinary and serum proteins have been intensively investigated as possible biomarkers for the early diagnosis of AKI. There are promising candidate biomarkers with the ability to detect an early and graded increase in tubular epithelial cell injury and distinguish pre-renal disease from acute tubular necrosis. In this review, new emerging biomarkers of AKI are presented and described in some clinical settings, such as cardiac surgery, contrast-induced nephropathy, delayed graft function and ICU/emergency departments, where biomarkers are urgently needed to diagnose, make prognoses and differentiate.

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Acute renal failure is a complex syndrome occurring in a variety of settings with clinical manifestations ranging from a minimal rise in serum creatinine to anuria and need for renal replacement therapy. The term acute kidney injury (AKI) is barely a few years old. The concept of AKI was based on the model that had been developed for chronic kidney disease (CKD), which incorporated the accrued epidemiologic data and presented it as a public health model of disease that is potentially preventable and treatable at early stages. AKI is diagnosed in 5% of all hospitalized patients and in up to 50% of all ICU patients. In the last years a dramatic rise in the prevalence of AKI has been observed with virtually no change in mortality, which reaches up to 50–80% of all dialyzed ICU patients (fig. 1, 2). Outcomes of AKI range from recovery to death and include the development of CKD, progression to end-stage renal disease and requirement of renal replacement therapy.

AKI: An Urgent Need for Early Detection

There is a direct correlation between the time of detection of kidney failure and mortality. In current clinical practice, AKI is typically diagnosed by measuring serum creatinine in timely intervals. However, as it was shown in human and animal studies that a steady state is reached

within days after the insult, the loss of kidney function in acute renal failure is most easily detected by measurement of the serum creatinine. Serum creatinine is also used to estimate the glomerular filtration rate (GFR), making estimated GFR (eGFR) useless in diagnosis and prognosis of AKI. Unfortunately, creatinine is an unreliable indicator during acute changes in kidney function [1]. First of all, using serum creatinine to estimate true renal function has well-recognized inaccuracies and limitations [2]. A marked reduction in GFR can be present before it is reflected in a rise in creatinine (up to 50% of kidney function has already been lost before creatinine might change). Second, creatinine does not reflect kidney function during acute changes until a steady state has been reached, which can take several days. Moreover, creatinine is a poor biomarker for AKI due principally to its inability to help diagnose early acute renal failure and complete inability to help differentiate among its various causes.

Since the early stages of AKI are often reversible, AKI should be prevented and/or treated by various approaches instituted as early as possible after the initiating insult, well before serum creatinine even begins to rise. The rise in serum creatinine is slow following the onset of AKI. By the time a change is observed in serum creatinine, a critical 'window of therapeutic opportunity' may have already been missed, particularly among those with acute tubular necrosis. Different urinary and serum proteins have been intensively investigated as possible biomarkers for the early diagnosis of AKI [3] (fig. 3, 4). There are promising candidate biomarkers with the ability to detect an early and graded increase in tubular epithelial cell injury and to distinguish prerenal disease from acute tubular necrosis. Biomarkers in AKI are needed for early diagnosis, to help monitor treatment (avoidance of nephrotoxic drugs, adequate volume monitoring), to help develop new forms of treatment, as secondary endpoints, as enrollment criteria, and for the assessment of the safety of drugs and/or potential nephrotoxicity, prognosis, and need for renal replacement therapy (fig. 5).

Neutrophil Gelatinase-Associated Lipocalin

Neutrophil gelatinase-associated lipocalin (NGAL) is a member of the lipocalin family [4], but it is also expressed at a low level in other human tissues including the kidney, prostate and epithelia of the respiratory and alimentary tracts [5]. NGAL is a 25-kDa monomer (polypeptide chain of 178 amino acids) associated with

neutrophil gelatinase [6]. NGAL is thought to be an acute-phase protein with upregulated expression in different inflammatory conditions as well as in different cancers [7]. Recently, it has been found that NGAL binds small iron-carrying molecules, so-called siderophores, and is critical in various states including bacterial infection and kidney injury [6, 7]. Because of its small molecular size (25 kDa) and its resistance to degradation, NGAL is readily excreted and detected in urine. During a 'fishing expedition', NGAL was found to be 1 of the 7 genes which were highly upregulated in the mouse model of ischemia-reperfusion injury [8]. NGAL is highly accumulated in human kidney cortical tubules, blood and urine after nephrotoxic and ischemic injury [9]. NGAL is synthesized systemically in response to kidney damage followed by glomerular filtration and tubular uptake; it could be produced locally by injured tubules. As a small molecule, NGAL is freely filtered by the glomerulus and then largely absorbed in the proximal tubules by efficient megalin-dependent endocytosis [10]. NGAL might be expressed by the damaged tubules to induce re-epithelialization and to reduce apoptosis, thereby protecting against AKI. This protective effect, as shown by Mori et al. [11], may be mediated by NGAL delivering iron to proximal tubules, and then in turn by iron upregulating heme oxygenase-1, an enzyme protecting tubular cells. A source of NGAL could be also activated neutrophils/macrophages or inflamed vasculature, frequently found in coronary artery disease, hypertension and CKD [12].

Initially, NGAL was detected by the Western blot technique. Today commercially available ELISA assay from Bioporto (Gentofte, Denmark) is an option. Moreover, a standardized point-of-care Triage[®] device (Inverness Inc., San Diego, Calif., USA) was designed to measure plasma NGAL. This assay is easy, with quantitative results available in 15 min, requiring only a microliter sample [13], which makes bedside testing feasible. Similarly, a urinary NGAL assay Architect[®] (Abbott Diagnostics, Abbott Park, Ill., USA) is also available for clinical application. This urinary assay, on the other hand, requires 150 μ l of urine and results are available within 35 min [14], making bedside diagnosis easy and noninvasive within a reasonable period of time. Both plasma and urinary NGAL assessed by Western blotting correlated well with values obtained using research ELISA [15].

