CHAPTER 27

Kidney Stress Biomarkers

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OBJECTIVES

This chapter will:

1. Demonstrate that Acute kidney stress is associated with activation of event cascades that result in excretion of IGFBP7 and TIMP2, stress biomarkers of AKI.
2. Reviews the literature to show stress biomarkers of AKI are able to detect patients at high risk for AKI and predict their long-term outcomes with high reliability and accuracy at least 12 hours before its clinical presentation.
3. Describes the two cutoffs defined for [TIMP-2]*[IGFBP7] – a high sensitivity cutoff at 0.3 and a high specificity cutoff at 2 (ng/mL)2/1,000.

Acute kidney injury (AKI) is a common complication of critical illnesses, and it is associated with significantly high mortality, morbidity, and healthcare cost.[1–3] Incidence of AKI has been reported between 20% and 67% among ward and intensive care unit (ICU) patients.[4–6] Despite the growing knowledge regarding the impact of AKI on the outcomes of critically ill patients, the number of therapeutic options that have been tested in humans is limited, and the majority of them have been found to be ineffective.[7] Delay in the diagnosis of AKI contributes to this lack of success. Current functional biomarkers of AKI, including serum creatinine levels, are often nonspecific and late in the detection of AKI. Often this delay results in missed opportunities to provide appropriate treatment while the therapeutic window is open. In addition, the inability to identify the site and intensity of injury by current biomarkers prevents investigators from testing novel therapeutic and preventive strategies in patient populations with specific causes and a varying range of AKI severity. Therefore improving the outcomes of AKI patients will require sensitive, cause-specific, and early biomarkers of kidney injury.[8]

In recent years, several sensitive biomarkers of AKI have been studied. Kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), urinary interleukin-18 (IL-18), and liver-type fatty acid binding protein (L-FABP) are among these new discoveries.[9–12]

These biomarkers are able to identify patients who will develop AKI within the next 12 to 24 hours based on serum creatinine levels. Despite this progress, a recent systematic review concluded that these biomarkers are effective only in a well-defined timed injury in the pediatric population. In adult patients with multiple comorbid conditions and nebulous time-course of injury, however, these biomarkers were found to be significantly less robust for early recognition of AKI.[13]

Recently, two novel biomarkers of kidney stress (insulin-like growth factor binding protein-7 [IGFBP7] and tissue inhibitor of metalloproteinase-2 [TIMP-2]) were validated in the clinical setting for prediction of AKI among critically ill patients.[14,15] This chapter reviews the physiology of kidney response to stress, the role of stress biomarkers in the pathophysiology of AKI, and then examines the current literature on their clinical applications.

STRESS RESPONSE

Stress refers to an environmental stimulus that disturbs homeostasis of the body and causes illness.[16] Upon disruption of physical equilibrium, the body responds by multi-organ systemic reactions. These responses cause a number of physiologic or pathologic changes involving intracellular signal transduction and gene regulation, with short- and long-term effects.[17] Vulnerability to the pathogenic effects of stress differs among individuals because of genetic factors and age at which the stress is experienced.[18] The body's response to the stress could be at the systemic, cellular, or organ (e.g., kidney) level.

Systemic Stress Response

Stress may prompt neuroendocrine reactions represented by the hypothalamic-pituitary-adrenocortical axis activity.[19,20]

During the stress process, the neuroendocrine alteration increases mobilization of the oxygen metabolism chain and promotes reactive oxygen species (ROS) generation.[21] Regardless of the type of stimuli, neuroendocrine stress responses have a direct impact on metabolism and energy production.

The immune system is affected greatly by these neuroendocrine responses, mostly manifested as increased susceptibility to a variety of viral infections.[22,23] Studies show that plasma interleukin 6 (IL-6) rises simultaneously with the elevation in plasma corticosterone. This observation may suggest the response by neuroendocrine systems would regulate the release of IL-6 into the plasma during stress.[24]

