

EDITORIAL COMMENT

Early Detection of Acute Kidney Injury With Neutrophil Gelatinase-Associated Lipocalin*

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Acute kidney injury (AKI) after cardiac procedures and in critically ill patients has been consistently associated with increased cost of care, complications, permanent kidney dysfunction, and higher mortality (1,2). There is an immediate and present need to detect acute AKI before the rise in serum creatinine (Cr) and the development of oliguria, because by that time patients are often resistant to therapeutic interventions (3).

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For decades, the rise in serum Cr has been the only detectable sign of a reduction in glomerular filtration. Creatinine has had the disadvantages of being linked to creatine and the overall body muscle mass—hence, differing according to body size in addition to the rate of renal elimination (4). Furthermore, the kidney both filters and secretes Cr. Finally, the assays used to measure Cr have not been standardized across laboratories; therefore, studies reporting values from multiple centers have inherent variation in values attributed to differences in measurement technique (5). Cystatin C is a cysteine protease inhibitor that is synthesized and released into the blood at a relatively constant rate by all nucleated cells. It is freely filtered by the glomerulus, completely reabsorbed by the proximal tubule, and not secreted into urine. Its blood levels are not affected by age, sex, race, or muscle mass; thus, it seems to be a better indicator of glomerular function than serum Cr in patients with CKD. However, in AKI, it rises only slightly before

serum Cr (6). The field of nephrology in the United States has been devoid of approved blood or urine markers of AKI, unlike cardiac biomarkers indicating myocardial injury and overload (troponin, creatine kinase myocardial band, and natriuretic peptides). The concept of measuring markers of the acute injury process is crucial to the early upstream identification of AKI before there is serious loss of organ function (7). Ideally, markers that give insight into the pathophysiologic mechanisms for AKI would be desirable, because there could be tandem development of therapeutic approaches.

Neutrophil gelatinase-associated lipocalin (NGAL), otherwise known as siderocalin or lipocalin-2, is normally secreted by renal tubular cells, lymphocytes, and cardiomyocytes and is a physiologic response to the presence of catalytic (labile, poorly liganded) iron in the cytosol or pericellular space that itself is liberated from organelles as a result of ischemic or toxic injury. It is a 25-kDa protein that acts as a natural siderophore that scavenges cellular and pericellular labile iron, thus reducing its availability for bacterial growth. Iron is the most common metal element in the human body, and there are elaborate transport (heme, transferrin, ferritin, ferroportin, electron transport chain) and management systems for its use in a variety of critical cellular systems, including oxygen transport and cellular respiration (8,9). It has been recently understood that the process of oxidative stress resulting in cell dysfunction, accelerated apoptosis, and death is reliant on the cytosolic and extracellular presence of labile or catalytic iron, liberated from its binding proteins. There are several steps in generation of reactive oxygen species. Oxygen might be reduced, forming superoxide anion, which can undergo reduction by superoxide dismutase to form hydrogen peroxide—which itself can then be reduced through several pathways. The net reaction is slow, and in the presence of reduced transition metals such as ferric iron (Fe^{+3}), a Haber–Weiss reaction results in the rapid formation of the highly damaging hydroxyl radical from the superoxide anion. Likewise, in the presence of ferrous iron (Fe^{+2}), a Fenton-type reaction converts hydrogen peroxide to the hydroxyl radical. It has been theorized that a common element to all forms of oxidative stress to the heart and kidneys involves the availability of unbound or poorly liganded iron (10). With the bleomycin detectable assay, Lele et al. (9) have recently demonstrated the release of catalytic iron into the blood in patients with acute coronary syndromes. In this study, the appearance of catalytic iron preceded the rise in serum troponin and had an area under the receiver operating characteristic curve for the detection of acute myocardial infarction over 0.90. Local cellular and tissue availability of catalytic iron might determine the degree and severity of organ injury in the setting of most hypoxic and other toxic insults (8). Therefore, a putative final common pathway for common sources of organ injury including ischemia, neurohormonal activation, chemotoxicity, and sepsis involves

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the loss of control over normal iron management and the transient tissue and organ system exposure to catalytic iron, which is attenuated by NGAL (11).

