Acute kidney injury (AKI) is an increasingly recognized syndrome associated with short-term and long-term morbidity and mortality. Recent studies have demonstrated that even mild AKI portends an increased risk of chronic kidney disease. There is thus great impetus to develop novel therapies for treatment of AKI. Several agents are currently being tested in phase I/II clinical trials. Despite these ongoing efforts, no effective therapeutic agents have yet emerged on the clinical scene. Some of the reasons for the lack of beneficial therapies in AKI include incomplete understanding of the pathogenesis of this disorder and the absence of early and reliable markers of AKI, which may enable treatment before irreversible injury ensues. The importance given to the development of novel biomarkers is high, as evidenced by the proclamation of the American Society of Nephrology to designate this research endeavor a top priority.

Biomarkers of AKI have represented an area of active research in the last decade. These biomarkers should identify those at risk, enable a timely diagnosis of AKI (i.e., before the alterations in traditional markers), stratify patients on the basis of prognosis, and improve understanding of the nephron segment(s) affected. Markers of renal dysfunction in current use lack both specificity and sensitivity. Serum creatinine is the most widely employed marker of AKI. However, it is a late marker, highly nonspecific to the site or type of injury. It predicts glomerular filtration rate (GFR) only in the steady state and varies with muscle mass and diet. Another biomarker used in the diagnosis of AKI is urine output. Urine output is unreliable except in monitored settings such as intensive care, and it can be altered by administration of fluids and diuretics. Despite their shortcomings, serum creatinine and urine output are the diagnostic biomarkers of AKI on which are based the RIFLE (Risk, Injury, Failure, Loss, and End-stage kidney disease), AKIN (Acute Kidney Injury Network), and KDIGO (Kidney Disease/Improving Global Outcomes) criteria. These traditional biomarkers are functional, because they become altered when GFR declines.

Novel functional biomarkers of AKI have emerged. Cystatin-C is one such marker. Cystatin-C is produced by all nucleated cells and is freely filtered and absorbed by the megalin receptor in proximal tubular epithelial cells (PTECs), where it is subsequently degraded. Its appearance in urine thus signifies deficient uptake or degradation by PTECs, making it a potential biomarker of AKI, particularly when the PTEC compartment is injured. Plasma cystatin-C has been validated as a sensitive marker of GFR decline in chronic kidney disease. Microalbuminuria is another functional marker of glomerular and/or tubular injury that may be informative in detecting AKI in pediatric patients. However, microalbuminuria is nonspecific, does not allow differentiation between acute and chronic injury, and can be physiologic or pathophysiologic. Other functional markers include β2-microglobulin, retinol-binding protein, and α1-microglobulin.

In most forms of AKI, structural changes precede functional impairment. Markers of structural damage may thus be more sensitive in heralding injury. A plethora of structural biomarkers has emerged, and the first group of these biomarkers is upregulated as a result of injury in either renal tubular or infiltrating immune cells. The second group of structural biomarkers includes proteins/enzymes constitutively expressed in the tubules and released from injured cells into the urine. The former group of biomarkers displays increased expression in response to injury and is detected due to protein release into urine or plasma. The most notable example includes neutrophil gelatinase-associated lipocalin (NGAL), one of the most studied biomarkers of AKI. NGAL is synthesized by tubular cells, neutrophils, and several other cell types. It is typically present in the circulation, then freely filtered across the glomerulus, and

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2 Nonstandard abbreviations: AKI, acute kidney injury; GFR, glomerular filtration rate; PTEC, proximal tubular epithelial cell; NGAL, neutrophil gelatinase-associated lipocalin; IL-18, interleukin-18; KIM-1, Kidney injury molecule-1; MIOX, myo-inositol oxygenase.
claimed by the PTECs. During AKI, its expression in the tubules is markedly increased. Animal studies demonstrated that intratubular expression of NGAL paralleled its urinary excretion in AKI (6), and that this protein may mediate protective effects. In clinical studies, NGAL appears to be a powerful predictor of AKI outcomes (7, 8). Similarly, interleukin-18 (IL-18), a cytokine involved in innate and adaptive immunity, has emerged as a promising biomarker predictive of mortality in critically ill patients with AKI (9). IL-18 is produced by immune cells and tubular epithelium. In vivo studies suggest that tubules secrete this cytokine into urine during AKI, and that IL-18 may have a functional role in ischemic injury (10). Kidney injury molecule-1 (KIM-1) is another potential biomarker of AKI, expressed by immune and tubular cells. KIM-1 was the most upregulated gene in PTECs following AKI in rodent disease models (11). Furthermore, its ectodomain was shed in the urine of rodents after injury. Its role may be in clearance of injured epithelial cells. In human AKI, KIM-1 appears to be an early marker of injury that increases before the rise in serum creatinine (12). Two other promising biomarkers4 of early AKI, as well as AKI prognosis, include insulin-like growth factor–binding protein-7 and tissue inhibitor of metalloproteinases-2 (13). These proteins are involved in cell-cycle arrest, which appears to be disturbed in AKI. The second group of structural biomarkers includes constitutively expressed proteins, such as alkaline phosphatase, γ-glutamyl-transpeptidase, α-gluthathione-S-transferase, and N-acetyl-β-glucosaminidase. These enzymes are stored in intracellular organelles and released into urine upon injury.