Cellular Stress Response

As a consequence of stress induced by environmental factors, a series of adaptations to cellular proteins, lipids, and DNA occurs, mainly through triggering specific signaling pathways. These pathways include the extracellular signal–regulated kinase (ERK), c-Jun amino-terminal kinase (JNK), and p38 mitogen-activated protein kinase (MAPK) signaling cascades, the phosphoinositide 3-kinase (PI3K)/Akt pathway, the nuclear factor (NF)-κB signaling system, p53 activation, and the heat shock response.[25] In general, the heat shock response and ERK, PI3K/Akt, and NF-κ B signaling pathways exert a prosurvival influence during oxidant injury, whereas activation of p53, JNK, and p38 more commonly is linked to apoptosis. The degree to which a given pathway is activated is highly dependent on the cell type, as well as the nature and duration of the stress.[21]

Cells have evolved an extensive reorganization of the gene expression program that can produce dynamic outcomes in response to stress. These gene expressions are tightly regulated and reversible, achieved by different molecular mechanisms that are highly dependent on the particular stress and the organism.[26] Depending on the nature of a specific stress and its severity, the amount of gene regulation may involve from 30% to 80% of all genes, with extremes of stress usually leading to greater changes in gene expression.[27,28] These stress responsive gene expressions encompass almost all general cell features, including metabolism regulation, mRNA synthesis, cell-type differentiation, cellular transport, and cytoskeleton organization.[29–32] The p53 tumor suppressor protein is one of the potent adaptive genes that has a robust transcriptional function. p53 activation plays a pivotal role in the cellular response to a range of environmental and intracellular stresses.[33–36] One of the biologic endpoints of p53 induction is growth arrest, which can be transient or permanent (senescence or differentiation). The other possible outcome of p53 activation is apoptosis.[37]

Individual variation in responses to stress result from interaction among genes, developmental plasticity, phenotypic flexibility, and the current environment.[38] A major part of personal variability may rest on variation in the genetic background.[39] Epigenetic changes also can constrain or limit immune responses in future generations based on each distinctive experience. Several studies also show that aging cells and organisms accumulate increased levels of oxidant-damaged nuclear DNA.[40,41] In addition, evidence suggests that flexibility in immune responses becomes constrained with age through the accumulation of memory cells at the cost of naive cells and decreased cellular functions involved in adaptive and innate immunity.[39]

Kidney Stress Response

The term "acute kidney stress" (AKS) is defined as the preinjury phase that leads to AKI.[42] After exposure to stress, depending on its intensity and type, the kidney reacts in several different ways. Mild stimulus, such as a brief episode of hypovolemia, does not generate significant stress at the cellular level in the kidney. In this case, after appropriate management of the underlying cause, kidney function recovers without any sequelae. When the intensity of stressor is higher but not injurious, cells enter a dormant phase by downregulating their metabolism, solute transport, and protein synthesis. In addition, they enter a cell cycle arrest phase to preserve their energy supplements.[43] When the stressor is more intense, cells remain in cell cycle arrest and develop a senescent phenotype, which can result in fibrosis.[44] Finally, when the stimuli are injurious, cells commence the necrosis, apoptosis, and autophagy cell death processes.[45]

The cell cycle arrest biomarkers IGFBP7 and TIMP-2 can quantify the stress imposed on the renal epithelial cells during critical illnesses and therefore can be used clinically to identify patients who have risk of AKI well before its clinical and laboratory manifestations.

Cell Cycle Arrest

The majority of renal tubular epithelial cells are in a quiescent phase during normal physiologic conditions (Gap 0 [G_0]).[46] After kidney stress, tubular cells start their cell cycle to replace any lost cells. Interphase is the first stage of the active cell cycle. In this phase, cells prepare themselves for the metaphase (mitosis). The interphase includes three distinct stages: gap 1 (G_1), synthesis (S), and finally gap 2 (G_2). During G_1 or the growth phase, cell biosynthesis increases significantly to prepare cells for doubling their chromatin content. Cells gather supplements required for replication of deoxyribonucleic acid (DNA) content, including proteins and organelles such as ribosomes and mitochondria. During the S phase, cell DNA content replicates. Before entering the mitosis phase, cells continue their growth within the G_2 phase. Mitosis is divided into four stages: condensation of chromatin to chromosomes (prophase); alignment of chromosomes at the equator of the cell (metaphase); splitting sister chromosomes to the opposite pole of the cell (anaphase); and finally formation of two daughter cells (telophase).[47–49]