The cutoff value for 2 h after cardiopulmonary bypass (CPB) plasma NGAL is 150 ng/ml with an AUC of 0.96, sensitivity of 84% and specificity of 94% in predicting AKI. The cutoff value for 2 h after CPB urinary NGAL is

100 mg/ml with an AUC of 0.95, sensitivity of 82% and specificity of 90% in predicting AKI. NGAL is a highly sensitive, but rather nonspecific, marker of renal injury as it is also elevated in CKD [16] and sepsis [17]. NGAL performs better in children than in adults, probably because they have less or even no comorbidities. As shown previously, NGAL was significantly elevated in hypertensive patients when compared to their normotensive counterparts [18]; similarly it was higher in diabetic patients compared to nondiabetic patients [19]. The effect of age should also be taken into account as NGAL is related to age and was proved to be higher in the elderly when compared to younger patients [20].

Interleukin-18

Interleukin-18 (IL-18) is a proinflammatory cytokine, induced in the proximal tubule after AKI, and after cleavage by caspase-1 appears in the urine. It may also act as a neutrophilic attractant [21]. IL-18 is both a mediator and biomarker of ischemic AKI as evidenced by how IL-18 expression rises in the kidney in AKI, how inhibition of IL-18 is protective against AKI in animal models and how IL-18 rises in the urine in both humans and animals after AKI. In a cross-sectional study, Parikh et al. [22] reported that IL-18 was significantly increased in patients with established AKI, but not in patients with urinary tract infections, CKD, nephritic syndrome or prerenal azotemia. On the other hand, Matsumoto and Kanmatsuse [23] found that urine IL-18 was significantly higher in patients with minimal-change nephrotic syndrome when compared to patients with IgA nephropathy. In addition, urinary IL-18 correlated with proteinuria and disease activity. There are only experimental assays available (no standardized), with no information about interferences, such as sepsis or SIRS. Data is only available on pilot studies on small patient cohorts. IL-18 did not differ between SIRS and non-SIRS patients, but increased 2–3 days before the diagnosis of post-traumatic multiple-organ dysfunction syndrome was made [24]. On the other hand, IL-18 did not predict sepsis in neonates [25].

Kidney Injury Molecule-1

Kidney injury molecule-1 (KIM-1) is a type-1 membrane protein with extracellular immunoglobulin and a highly O-glycosylated mucin subdomain as well as mul-

tiply N-glycosylation sites and a relatively short cytoplasmatic tail [26, 27]. This putative epithelial cell adhesion molecule [also known as Tim-1 (T cell immunoglobulin and mucin) containing molecule] is not detectable in healthy kidney tissue. Transcript levels for the genes that encode KIM-1 are strongly upregulated in dedifferentiated proximal tubule epithelial cells in kidney after ischemic or toxic injury. It may also play a role in epithelial adhesion, growth and differentiation. KIM-1 is cleaved from the surface of activated tubular cells and released into the urine by metalloproteinase. This process is closely related to the KIM-1 expression in the tissue and urinary excretion. KIM-1 (Tim-1) is one of the members of a family of related molecules with three members in humans, encoded by genes adjacent to the IL-4, IL-5 and IL-13 cluster on human chromosome 5q33.2 [28]. Tim-1 is expressed predominantly on Th2 cells and Tim-4, a natural ligand for Tim-1, is expressed on macrophages and dendritic cells [29]. In kidney, KIM-1/Tim-1 is a phosphatidylserine receptor that confers a phagocytic phenotype on epithelial cells [30]. This receptor is responsible for the uptake of apoptotic cells and exosomes [31]. Ichimura et al. [29] demonstrated that KIM-1 is responsible for the clearance of debris from damaged renal tubules. KIM-1 can be expressed and excreted in urine within 12 h after the initial ischemic insult, before regeneration of the epithelium, and persists over time. Urinary KIM-1 was reported to be a noninvasive, rapid, sensitive and reproducible biomarker of nephrotoxic and ischemic AKI in animal models. However, on the other hand, van Timmeren et al. [32] reported that renal KIM-1 expression was significantly increased in human kidney tissue in patients with a wide range of kidney diseases including FSGS, IgA nephropathy, MPGN, MGN, SLE, chronic allograft nephropathy, acute rejection hypertension and Wegener's granulomatosis. Both renal and urinary KIM-1 correlated with kidney damage and negatively with kidney function, but not with proteinuria. This study showed that KIM-1 is associated with renal fibrosis and inflammation since KIM-1 was primarily expressed at the luminal side of dedifferentiated proximal tubules in areas with fibrosis and in macrophages in areas of inflammation. In a recent review, Huo et al. [33] stressed that KIM-1 could be both a marker of AKI as well as of CKD. Moreover, there are no standardized assays for KIM-1 assessment: there is one dipstick test developed in the USA by BioAssay (Renastick) [34] and some ELISA-based assays for urinary KIM-1 [26, 35].

Fig. 1. Treatment of acute myocardial infarction and AKI in the 1960s.

Fig. 2. Treatment of acute myocardial infarction and AKI in 2010. ASA = Acetylsalicylic acid.

Fig. 3. Biomarker localization in the nephron, 1960–1970. AST = Aspartate aminotransferase.

Fig. 4. Biomarker localization in the nephron, 2010. AST = Aspartate aminotransferase; CK = creatine kinase; CKMB = creatine kinase myocardial fraction; BNP = brain natriuretic peptide.