The problem with implementing any of these structural biomarkers into clinical practice is multifold. First, they perform differently in distinct patient populations. In addition, their use is problematic in patients with multiple comorbidities (likely because their expression is not kidney specific) and with underlying renal dysfunction, and their analytical properties vary with the type of insult. Finally, they currently do not impact clinical care, in the absence of effective therapeutic strategies.

In this issue of Clinical Chemistry, Gaut and colleagues report on a potential novel structural biomarker of AKI, myo-inositol oxygenase (MIOX) (14). These researchers used an innovative approach to discover a kidney-specific protein that could be released from PTECs during AKI. Analogous to a cancer-biomarker approach of searching for tissue-specific molecule expression, they examined genes with high, kidney-specific endogenous expression in mice. The gene encoding MIOX was the only such kidney-specific gene. Gaut and colleagues postulated that MIOX protein could represent a valuable biomarker of kidney injury in mice and humans in vivo. Driven by this hypothesis, they developed an immunoassay for quantifying MIOX in mice and humans. They confirmed the specificity of the antibodies for detecting human MIOX by Western blot and performed epitope mapping. The assay demonstrated good intra- and interassay CVs of <10% and <20%, respectively. The limit of detection was reasonable at 115 pg/mL. The assay demonstrated that MIOX protein was expressed only in human kidneys, and that its renal expression was likely localized in the PTECs. In a murine model of ischemic renal injury, serum MIOX was increased 24 h postinjury, at the time when histologic injury was profound. In critically ill patients, plasma MIOX was increased 54 h earlier than creatinine, although the timing of injury was unclear. Furthermore, it seemed that plasma MIOX was able to segregate patients with oliguric (traditionally more severe) and nonoliguric AKI. MIOX might thus fit several requirements of novel biomarker development, including an early increase, and specificity to renal tissue and nephron segments.

Unlike most structural biomarkers described above that are upregulated in kidney tissue during AKI, MIOX is constitutively expressed uniquely in the PTECs and is then released into circulation during renal injury. In this regard, MIOX resembles structural enzyme biomarkers such as N-acetyl-β-glucosaminidase, alkaline phosphatase, γ-glutamyltranspeptidase, and α-gluthathione-S-transferase. However, MIOX appears to be uniquely expressed in the kidney, unlike any of the other enzymes. Thus its release into the circulation, and potentially into the urine, may represent a specific and early measure of injury. One caveat is that although the expression of this intriguing enzyme is strictly renal under baseline conditions, in cases of multiorgan failure this protein may be more broadly expressed, and future research should address this issue.

Prior studies found corroborating data concerning the kidney-specific expression of MIOX in animal disease models. MIOX catabolizes myo-inositol, a metabolite depleted in diabetes mellitus. MIOX is upregulated during oxidative stress and hyperglycemia in vitro and in vivo, and this upregulation aggravates tubulointerstitial injury (15, 16). Curiously, an older study demonstrated decreased MIOX mRNA expression in kidneys of rodents with AKI (17). It is possible that tubular injury was severe in this study, and injured PTECs could not synthesize their housekeeping proteins. Animal models of ischemic renal injury provide an opportunity to further explore unanswered questions, such as how the renal MIOX gene and protein are expressed after injury and what dynamic changes occur in MIOX in plasma and urine following injury. These studies should establish the timing of release of this
enzyme in relation to injury and to other biomarkers of AKI, including serum creatinine.

Although the data presented by Gaut and colleagues are compelling, many questions pertaining to human AKI remain unanswered. For example, the current study was retrospective, and pertinent patient data were missing. It remains unclear how plasma MIOX relates to the onset of AKI, whether lower plasma MIOX in nonoliguric patients reflects a lower degree of injury, whether MIOX concentrations are specific to a type of renal injury, and whether measurement of urine MIOX excretion would be equally (or more) informative. Finally, MIOX should be compared to other traditional and emerging markers of AKI to determine whether its measurement has additive value.

In summary, MIOX is a promising, kidney-specific protein that may represent a coveted marker of AKI. It remains to be seen whether this intriguing enzyme can fulfill rigorous criteria for translation into clinical practice and whether further studies examining its biology will yield novel insights into potential therapeutic targets.

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