Eukaryotic cells are able to perform a rigorous self-examination to ensure the fidelity of DNA content before and during replication. This process happens at cell cycle arrest checkpoints. There are at least three checkpoints that are well recognized in each cell cycle. The first checkpoint, also known as the restriction point, happens at G_1/S immediately before cells enter the S phase. Cyclin-dependent protein kinase (CDK) inhibitors, including P21, P16, and P53, halt the progression of the cell cycle from G_1 to S phase by inhibiting the CDK complexes (CyclD-CDK4 and CyclE-CDK2).[50–53] The second checkpoint is prior to the beginning of mitosis at G_2 phase. The inability to pass this checkpoint is a known promoter of fibrogenesis, which occurs during "maladaptive" recovery after AKI.[44] The final checkpoint is at the metaphase to evaluate the tension in bipolar attachments among chromosomes.

The outcome of self-evaluation of cells in checkpoints could be summarized as (1) transient arrest, repair, and return to cell cycle; (2) defective repair typically leading

to apoptosis or cell senescence; or (3) direct apoptosis when the damage is very severe.[54]

Cell Cycle Arrest and Acute Kidney Injury

AKI is a clinical syndrome that results from a combination of inflammatory injuries to endothelial and epithelial cells.[55] When stimuli damage DNA, the DDR (DNA damage response) network and P53 are activated to determine the ultimate fate of an injured cell.

In a cecal ligation and puncture (CLP) murine model, G_1 cell cycle arrest preceded the development of AKI.[56] Within 6 hours of insult, the number of cells in G_1 significantly increased, and those in S phase decreased. High G_1:S ratio subsided after 24 hours, and 72 hours later the number of cells in S phase increased. This was followed by clinical recovery of kidney function. Significant upregulation of the cell cycle arrest indicators (p53 and p21) during the first 24 hours, the S phase indicators (CDK) after the first 24 hours, and proliferation indicator (retinoblastoma) after 72 hours were noted in this study. In the renal arteries ischemic murine model, investigators found successive changes of injury (clusterin), G/S transition (proliferating cell nuclear antigen [PCNA]), and differentiation markers (vimentin) in the S_3 segment of proximal tubules.[57] In the cisplatin, ischemia-reperfusion, and ureteral obstruction models of AKI, rapid induction of p21 in proximal and distal tubular cells is demonstrated, which are involved heavily in the G_1/S cell cycle arrest.[58] The current evidence indicates that cell cycle arrest happens early during the process of AKI; therefore biomarkers of cell cycle arrest may be able to predict the impending development of AKI.

In early phases of AKI, ROS, pathogen-associated molecular pattern (PAMP) and damage-associated molecular pattern (DAMP) molecules induce initiation of the cell cycle in renal epithelial cells. Before completion of the cell cycle, these cells enter a temporary phase of arrest in the checkpoints, which are highly regulated by several factors including IGFBP7 and TIMP-2. Expression of p53 and p21 is directly induced by IGFBP7 and p27 by TIMP-2. The cell cycle promotion complexes CyclD-CDK4, and CyclE-CDK2 are directly blocked by p-proteins, which results in the initiation of a transient G_1/S cell cycle arrest.

The role of cell cycle arrest in the outcomes of AKI is not very well known. Although prolonged cell cycle arrest is associated with maladaptive repair of AKI, CKD, and senescence of tubular cells, in the early phases of kidney stress it plays a protective role. Cell cycle arrest can not only help cells avoid the disastrous consequences of entering cell division with damaged DNA but also assist in preserving energy during stress when there are insufficient bioenergetic resources. In a recent randomized trial using remote ischemic preconditioning to prevent AKI after cardiopulmonary bypass surgeries, patients within the intervention arm showed a significant postintervention, preoperation increase in the cell cycle arrest biomarkers. This group had a significantly lower incidence of AKI. Conversely, in the control group for whom there was no increase in the urinary level of stress biomarkers in the postintervention, preoperation period, authors found a significantly higher incidence of AKI.[59]

Kidney Stress Biomarkers

In this section, we describe characteristics of these two proteins and their relationship with the cell cycle arrest during AKI.