Liver-Type Fatty Acid Binding Proteins

Mammalian intracellular fatty acid binding proteins (FABP) are expressed from a large multigene family and encode 14-kDa proteins that are members of the superfamily of lipid-binding proteins. FABPs are tissue-specific. There are nine FABPs: liver, intestinal, heart muscle, adipocyte, epidermal, ileal, brain, myelin and testis. However, not much is known about their exact biological role and mechanism of action [36]. Liver-type (L)-FABP is a 14-kDa protein synthesized mainly in the liver, with its gene located on chromosome 2p11 in humans. L-FABP is expressed in hepatocytes and the crypt-to-villus

tip of the intestines from the duodenum to the colon. It can be filtered through the glomerulus due to its small molecular size similar to cystatin C, but it is reabsorbed in proximal tubule epithelial cells like other small proteins. As shown by Portilla et al. [37] L-FABP is expressed in human kidney predominantly in the proximal tubules, in the nephron segment which utilizes fatty acids as a major source of energy metabolism.

There are two types of FABPs in the renal tubule cells: L-FABP (FABP1) and heart muscle-type FABP (FABP3). L-FABP is found in the cytoplasmic region of the proximal tubules, whereas FABP3 is found in the cytoplasmic region of the distal tubules except the macula densa [38,

- NGAL (plasma, urine)
- KIM-1 (urine)
- L-FABP (urine)
- IL-18 (urine)
- Cystatin C (plasma, urine)
- Albumin (urine)
- NAG (urine)
- GST- α and - π (urine)
- GGT (urine)
- Beta-2-microglobulin (urine)

Fig. 5. New biomarkers. GST = Glutathione transferase; GGT = γ -glutamyl transferase.

39]. The promoter region of L-FABP contains the binding site for hepatocyte nuclear factor, hypoxia-inducible factor-1 and peroxisome proliferator-activated receptors. Expression of the erythropoietin gene is also controlled by oxygen-sensitive transcription factor hypoxia-inducible factor-1. It might be that the mechanism of hypoxic regulation of L-FABP is transcriptional, via the common oxygen-sensing regulatory pathway, and that L-FABP could serve as a marker of renal hypoxia. There are also no standardized assays (only experimental) available for L-FABP assessment.

Cystatin C: A Marker of Glomerular Filtration

In recent years, there has been an interest in exploring other biomarkers for estimating renal function. The most promising agent seems to be cystatin C, a basic low molecular mass protein (13,359) freely filtered by the glomerulus and subsequently reabsorbed by the proximal tubule where it is catabolized [40]. It is not secreted by the renal tubules as creatinine [41]; therefore, some limitation of creatinine (effects of muscle mass, diet, sex, tubular secretion) may not be a problem with cystatin C. It is a polypeptide chain with 120 amino acid residues. A review of reports where cystatin C is compared to gold standard markers has resulted in mixed reviews concerning its potential usefulness [42]. In addition, cystatin C is affected by hyper- and hypothyroidism [43, 44]. Levels of CRP may also increase cystatin C, suggesting that it could be a marker of inflammation [45].

Fig. 6. Biomarker localization in the nephron.

Biomarkers of AKI in Different Clinical Settings

In the meeting of the Acute Kidney Injury Network (AKIN) in 2008, seven markers (urinary KIM-1, urinary NGAL, urinary IL-18, plasma IL-6, urine and plasma cystatin C, urinary NGAL, and urinary L-FABP) were listed as being on the leading edge of predictive biomarkers of AKI [46]. However, it might be a good strategy to develop a panel of several renal biomarkers since AKI is a complex disease with multiple causes (fig. 6). On the other hand, we should be fully aware of the limitation of these emerging biomarkers in various clinical settings.

Cardiopulmonary Bypass

CPB could be an 'ideal' model of human AKI due to the known time of the renal insult. Pediatric cardiac surgery, in particular, is recognized as an ideal clinical setting to study biomarkers due to minimal comorbidities and known timing of kidney injury [47]. Therefore, it could be easier to study potential new biomarkers and their predictive value in patients after CPB. Biomarkers should also be further evaluated in adults and ICU/emergency departments in populations which are more heterogeneous with more comorbidities and have more complicated and/or multiple renal insults to confirm the possibility of clinical application. CPB or cardiac artery bypass grafting (CABG) could be potentially complicated by AKI due to a variety of causes, including intraoperative hypotension, postoperative cardiac complications

that impair renal perfusion, atheroemboli and exposure to contrast media [48].

The incidence of AKI after CPB is dependent upon the variable definitions used and population studied (pediatric without comorbidities, adults with many comorbidities, urgent vs. elective procedure, etc.) [49]. In two studies of 843 and 649 patients undergoing cardiac surgery (mostly CABG), the incidence of AKI (defined as a rise in serum creatinine of only 25%) was 17 and 24%, respectively [50, 51]. When AKI is defined as either an increase of serum creatinine >2 mg/dl ($177 \mu\text{mol/l}$) with a minimum doubling of the preoperative value, or a new requirement for renal replacement therapy [52, 53], the overall rate of AKI ranged from 3.6 to 5% and did not vary over time in two large series. A higher rate of 7.5 and 12.9% was reported when CABG was combined with an aortic or mitral valve replacement, respectively [53]. Both the development and severity of AKI are independent predictors of mortality after CABG [54, 55]. There was a progressive increase in risk of mortality together with a decrease in GFR [56].

