Neutrophil gelatinase-associated lipocalin (NGAL): the clinician’s perspective

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Introduction

Neutrophil gelatinase-associated lipocalin (NGAL), originally discovered in the leukocyte neutrophil and than extensively studied in renal tissues, is now under focus as a “prognostic” biomarker, potentially useful in risk stratification of a variety of cardiovascular as well as neoplastic and inflammatory conditions. The same journey (i.e., from diagnostic to prognostic marker) has been covered by other biomarkers, including cardiac troponins (1), natriuretic peptides (2), copeptin (3), C reactive protein (4) and procalcitonin (5) among others.

Acute renal failure (ARF), also known as acute kidney injury (AKI) as now referred to in the scientific literature, is defined as an abrupt or rapid decline in renal filtration function. This condition is usually mirrored by an increase of serum creatinine concentration or azotemia [i.e., an elevation of blood urea nitrogen (BUN) concentration] (6). Nevertheless, immediately after kidney injury, BUN or creatinine levels may be normal, and the only sign of a kidney injury may be a reduced urine output (7). An increase of serum creatinine concentration may result from several medications (e.g., cimetidine, trimethoprim) that inhibit the kidney tubular secretion, whereas an elevation of serum BUN concentration may be due to increased production (i.e., gastrointestinal bleeding, steroid use, or protein loading), which may occur without concomitant renal injury, so that careful investigation must be performed before assessing as to whether kidney injury is really present (7).

The RIFLE system

In 2004, the Acute Dialysis Quality Initiative work group proposed a new classification for acute renal failure, described by the acronym RIFLE (Risk of renal dysfunction, Injury to the kidney, Failure or Loss of kidney function, and End-stage kidney disease) (7). Investigators have since applied the RIFLE system to the clinical evaluation of AKI, although it was not originally intended for that purpose (Figure 1 shows a new concept of AKI, which is obtained coupling functional and cellular damage biomarkers). A large number of multiorgan complications can be observed in patients with AKI.

Cardiovascular complications

Cardiovascular complications [e.g., congestive heart failure (CHF), myocardial infarction, arrhythmias, cardiac arrest] have been described in as many as 35% of patients with AKI. Fluid overload secondary to oliguric AKI is a particular risk for elderly patients with modest cardiac reservoir. Cardiac arrest in a patient with AKI always should raise the suspicion of hyperkalemia.

Pulmonary complications

Pulmonary complications have been reported in approximately 54% of patients with AKI, and represent the single
most significant risk factors for death in these patients. Several diseases may frequently onset with simultaneous pulmonary and renal involvement, including pulmonary/renal syndromes (e.g., Goodpasture syndrome, Wegener granulomatosis, polyarteritis nodosa, cryoglobulinemia, sarcoidosis).

Gastrointestinal symptoms

Gastrointestinal symptoms of nausea, vomiting, and anorexia are frequent complications of AKI and represent one of the key feature of uremia. Gastrointestinal bleeding occurs in approximately one third of patients with AKI. Most episodes would be mild, but still account for 3%–8% of deaths in these patients. Mild hyperamylasemia (i.e., 2–3 times above the upper limit of the reference range) may be frequent in AKI and elevation of baseline amylase can complicate the diagnosis of pancreatitis (8). Jaundice, which recognizes hepatic congestion, blood transfusions and sepsis among the potential causes, may complicate AKI in approximately 40% of cases.

Infections

Infections may frequently complicate the course of AKI, occurring in as many as one third of patients. The more frequent sites include the pulmonary and urinary tracts. Indeed, infections represent the leading cause of morbidity or death in AKI patients, and various studies have reported mortality rates ranging from 11% to 72%.

Neurological signs

Neurological signs of uremia, including include lethargy, somnolence, reversal of the sleep-wake cycle, and cognitive
or memory deficits, are reported in approximately 38% of patients. The pathophysiology is still unknown, although neurological involvement is not associated with serum levels of either BUN or creatinine.

It is not surprising that the high degree of complexity and the high morbidity and mortality rate, along with the high degree of time-dependence, have led the scientists to coin the term “kidney attack” in analogy with the widely accepted terms of “heart attack” and “brain attack” (9).

The search for the “ideal” biomarker of AKI

Serum creatinine levels or GFR, as well as urinary output, are the most commonly used markers of renal function and are useful for assessing the degree of renal injury. As aforementioned, however, these measures are neither perfect, nor early. Moreover, they cannot be used to distinguish prerenal from intrinsic or obstructive AKI. Similarly, changes in circulating volume can significantly influence the levels of serum creatinine, further minimizing the “true” relative change of renal function (10). With deep knowledge of the limitations of the currently used kidney function measures, it is hence obvious that their assessment is inefficient to detect any acute injury or process, so that more accurate kidney function and/or damage biomarkers have been extensively investigated (11, 12).

The search for the Holy Grail of AKI – see also the sister commentary by Lippi and Plebani in this issue (13) – is actually the search for the “kidney troponin”. The ideal kidney biomarker should permit early diagnoses of AKI, as well as provide meaningful information for treatments before a permanent damage has occurred. The recent research has led to the discovery of several potential biomarkers, including kidney injury molecule 1 (KIM-1), human neutrophil gelatinase-associated lipocalin (NGAL), interluekin-18 (IL-18), cystatin C, clusterin, fatty acid binding protein, osteopontin, monocyte chemotactic peptide-1 (MCP-1), netrin-1, liver-type fatty acid binding proteins (L-FABP) (11, 12).