IGFBP7

IGFBP7 is a 27 kDa protein that is expressed in the renal epithelial cells.[60,61] p53 enhances IGFBP7 expression after DNA injury induced by retinoic acid, transforming growth factor-β (TGF-β), glucocorticoids, or ROS.[62–64] After expression, it regulates insulin-like growth factors, cell adhesion,[65] and cell repair. In the kidney, IGFBP7 is induced in microvasculature after ischemia[66] and is involved in cell senescence.[67–69]

In an in vitro experiment with human melanoma cell lines, recombinant IGFBP7 decreased cell proliferation and increased apoptosis.[70] In MCF-7 breast cancer cells, transfecting cells with IGFBP7-induced senescent phenotypes including decreased cell proliferation, increased the G_1/S cell cycle arrest cells, altered cell morphology, and increased senescence-associated beta-galactosidase (SA-β-gal) activity.[71]

Tissue Inhibitor of Metalloproteinase-2

TIMP-2 is a 21 kDa protein expressed in melanoma and renal tubular cells. TIMP-2 irreversibly inactivates metalloproteinases (MMP) by binding to their catalytic zinc cofactor.[72,73] TIMP-2 expression is induced by cytokines and chemokines and proliferation (βFGF and EGF) and differentiation (retinoic acid and NGF) factors.[74] TIMP-2 induces G1 cell cycle arrest by binding to human endothelial cells through integrin α3/β1 (ITG α3 β1).[75–79] TIMP-2 is induced by ROS, differentiation signals (retinoic acid), and proliferation signals (EGF). The involvement of TIMP-2 in AKI includes its role in the innate immunity, such as structural changes influencing leukocyte transmigration from the capillaries to areas of injury in the renal tubule,[80,81] changes in endothelial permeability,[82] and modulation of the inflammatory response,[83,84] apoptosis (cell death),[85,86] and finally loss of cell-cell adhesion and sloughing of tubular epithelial cells.[82,83,87] TIMP-2 decreases endothelial cell proliferation via a mechanism that is independent of MMP inhibition.[88]

Kidney Stress Biomarkers: Clinical Studies

Recently, IGFBP7 and TIMP-2 were validated as predictors of AKI in studies involving more than 2000 patients.[14,15] As the result, on September 5, 2014, the US Food and Drug Administration (FDA) approved [TIMP2]•[IGFBP7] for assessment of AKI risk in adult (≥21 years old) critically ill patients under the brand name NephroCheck.[89] NephroCheck showed the coefficient of variation of 10% at the 0.3 $(ng/mL)^2/1000$ cutoff, and its limit of quantitation was 0.002. Albumin, conjugated bilirubin, and methylene blue interfered with test results when their levels were higher than 1250 mg/L, 72 mg/L, and 0.49 mg/L, respectively. These biomarkers were found to be stable for 6 hours at room temperature, 24 hours refrigerated, and not affected by sample centrifugation temperature.[90] The technical aspects of the stress biomarkers clinical implementation and its cost have been discussed elsewhere.[91]

Discovery and Validation

The Sapphire trial included 522 high-risk patients who did not have AKI at the time of enrollment. After timed blood and urine sample collection, more than 340 proteins were identified and measured in these samples.[14] Among the

measured biomarkers, IGFBP7 and TIMP-2 exhibited superior performance in the early detection of AKI.[14] Subsequently, these biomarkers were validated in animal and human studies. In a study of 60 Sprague-Dawley rats undergoing CLP, area under the receiver operating characteristic curve (AUROC) of [TIMP2]•[IGFBP7] for moderate-to-severe AKI was 0.89 (95% CI, 0.80–0.98).[92] The human clinical validation phase involved a large-scale multicenter study of 728 patients from 35 medical centers in North America and Europe. These patients were critically ill adults more than 21 years of age who were admitted to the ICU. Patients with AKI stage II or III were excluded from the screening process. In comparison with previously known AKI biomarkers such as KIM-1 and NGAL, IGFBP7 and TIMP-2 performed better in the early detection of AKI. It also was noted that IGFBP7 performed better in surgical patients, whereas TIMP-2 had a superior performance in patients with sepsis. The product of these two markers ([TIMP-2]•[IGFBP7]) was selected as a biomarker panel for AKI risk stratification.[14]