Mishra et al. [15] for the first time highlighted the value of NGAL as a novel marker for early detection of AKI in 71 children undergoing CPB. Both urinary and plasma NGALs at 2 h after the procedure were found to be powerful independent predictors of AKI with extraordinary AUC for urinary NGAL (0.998) and plasma NGAL (0.91). Similar findings were reported by Tuladhar et al. [57]. In 426 adult patients undergoing cardiac surgery, Wagener et al. [58] found that elevation of urinary NGAL correlated significantly with time of CPB and aortic cross-clamp time, a well-established risk factor for AKI in this population [59]. Moreover, Dent et al. [60] reported that plasma NGAL 2 h after CPB was a predictor of duration of AKI and length of hospitalization, whereas plasma NGAL 12 h after CPB was a predictor of mortality in children undergoing CPB. Similarly, Bennett et al. [61] in a study on 196 children undergoing CPB found that urinary NGAL 2 h after the procedure predicted severity and duration of AKI, length of hospitalization, requirement for renal replacement therapy and mortality. However, Xin et al. [62] found that urinary NGAL 2 h after CPB was predictive of AKI, whereas serum NGAL did not change significantly in the group of 33 adults during the study.

In a recent study of Liangos et al. [63], NGAL was neither specific nor predictive of AKI in the group of 103 patients undergoing cardiac surgery, whereas McIlroy et al. [64] reported that postoperative NGAL best identified AKI in patients with a baseline of eGFR 90–120 ml/min.

In patients with a baseline eGFR <60 ml/min, urinary NGAL did not differ at any time between those who did and those who did not develop AKI [65]. In a study by Parikh et al. [66], urine IL-18 increased 4–6 h after CPB, peaked at over 25-fold at 12 h, and remained markedly elevated up to 48 h after CPB in patients who developed AKI diagnosed 2 days later by creatinine criteria. They suggested that IL-18 is an early predictive biomarker of AKI after CPB, and that NGAL and IL-18 are increased in tandem after CPB. However, in another study from Europe by Haase et al. [67], IL-18 did not predict AKI in the group of 100 patients undergoing cardiac surgery.

Han et al. [68] assessed the diagnostic utility of urinary KIM-1, N-acetyl- β -D-glucosaminidase (NAG) and NGAL for the early detection of postoperative AKI in a prospective study of 90 adults undergoing cardiac surgery. The AUCs for KIM-1 to predict AKI immediately and 3 h after operation were 0.68 and 0.65; 0.61 and 0.63 for NAG; and 0.59 and 0.65 for NGAL, respectively. Similarly, Liangos et al. [63] studied the performance of six candidate urinary biomarkers, KIM-1, NAG, NGAL, IL-18, cystatin C and alpha-1-microglobulin, in 103 subjects undergoing cardiac surgery. Urinary KIM-1 2 h postoperatively achieved the highest AUC (0.78, 95% CI: 0.64–0.91), followed by IL-18 and NAG. Only urinary KIM-1 remained independently associated with AKI after adjustment for a preoperative AKI prediction score (Cleveland Clinic Foundation score; $p = 0.02$), or CPB perfusion time ($p = 0.006$), and performed best as an early biomarker for AKI. In the study on adults undergoing CPB, urinary L-FABP increased significantly 4 h following surgery, whereas serum L-FABP started to increase 12 h postoperatively [37], indicating that urinary L-FABP was mostly determined by proximal tubule injury. AUC of urinary L-FABP was 0.81, and urinary L-FABP at 4 h after surgery was an independent predictor of AKI. Haase et al. [69] reported that NGAL, cystatin C and their combination on arrival in intensive care after cardiac surgery correlated with subsequent AKI duration (all $p < 0.01$) and severity (all $p < 0.001$). The AUC for AKI prediction was 0.77 (95% CI: 0.63–0.91) for NGAL and 0.76 (95% CI: 0.61–0.91) for cystatin C on arrival in intensive care.

Both markers also correlated with length of stay in intensive care ($p = 0.037$; $p = 0.001$). NGAL and cystatin C were independent predictors of AKI duration and severity, and length of stay in intensive care (all $p < 0.05$). The value of cystatin C on arrival in intensive care appeared to be due to a carry-over effect from preoperative values. Moreover, Haase-Fielitz et al. [70] also reported that plasma NGAL and serum cystatin C were superior to conven-

tional biomarkers in the prediction of AKI in patients after cardiac surgery and that they were also of prognostic value in this setting. On the other hand, Koyner et al. [71] found that plasma cystatin C and NGAL were not useful predictors of AKI within the first 6 h following surgery. In contrast, both urinary cystatin C and NGAL were elevated in the 34 patients who later developed AKI, compared to those with no injury. The urinary NGAL at the time of ICU arrival and the urinary cystatin C level 6 h after ICU admission were most useful for predicting AKI. A composite time point consisting of the maximum urinary cystatin C achieved in the first 6 h following surgery outperformed all individual time points. They suggested that urinary cystatin C and NGAL were superior to conventional and novel plasma markers in the early diagnosis of AKI following adult cardiac surgery. On the other hand, Felicio et al. [65] studied 50 patients after cardiac surgery and found that there was an increase in cystatin C on the 1st and 5th day after surgery, with it being significantly different on the 5th postoperative day ($p < 0.01$). Cystatin C and the cystatin-GFR showed significant changes after cardiac surgery when compared with creatinine and the respective GFR calculated by the Cockcroft-Gault and MDRD formulas. They further stressed the uselessness of eGFR in assessing kidney function in unstable patients, particularly after surgery.