In this issue of the *Journal*, Haase et al. (12), as part of a previous larger meta-analysis, identified and invited authors of 18 published studies of clinical outcomes of AKI patients according to NGAL (measured either in the blood or urine) and Cr status. Of those, 7 authors returned complete datasets consisting of 1,217 patients (11). The major findings were that 41.1% of patients diagnosed with AKI would have been missed with Cr alone. Compared with patients declared negative with respect to both NGAL and Cr results, those positive for both had a 2-fold longer stay in the ICU and in-hospital, more dialysis initiation, and a tripled in-hospital mortality. In the discordant groups, NGAL tended to identify increased risk when the Cr was not significantly changed, with the greatest value being in the NGAL(+) but Cr(-) group. These data suggest that a marker of the renal response to oxidative stress (NGAL) and damage in addition to a marker of decreased renal filtration function (Cr) are complementary in the diagnosis and prognosis of AKI across a variety of hospitalized populations.

The reason this study is important is, first, because it consistently showed prognostic significance of NGAL value in a large number of AKI patients with wide range of clinical situations, including critically ill patients and post-cardiac surgery patients as well as pediatric and adult population. Neutrophil gelatinase-associated lipocalin typically detected AKI 36 to 48 h earlier than Cr, thus opening up a possibility of therapeutic interventions of AKI. Second, this analysis identified new important subgroups of AKI patients according to NGAL and Cr values, which seem to be complementary. Specifically, NGAL(-)/Cr(+) patients might have pre-renal azotemia or tubulo-glomerular feedback mechanisms at work without acute tubular necrosis, which might require totally a different therapeutic approach, as opposed to those who have started to secrete increased quantities of NGAL. Conversely, identification of the NGAL(+)/Cr(-) patient is a critical step forward, because the production of NGAL signals a response to catalytic iron-dependent oxidative stress before there is a measurable decrement in organ function. We recognize that a shortcoming of NGAL as a singular test is that baseline levels rise with chronic renal disease, and thus it becomes a marker of disease progression and might lose its ability to detect AKI from an elevated baseline in more seriously ill populations (13,14).

With the advent of NGAL and the detection of AKI within a few hours of initiation, it is now conceivable to test preventive or immediate therapeutic interventions. The change in NGAL might serve as an experimental marker in phase 2 trials of preventive approaches including catalytic iron chelators (e.g., deferoxamine), other antioxidant agents such as N-acetylcysteine (for contrast exposure), or B-type natriuretic peptide in the perioperative period after cardiac

surgery (15,16). Once NGAL has started to rise in critical care and post-surgical scenarios, treatment trials could be organized to test forms of continuous renal replacement therapy (CRRT) in the period of time surrounding the renal insult. Conceptually, use of CRRT would provide 3 important protective mechanisms that cannot be achieved pharmacologically: 1) ensures euolemia and avoids hypo- or hyper-volemia; 2) provides sodium and solute (nitrogenous waste products) removal; and 3) by both aforementioned mechanisms, it might work to avoid both passive renal congestion and a toxic environment for the kidneys and allow their optimal function during a systemically vulnerable period (17). Despite these advantages, there remains a lack of clinical trial data supporting CRRT or other forms of extracorporeal solute removal, largely because the intervention is applied too late in the clinical course where the detection of AKI is dependent on the rise in serum Cr and development of volume overload.

The identification of NGAL as a protective siderophore and its role in limiting the ability of catalytic iron to propagate oxidative stress reactions in acute organ injury syndromes seems to be a major advance. Additional biomarkers, including kidney injury molecule-1, interleukin 18, liver-type fatty acid binding protein, renal tubular enzymes, and multiple proprietary markers under investigation, might add to the internal validity and assist in understanding the time course and recovery of AKI. These markers used together as a panel might be particularly valuable in the population where Cr has not yet changed. The discovery of NGAL and its clinical introduction in Europe last year has emerged as an ideal circumstance in the biomarker field, where both diagnostic and therapeutic advances are on the horizon.

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