In the follow-up validation study (Topaz trial), investigators enrolled 420 patients in 23 centers in the United States within the first 24 hours of ICU admission. Investigators excluded patients with AKI stage II or III. A panel of three independent clinical experts adjudicated AKI within 12 hours of enrollment. Investigators used 0.3 $(ng/mL)^2/1000$ for the [TIMP-2]•[IGFBP7] cutoff. In this study, the performance of a clinical model to predict AKI (AUC 0.70 [95% CI, 0.63–0.76]) significantly improved when the urinary [TIMP-2]•[IGFBP7] was added to the model (AUC 0.86 [95% CI, 0.80–0.90]).[15]

Cutoff Levels

Cutoffs for [TIMP-2]•[IGFBP7] for clinical use were validated in another follow-up investigation (Opal trial). In this study 154 patients from six sites in the United States were enrolled. Unlike the previous studies that used a central laboratory and ELISA, each site used the commercial platform NephroCheck (Astute Medical, Inc.) to measure the urinary [TIMP2]•[IGFBP7] levels locally. Eligibility criteria for enrollment were similar to the earlier studies. Two previously determined thresholds from the Sapphire trial (0.3 and 2 $[ng/mL]^2/1000$) were validated as the sensitivity and specificity cutoffs, respectively. Investigators found a sensitivity of 89% for 0.3 $(ng/mL)^2/1000$ and a specificity of 90% for 2 $(ng/mL)^2/1000$ as the cutoff. The lower cutoff could be used for screening and risk stratification processes, whereas the higher cutoff could be used to identify patients with the very high likelihood of developing AKI.[93] A subsequent analysis of data from the Sapphire trial revealed that these cutoffs were able to accurately predict 9-month death or dialysis in ICU patients developing AKI.[94]

Subgroups Analyses of Validation Studies

In a posthoc analysis of Sapphire trial data, among 375 postsurgical patients, the performance of [TIMP-2]•[IGFBP7] in prediction of postoperative AKI within 12 hours remained excellent (AUC 0.84, 95% CI, 0.76–0.90; $p < .0001$). In addition, these stress biomarkers improved the performance of AKI clinical models.[95] In a separate study of 107 postsurgical patients, the [TIMP-2]•[IGFBP7] with a cutoff of more than 0.3 $(ng/mL)^2/1000$ was found to have an AUC of 0.85 for the risk of any AKI, 0.83 for early use of dialysis, and 0.77 for 28-day mortality. The authors also showed that these biomarkers were the strongest predictor of AKI in a multivariate model.[96] In a similar analysis of Sapphire and Topaz study cohorts, AUC of [TIMP-2]•[IGFBP7] with a cutoff of more than 0.3 $(ng/mL)^2/1000$ among patients with chronic kidney disease and chronic heart failure were found to be 0.91 and 0.89, respectively.[97] In the same collated cohort, among 232 patients with sepsis the AUC of [TIMP-2]•[IGFBP7] with a cutoff of more than 0.3 $(ng/mL)^2/1000$ was 0.84 (0.73–0.92) and 0.85 (0.76–0.94) in patients with low and high nonrenal sequential organ failure assessment (SOFA) score subgroups, respectively.[98] In validation studies and follow-up subgroups analyses adding [TIMP-2]•[IGFBP7] to the clinical models of AKI prediction, improved their performances significantly.

Follow-Up Studies in Different Cohorts

Among patients who underwent cardiac surgery, serial urinary samples of [TIMP-2]•[IGFBP7] were found to be predictive of postoperative AKI. In this cohort of 50 post-cardiac surgery patients, 52% developed AKI. The maximum urinary [TIMP-2]•[IGFBP7] concentration in the first 24 hours after cardiopulmonary bypass was predictive of AKI with an AUC of 0.84, sensitivity of 92% and specificity of 81% at a cutoff of 0.5 $(ng/mL)^2/1000$.[99]

In a cohort of 298 emergency room patients, one measurement of [TIMP-2]•[IGFBP7] was predictive of AKI development in the hospital, and patients with [TIMP-2]•[IGFBP7] more than 2 $(ng/mL)^2/1000$ compared with less than 0.3 $(ng/mL)^2/1000$ had an odds ratio of 2.5 to develop AKI. As had been observed in previous studies, in this cohort adding the stress biomarkers improved performance of the clinical AKI prediction model (AUC 0.67 [95% CI, 0.61 to 0.78] to 0.77 [95% CI, 0.72–0.86] for clinical model vs. clinical model with added [TIMP-2]•[IGFBP7], respectively [$p = .001$]).[100]