Contrast-Induced Nephropathy

Approximately 5 billion doses of contrast agents are used annually in the US. The administration of contrast media can lead to a usually reversible form of acute renal failure called contrast-induced nephropathy (CIN) that begins soon after the contrast is administered [72]. The underlying pathology of CIN is acute tubular necrosis, with renal vasoconstriction resulting in medullary hypoxemia and direct cytotoxic effects of the contrast agents considered, although the mechanism by which this occurs is not well understood [73]. Other postulated contributors include rheologic alterations, activation of the tubuloglomerular feedback mechanism, regional hypoxia and production of reactive oxygen species [73]. The nephrotoxic properties of these agents appear to vary, with low- and iso-osmolar agents being associated with a relatively lower incidence of renal injury among high-risk patients. The reported incidence of CIN varies widely, ranging from 0 to over 50% [74]. The risk is greatest in patients with moderate to severe renal insufficiency and diabetes. This variability results from differences in the

presence or absence of risk factors (primarily CKD), definition of CIN, amount and type of agent administered, prospective or retrospective design, the exact radiologic procedure, and whether other causes of acute renal failure unrelated to contrast media were excluded (e.g. atheroemboli during arteriography) [74]. In comparison to a percutaneous coronary intervention (PCI), the risk of CIN is low following intravenous contrast administration, even in patients with CKD [75]. Most commonly, CIN is defined as an acute impairment of renal function manifested by an absolute increase in serum creatinine of at least 0.5 mg/dl or by relative increase by at least 25% from the baseline levels [73, 76]. Peak creatinine typically occurs 3–5 days after contrast administration and returns to baseline (or a new baseline) within 1–3 weeks [76]. The renal failure is nonoliguric for the vast majority of patients. In almost all cases, the decline in renal function is mild and transient. The most common causes are hemodynamic instability, radiocontrast toxicity and atheroembolism. Since interventional cardiologists are being asked more frequently to perform PCI on increasing numbers of patients with significant comorbidities such as CKD and/or diabetes, contrast nephropathy is a potentially serious complication of PCI [76].

In our study on low risk patients with and without diabetes undergoing PCI, we found a significant rise in serum NGAL after 2, 4 and 8 h, and in urinary NGAL 4, 8 and 24 h after the procedure in both groups [19]. Serum NGAL was significantly higher in diabetic patients 2, 4, 8 and 24 h after PCI, whereas urinary NGAL was significantly higher 4, 8 and 24 h after PCI. We found a significant rise in serum NGAL after 2, 4 and 8 h, and in urinary NGAL 4, 8 and 24 h after PCI in nondiabetic patients. NGAL levels were significantly higher in patients with CIN starting 2 h (serum NGAL) or 4 h (urinary NGAL) after PCI. Even after 48 h, serum and urinary NGALs were significantly higher in patients with CIN when compared to patients without CIN. Cystatin C was higher only 8 and 24 h after cardiac catheterization in patients with CIN. IL-18 followed the cystatin C pattern and was significantly higher in patients with CIN 8 and 24 h after cardiac catheterization, whereas L-FABP was significantly higher only 24 h after the procedure. KIM-1 tended to be statistically higher after 24 and 48 h, but the difference did not reach statistical significance. Similarly, Rickli et al. [77] observed that the rise in cystatin C achieved a maximum at 24 h after the application of the contrast agent. In a prospective study on NGAL and L-FABP in patients with normal serum creatinine undergoing PCI due to unstable angina, a significant rise in serum

NGAL after 2 and 4 h was found [78]. Urinary NGAL and urinary L-FABP followed the same pattern. They increased significantly after 4 h and remained elevated up to 48 h after PCI. In the study by Hirsch et al. [79], performed in 91 children with congenital heart disease undergoing elective cardiac catheterization and angiography with contrast administration, significant elevation of NGAL concentrations in urine and plasma were noted within 2 h after contrast administration with an AUC for urinary NGAL of 0.92 and for plasma NGAL of 0.91. They reported that patient demographics and contrast volume were not predictive of CIN. Diabetic children were enrolled in their study, but their findings are in line with our results. In the study published by Ling et al. [80] on patients undergoing coronary angiography using low-osmolar contrast medium, urine samples for NGAL and IL-18 assessment were collected before and 24 h after coronary angiography. At 24 h after the procedure, the urinary IL-18 and NGAL levels were significantly increased in the CIN group (13 patients out of 150, prevalence of CIN: 8.7%), but not in the control group (27 patients; $p < 0.05$). The predictable time of AKI onset determined by IL-18 was 24 h earlier than that determined by serum creatinine ($p < 0.01$). Bulent Gul et al. [81] studied urinary IL-18 values before and 24 and 72 h after PCI. They found no statistically significant differences in urine IL-18 between cases of CIN ($n = 15$) and controls ($n = 36$), or between the patient samples obtained before PCI and after the invasive procedure in both study groups. They concluded that their findings argued against the hypothesis that urine IL-18 might be clinically useful as a biomarker of CIN after radiological procedures requiring intravascular administration of iodinated contrast media. Kato et al. [82] studied changes in creatinine, cystatin C, α - and β -microglobulins, NAG and L-FABP in 87 patients undergoing elective catheterization with or without PCI. They found that L-FABP increased on days 1 and 2 after the procedure in 31 Japanese patients with stage 3 CKD, where the prevalence of CIN was 42%. They also found a rise in L-FABP 1 day after the procedure in 41 patients with mild renal disease defined as eGFR 89–60 ml/min (modified MDRD equation for Japanese). In our population we observed a significant rise in L-FABP 24 and 48 h after PCI in both the diabetic and nondiabetic group without any significant changes 2–8 h after the procedure. Nakamura et al. [83] studied 66 patients with serum creatinine between 1.2 and 2.5 mg/dl. They defined contrast medium nephropathy as an increase in serum creatinine level >0.5 mg/dl or a relative increase of more than 25% at 2–5 days after the procedure, and found that

the prevalence of CIN was almost 20%. They observed a rise in urinary L-FABP levels the next day and 2 days after angiography in a CIN group. After 14 days, serum creatinine returned to the baseline level, but the urinary L-FABP level remained high. However, urinary L-FABP levels in the non-CIN group changed little throughout the experimental period.