Among these, there is universal agreement to conclude that NGAL may be the more reliable AKI biomarker (14–17), as well as a useful “prognostic” biomarker for risk stratification of several cardiovascular, neoplastic and inflammatory conditions (18–21) as also comprehensively described by Cruz et al. in this issue of the journal (22). Another important advantage, as also highlighted otherwise in this issue (13), is the availability of fully-automated commercial methods for stat assessment (23). Due to neutrophil granulocyte production (24), it has been argued, however, that the prognostic value of NGAL – at least when assessed in serum, plasma or blood – might be at least partially mediated by leukocytosis in cardiovascular as well as renal morbidity and mortality (25, 26).

The exact physiologic role played by NGAL in the kidney remains a mystery. One possibility is that it may be involved in renal morphogenesis, such as induction of repair and re-epithelialization. The expression of the NGAL messenger ribonucleic acid (mRNA) and protein in the kidney is significantly increased in the renal tubules of patients with ischemic, septic, or post-transplantation AKI (27) as well as within 2–6 h post-cardiopulmonary bypass surgery (15), at frequent intervals for 24 h post-cardiopulmonary bypass surgery in children (28), and even following contrast media administration (29).

Using samples from the Translational Research Investigating Biomarker Endpoints in AKI study (TRIBE-AKI), researchers aimed to determine whether biomarkers measured at the time of first clinical diagnosis of early AKI after cardiac surgery can potentially predict AKI severity. Biomarkers such as urinary IL-18, urinary albumin-to-creatinine ratio (ACR), and urinary and plasma NGAL improve risk classification compared with the clinical model alone, with plasma NGAL performing as the best measure (category-free net reclassification improvement of 0.69, p<0.0001). The authors concluded that biomarkers measured on the day of AKI diagnosis may improve risk stratification and allow to identify patients at higher risk for progression of AKI and worse outcomes (30). Unfortunately, there are several analytical issues that limit the clinical usefulness of NGAL, as extensively underlined by Lippi and Plebani (13). Moreover, most studies have used complex techniques that are recognized as unsuitable for rapid clinical decision-making (31). The main unresolved issue in the clinical assessment of NGAL also includes the lack of consensus on the ideal sample matrix for testing (i.e., blood, serum, plasma or urine), the diagnostic thresholds and the inefficiency of the current assays to discriminate between the NGAL produced by the renal tubular cells from that released by the leukocytes. Being so many doubts still on debate, some authors have recently argued in a some provocative way that a thorough urinanalysis could still be better for diagnosing AKI (32).

At present, it seems reasonable to conclude that the urinary immunoassay displays the best analytical performance (33), but more evidence is needed. The new double-ELISA assay, which is arguably able to distinguish NGAL produced by the tubular cells of the kidney from that released by neutrophils, has been developed and preliminarily validated (34). Other potential areas of interest include the use of NGAL as a diagnostic aid in other biological fluids. Given its remarkable production by neutrophils, it would be interesting to investigate as to whether NGAL assessment in cerebrospinal, pulmonary, peritoneal as well as pancreatic cyst fluids may be useful for diagnosis of acute bacterial infections.

In the review by Cruz et al. in this issue of the journal, the authors have reviewed 21 studies on NGAL biology, providing reliable evidence that this protein is highly expressed in the heart and especially in atherosclerotic plaques. It is hence noteworthy in animal studies that have explored the role of NGAL in cardiovascular disease, have shown atherosclerotic mice display consistent production of NGAL mRNA, which is further induced by hypoxic stress, a process that has considered important for the development of myocardial infarction. In studies on human tissues, the strongest NGAL immunostaining has been reported in cardiomyocytes within failing myocardium, with modest immunoreactivity also found in vascular smooth muscle and endothelial cells. It is also noteworthy that atherosclerotic plaque from both carotid artery and abdominal aortic aneurysm demonstrated co-localization of NGAL and Matrix metalloproteinase 9 (MMP-9)
in the lipid core of the plaque. The concentration of NGAL increased in parallel with the clinical severity of CHF (35, 36) and was significantly correlated with natriuretic peptide levels. However, other studies failed to find a significant correlation between plasma and urine NGAL and echocardiographic indices of left ventricular cardiac structure or right ventricular systolic function (37).

Although the discovery of novel biomarkers may revolutionize our current knowledge of AKI, we still lack prospective clinical trials that have compared their diagnostic or prognostic performance over long period of times. As these new biomarkers evolve, so will our understanding of AKI. A critical issue is, e.g., the differentiation between pre-renal AKI from intrinsic and/or obstructive AKI. Parikh et al. have shown that IL-18 might enable the distinction between pre-renal azotemia, ATN, and other glomerular disorders (38). Ultimately, the goal of biomarker research is the early diagnosis of AKI (within hours, rather than within days or weeks) as well as the differential diagnosis of the mechanisms involved. It is hence sorrowful to conclude that the current commercial immunoassays for NGAL do not end the mythical search of the Holy Grail of AKI!

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