In the pediatric age group with AKI, the [TIMP-2]•[IGFBP7] was found to have an AUC of 0.79 (95% CI, 0.61–0.97), 0.84 (95% CI, 0.67–0.99), and 0.67 (95% CI, 0.50–0.84) for prediction of 30-day and 3-month mortality and dialysis, respectively.[101]

In a study of 56 postkidney transplant patients, TIMP-2 significantly enhanced the delayed graft function prediction when it was measured 4 and 12 hours after the operation.[102]

Although in the overwhelming majority of the studies the performance of these biomarkers in prediction of AKI was found to be excellent, a few investigations indicated the need for cautious use of these biomarkers in the patients who were stratified clinically in the low AKI risk striae.[103,104]

KIDNEY STRESS BIOMARKERS: CLINICAL APPLICATIONS AND SUMMARY

The kidney stress biomarkers, as outlined above, are able to identify adult (≥21 years old) critically ill patients at risk to develop AKI within the next 12 hours. These biomarkers, with a more than 0.3 $(ng/mL)^2/1000$ cutoff, are approved by the FDA for such indication. This cutoff provides sensitivity of 92% and specificity of 46% (95% CI, 41%–52%). Therefore the negative test result (≤0.3 $[ng/mL]^2/1000$) has a very high predictive value of being low risk for AKI. By using the test in the patient population that has higher pretest probability of AKI (higher incidence of AKI), clinicians can expect improvement in the performance of the

test positive predictive value. In addition, this test should be used in conjunction with functional biomarkers of AKI (serum creatinine, cystatin C, urine output) to assist with clinical decision-making trees (Figs. 27.1 and 27.2). Based on the Opal trial results, the cutoff of more than 2 (ng/mL)^2/1000 could be used to identify patients who have a very significant chance of moderate to severe AKI mainly to be steered toward more invasive/expensive AKI therapeutic or investigative interventions.[93]

The impact of interventions based on kidney stress biomarkers on clinical outcomes are not very well described. There are, however, studies to test hypotheses that using kidney stress biomarker-guided interventions can affect patient outcomes. In one of these studies (http://apps.who.int/trialsearch/; DRKS00006139), authors included patients with [TIMP-2]×•[IGFBP7] ≥ 0.3 (ng/mL)^2/1000 in a randomized clinical trial to evaluate the impact of standard of care versus goal-directed management of postcardiac surgery patients on the incidence of AKI within 3 postoperative days. The results of this trial along with similar studies can delineate how these markers could be used in the clinical setting in the near future.

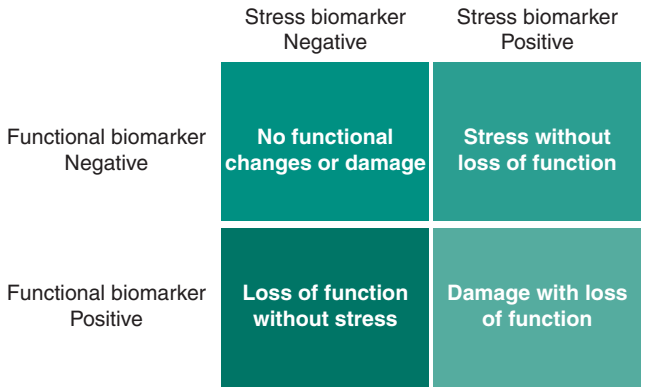

FIGURE 27.1 Incorporating stress biomarkers in conjunction with functional biomarkers. (Modified from Murray PT, et al. Potential use of biomarkers in acute kidney injury: report and summary of recommendations from the 10th Acute Dialysis Quality Initiative consensus conference. *Kidney Int.* 2014;85[3]:513–521.)

SUMMARY

In conclusion, AKI is a deadly syndrome that affects millions of patients around the world. Damaged renal epithelial cells enter the cell cycle shortly after injury. There is a cell cycle arrest immediately before S phase, and IGFBP7 and TIMP-2 are among the mediators of this process. These two proteins are sensitive and specific markers for the prediction of AKI and have now been validated in several large-scale investigations. [TIMP-2]•[IGFBP7] is currently FDA approved for clinical use to allow accurate assessment of the risk of developing AKI.