Kidney Transplantation: Delayed Graft Function

Delayed graft function (DGF) is generally defined as renal failure persisting after transplantation. The incidence is markedly higher among extended criteria donor kidneys and nonheart beating donor kidneys, compared with standard kidneys [84]. This most likely reflects inherent donor disease with a predisposition to atherosclerosis and acute tubular necrosis. The definition of DGF varies in different studies, but generally refers to oliguria or the requirement for dialysis in the first week after transplantation. The two major prerenal causes immediately after transplantation are hypotension and volume depletion. Postischemic acute tubular necrosis or reperfusion injury is usually the most common cause of DGF [85].

Parikh et al. [86], in a small group of kidney transplant recipients, reported that urinary NGAL may represent early predictive biomarker of DGF, another model of ischemia-reperfusion injury. They found, that in patients with DGF, peak postoperative serum creatinine requiring dialysis typically occurred 2–4 days after transplantation, whereas urine NGAL and IL-18 values on day 0 were maximally elevated in the DGF group when compared to the living donor kidney and deceased donor kidney groups with prompt graft function. In the previous study of this group, NGAL staining intensity in early protocol biopsies was suggested to be a novel predictive biomarker of AKI following transplantation [87]. They obtained protocol biopsies 1 h after anastomosis and found that the immunochemical staining intensity for NGAL was correlated with cold ischemia time, peak postoperative serum creatinine and dialysis requirement. Four patients developed DGF requiring dialysis during the first week after transplantation; all of these patients displayed the most intense NGAL staining in their first protocol biopsies. NGAL staining in early biopsies from patients receiving a cadaveric kidney was strongly correlated with peak postoperative serum creatinine, which occurred days later. In a recent study, Kusaka et al. [88] suggested that monitoring of serum NGAL

levels might allow for the prediction of graft recovery and the need for hemodialysis after a kidney transplantation from a donor after cardiac death. They observed a dramatic fall in serum NGAL on the first postoperative day after transplantation from a living related donor reaching normal range on the 10th postoperative day. All of the patients after cadaveric kidney transplantation (kidney procured after cardiac death) required hemodialysis for 5–22 days [88]. In the study of Schaub et al. [89] the stable transplant with the subclinical tubulitis group had slightly higher levels of NGAL ($p = 0.06$) than the stable transplant with the normal tubular histology group with a substantial overlap. The clinical tubulitis Ia/Ib and the other clinical tubular pathology groups had significantly higher levels of NGAL than stable transplants with normal tubular histology or stable transplants with subclinical tubulitis ($p < 0.002$). In our study we found that serum NGAL decreased significantly as early as 1 day after kidney transplantation, prior to a fall in cystatin C and creatinine [90]. In 4 patients, we observed a DGF. We did not observe a fall in serum NGAL, creatinine or cystatin C in any of these patients. Serum NGAL, cystatin C and creatinine were always significantly higher in patients with DGF when compared to patients without DGF. Urinary NGAL, however, has limited usefulness in anuric patients. In a recent study, Hall et al. [91] collected serial urine samples for 3 days after transplantation and analyzed levels of urinary NGAL, IL-18 and KIM-1. ROC curve analysis suggested that the abilities of NGAL and IL-18 to predict dialysis within 1 week were moderately accurate when measured on the first postoperative day, whereas the fall in serum creatinine was not predictive. In addition, NGAL and IL-18 quintiles also predicted graft recovery up to 3 months later. They concluded that urinary NGAL and IL-18 were early, noninvasive and accurate predictors of both the need for dialysis within the first week of kidney transplantation and 3-month recovery of graft function. In biopsies of 25 kidney transplant recipients, KIM-1 staining identified proximal tubular injury and correlated with renal dysfunction [92] as well as indicated the potential of recovery of kidney function, whereas in 145 kidney allograft recipients, urinary KIM-1 predicted graft loss independent of creatinine clearance, proteinuria and donor age. Yamamoto et al. [93] studied urinary L-FABP in living-related kidney transplants. They reported that urinary L-FABP correlated inversely with peritubular capillary flow and positively with total ischemia time of the kidney, i.e. defined as the time from clamp of the donor's renal artery to the appearance of the first urine from recipient's ureter. They

suggested that urinary L-FABP reflected hypoxic conditions resulting from decreased peritubular capillary flow in the kidney.

ICU and Emergency Department Settings

In the ICU setting, the main problem is to establish the time of renal insult. Often, the baseline kidney function also remains unknown. Among patients who develop acute renal failure in the hospital, the date of onset can be precisely timed in patients in whom the plasma creatinine concentration is measured frequently as part of routine blood testing. Suppose, for example, that a patient has had a stable plasma creatinine concentration which then begins to rise progressively on day 7. In such a patient, there must have been some insult on day 6 or even 5, or a cumulative insult that has become clinically apparent (such as aminoglycoside therapy, a hypotensive episode or administration of radiographic contrast media). Careful perusal of the patient's chart may identify the precipitating event on days 5 and 6 (e.g. hypotension, radiocontrast exposure or cessation of intravenous hydration in a patient treated with intravenous acyclovir). NGAL was tested as a potential biomarker of AKI in 143 critically ill children with systemic inflammatory response syndrome or septic shock within 24 h of admission to ICU. Wheeler et al. [94] found that plasma NGAL was highly sensitive (84%), but not specific (39%), in diagnosing AKI in this setting. It correlated with the severity of systemic disease with higher values in patients with septic shock relative to SIRS and healthy volunteers. In another study, Nickolas et al. [14] reported that a single measurement of NGAL upon admission to the emergency department predicted the need for nephrology consultation, ICU admission and renal replacement therapy. NGAL was found to be highly sensitive (90%) and specific (99%) in diagnosing AKI in this population. Moreover, values of urinary NGAL were helpful to distinguish between AKI and CKD. Similarly, in the study of Makris et al. [95] on critically ill polytrauma patients, urinary NGAL was found to be an early marker of AKI. In two recent studies on the NGAL as an early predictor of AKI in adult ICU population, Cruz et al. [96] showed that plasma NGAL allowed the diagnosis of AKI up to 48 h prior to a clinical diagnosis. Additionally, NGAL predicted the need for renal replacement therapy and correlated with AKI severity. Moreover, Bagshaw et al. [97] reported in the same issue of *Critical Care Medicine* that septic AKI patients had higher values than nonseptic AKI patients