Key Points

1. Acute kidney injury (AKI) is common among critically ill patients and is associated with higher mortality, morbidity, and cost.
2. Early diagnosis of AKI and its causes can assist clinicians to provide appropriate preventative measures and allows investigators to test novel therapeutic interventions for this lethal syndrome.
3. Acute kidney stress is associated with activation of event cascades that result in excretion of IGFBP7 and TIMP-2, stress biomarkers of AKI.
4. Stress biomarkers of AKI are able to detect patients at high risk for AKI and predict their long-term outcomes with high reliability and accuracy.

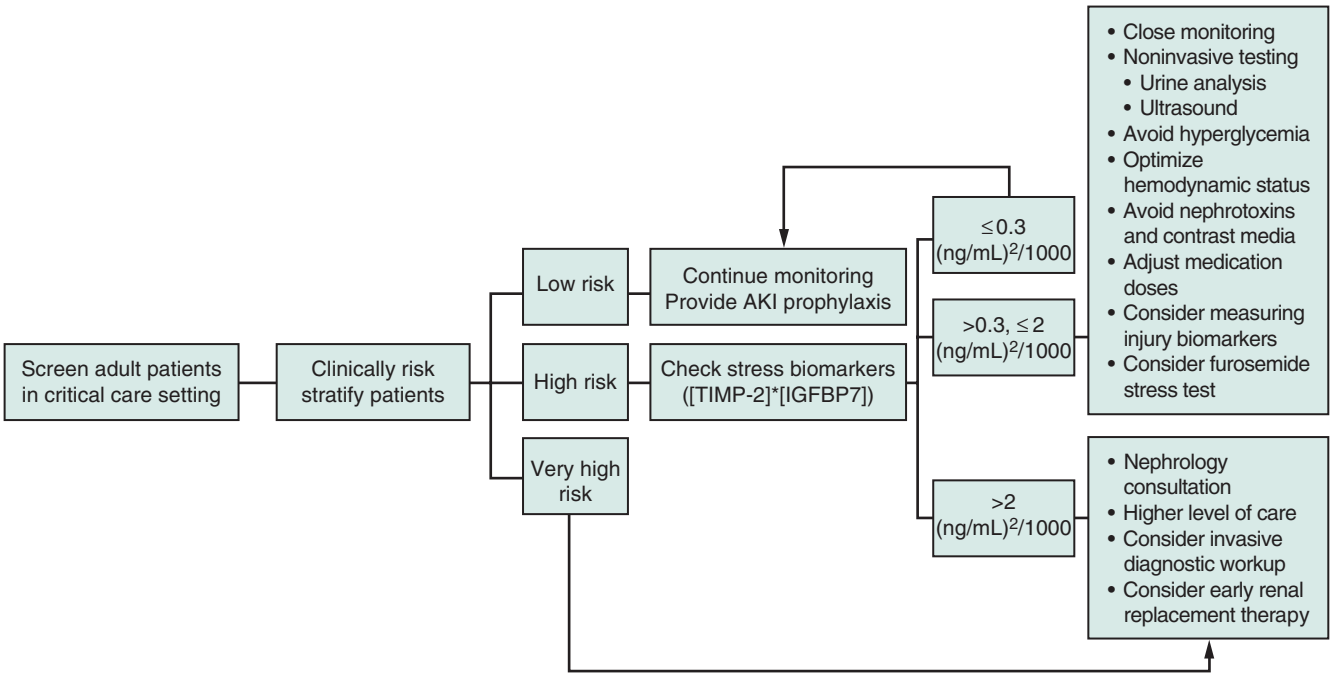

FIGURE 27.2 Clinical decision tree using cell cycle arrest biomarkers.

5. Studies to evaluate AKI preventive and therapeutic measures based on kidney stress biomarkers are ongoing.
6. Kidney stress biomarkers (i.e., cell cycle arrest biomarkers) can predict moderate to severe AKI at least 12 hours before its clinical presentation.
7. There are two cutoffs defined for [TIMP-2]•[IGFBP7]: a high sensitivity cutoff at 0.3 and a high-specificity cutoff at 2 $(ng/mL)^2/1000$.

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