and that this difference was diagnostically and clinically relevant with pathogenetic implications. Moreover, in a recent study, Kumpers et al. [98] reported that serum NGAL was an independent predictor of 28-day mortality in ICU patients (with systemic inflammatory response syndrome or critically ill patients with sepsis) with dialysis-dependent AKI. On the other hand, Trachtman et al. [99] studied urinary NGAL within 5 days of hospitalization due to diarrhea-associated hemolytic-uremic syndrome in 34 children as an adjunctive marker that defined severe renal involvement. However, despite differences in urinary NGAL within 5 days of hospitalization (no mean values were provided, only a description that normal was below 200 ng/ml in 14 children and high was over 200 ng/ml in 20 children), initial serum creatinine did not differ significantly between these groups. Plasma NGAL levels were unable to discriminate between children with less severe diarrhea-associated hemolytic-uremic syndrome and those requiring dialysis support similar to the recent data on NGAL in adult septic shock with and without AKI [17]. Mårtensson et al. [17] concluded that since plasma NGAL was elevated in patients with SIRS, severe sepsis and septic shock, it should be used with caution as a marker of AKI in the ICU. Urinary NGAL was more useful in predicting AKI as the levels were not elevated in septic patients without AKI [24]. IL-18 was found to predict AKI in patients with adult respiratory distress syndrome with an AUC of 0.73, and it was found to be an independent predictor of mortality in this group [100]. Liangos et al. [63] reported that NAG or KIM-1 in combination with the covariates cirrhosis, sepsis, oliguria and mechanical ventilation yielded an AUC of 0.78 (95% CI: 0.71–0.84) in predicting the composite outcome in patients with acute renal failure. In a small, cross-sectional study, Han et al. [26] reported that KIM-1 was markedly induced in proximal tubules in kidney biopsies from patients with established AKI (primarily ischemic) and that urinary KIM-1 was more elevated in patients with ischemic AKI than in prerenal azotemia or CKI. A rise in KIM-1 by 1 unit was associated with a more than 12-fold increased risk for AKI after adjustment for age, sex, and length of time delay between initial insult and urine sampling. In a recent study by Ferguson et al. [101] in hospitalized patients, the diagnostic performance of urinary L-FABP for AKI, assessed by the AUC, was 0.93. This compares favorably with other established biomarkers of AKI such as KIM-1, NGAL, NAG and IL-18. They found that age-adjusted urinary L-FABP levels were significantly higher in patients with poor outcome, defined as the requirement for renal replacement therapy or

the composite endpoint of death or renal replacement therapy. Previously, Nakamura et al. [83] showed that among the surviving patients after septic shock, urinary L-FABP was reduced by treatment, whereas nonsurvivors had higher urinary L-FABP with a smaller fall during the treatment. They suggested that urinary L-FABP might be able not only to reflect the severity of sepsis, but also to help monitor the effectiveness of treatment. Herget-Rosenthal et al. [102] reported that serum cystatin C is a useful detection marker of acute kidney failure and might detect it 1 or 2 days earlier than creatinine. However, in the recent study by Perianayagam et al. [103] of 200 patients with AKI (from two academic medical centers), the serum cystatin C level performed similarly to the serum creatinine level, serum urea nitrogen level and urine output for predicting dialysis requirement or in-hospital death. Bell et al. [104], in a recent study, evaluated a possible relation between cystatin C and mortality in 845 ICU patients. They found that in AKI patients, the HR comparing cystatin C above and below the median more than doubled from the second year on compared to the first year follow-up. Cystatin C correlated with mortality independently of renal function measured by creatinine in patients entering the general ICU. In a recent study by Nejat et al. [105], plasma cystatin C was an effective and earlier surrogate marker of decreased renal function than serum creatinine in a general ICU population (n = 442). Plasma cystatin C predicted sustained AKI with an AUC of 0.80 (95% CI: 0.71–0.88). On the other hand, both plasma cystatin C and serum creatinine were similarly moderately predictive of death or dialysis with AUCs of 0.61 (95% CI: 0.53–0.68) and 0.60 (95% CI: 0.51–0.67), respectively.

Evaluation of Biomarkers

We can evaluate the biomarkers on the basis of the AUC of the different tests (predictive values of markers: <0.60 = useless marker, 0.60–0.69 = poor, 0.70–0.79 = fair, 0.80–0.89 = good, 0.90–1.00 = excellent) and the time when the respective test indicated AKI prior to serum creatinine. In a landmark study by Mishra et al. [15], plasma and urinary NGAL predicted development of AKI within 2 h after surgery far earlier (2–3 days) than serum creatinine, with an excellent AUC of 0.998 for urinary NGAL with a sensitivity of 1.00 and a specificity of 0.98 for a cutoff value of 50 µg/l. In adults, the predictive accuracy of NGAL was somewhat diminished with an AUC of 0.77–0.96. In a recent study by Perry et al. [106], an

early increase of post-CPB plasma NGAL was associated with AKI in adult patients undergoing CABG surgery, although the sensitivity was low. Therefore, the authors concluded that assessing early plasma NGAL alone had limited utility for predicting AKI in this patient population. In the study of Yang et al. [107], initial urine NGAL was identified as an independent predictor of in-hospital mortality and persistent loss of renal function. In the analysis of predictive performance of urine NGAL, the AUC was 0.882 and a cutoff value of 298.28 ng/ml predicted loss of renal function with 88.2% sensitivity and 81.0% specificity. On the other hand, in hospitalized patients, the diagnostic performance of urinary L-FABP for AKI, assessed by the AUC, was 0.93 [102]. This compares favorably with other established biomarkers of AKI such as KIM-1, NGAL, N-acetyl- β -glucosaminidase and IL-18 as reported by Ferguson et al. [101]. However, in a study by Siew et al. [108], urinary IL-18 did not reliably predict AKI development, but did predict poor clinical outcomes in a broadly selected, critically ill adult population. Unfortunately, there are still many inconsistencies in the field of biomarkers. In most studies on KIM-1, time was not even mentioned, which is essential to judge the value for early detection. It should also be mentioned that the AUC in ROC for serum creatinine in the study by Nickolas et al. [14] was almost as good as that for urinary NGAL. This outweighs any advantage of urinary NGAL, as urinary NGAL measurement currently costs in most countries around 24 EUR, compared to 3–25 cents for creatinine measurement. On the other hand, in a recent paper by Soto et al. [109] serum cystatin C was an early predictive biomarker of AKI, which outperformed serum creatinine in the heterogeneous emergency department setting. Neither biomarker discriminated between AKI and CKD. However, as the authors also stated, the most significant result was the excellent power of serum cystatin C for predicting AKI even in the early time points (at presentation and at 6 h), with AUC values greater than 0.87. Surprisingly and contrasting with their previous statement, these AUC values are comparable to those for serum creatinine, the current and much cheaper standard marker for AKI. To date, there have been no data on the cost-effectiveness of biomarker use in AKI recognition, initiation of renal replacement therapy, hospitalization and other outcomes.

In the recent meta-analysis data from 19 studies and 8 countries involving 2,538 patients, 487 (19.2%) developed AKI, which was defined as an increase in serum creatinine level >50% from baseline within 7 days or CIN (creatinine increase >25% or concentration >0.5 mg/dl in

adults or >50% increase in children within 48 h) [110]. Other outcomes predicted using NGAL were renal replacement therapy initiation and in-hospital mortality. The diagnostic accuracy of plasma/serum NGAL [17.9 (95% CI: 6.0–53.7)/0.775 (95% CI: 0.679–0.869)] was similar to that of urine NGAL [18.6 (95% CI: 7.2–48.4)/0.837 (95% CI: 0.762–0.906)]. NGAL level was a useful prognostic tool with regard to the prediction of renal replacement therapy initiation [12.9 (95% CI: 4.9–33.9)/0.782 (95% CI: 0.648–0.917)] and in-hospital mortality [8.8 (95% CI: 1.9–40.8)/0.706 (95% CI: 0.530–0.747)]. The authors concluded that NGAL level appears to be of diagnostic and prognostic value for AKI. There are no meta-analyses on the other AKI biomarkers.

Final Remarks

There is an urgent need for new sensitive biomarkers of AKI to answer the question of what the right time is for diagnosing AKI, prognosis, need for renal replacement therapy and of course cost-effectiveness of renal replacement therapy. Diagnosis of AKI in the ICU increases the cost of hospitalization manifold, a small rise in creatinine by only 0.3 mg/dl is responsible for a rise in costs by 4,886 USD, a rise by 0.5 mg/dl increases the cost by 7,499 USD, whereas a rise by 2 mg/dl increases the cost by 22,023 USD according to Himmelfarb and Ikizler [111]. In critically ill patients with fluid shifts who are not in a steady state, serum creatinine does not accurately reflect the GFR. In addition, sepsis also decreases creatinine production [112]. Therefore, the search for a sensitive and specific biomarker of AKI has become a top priority, not only for nephrologists but for other specialties as well. It should be taken into account that NGAL is the earliest biomarker of AKI, followed by IL-18, KIM-1 and L-FABP, while cystatin C is more a marker of GFR than kidney injury. The limitations of current biomarkers include: the lack of an ideal single biomarker of AKI; all have strengths and weaknesses; and combinations of biomarkers are probably needed, but they are not well characterized and they are expensive to develop and validate. Furthermore, recent studies have only been performed on relatively small cohorts, are from a single center, had a small number of events, the standardized laboratory platform of biomarkers were not widely used, and the studies were performed on frozen samples (problems with protein degradation and freeze/thaw cycles). Moreover, hardly any marker has been externally validated (many RCTs on NGAL as a marker of AKI are ongoing) or external vali-

dation could not demonstrate results as good as the initial studies. Thus, more clinical studies, and then meta-analyses, are needed to validate new biomarkers of AKI, detect AKI earlier than a rise in serum creatinine does and prove the cost-effectiveness of a new biomarker or panel of biomarkers. We also should answer the question regarding the future of biomarkers: which biomarkers are the most promising? Which aims in biomarker development are the most important in human AKI? What is the cutoff value for AKI biomarkers? What is the predictive value of biomarkers in regard to AKI severity, need for

renal replacement therapy, mortality and progression to CKD? When should new AKI biomarkers be employed to allow timely and early intervention? Revalidation of AKI definition using new biomarkers – a paradigm shift – should be taken into account. The target population and type of intervention should also be defined. In addition, earlier detection of AKI with kidney-specific biomarkers may result in earlier nephrology consultation, more optimal drug dosing, avoidance of nephrotoxic drugs, or even earlier specific therapies (including renal replacement therapy) to repair the injured kidney.

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