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The cell cycle biomarkers: promising research, but do not oversell them

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Abstract

This review focuses on the most recent scientific and clinical information on the development and clinical applicability of the cell cycle biomarkers TIMP-2 and IGFBP-7 in the diagnosis and prognosis of patients at risk for and suffering from acute kidney injury (AKI). A number of evaluation studies have demonstrated that compared with existing biomarkers, urinary excretion of the product of both biomarkers, [TIMP-2]•[IGFBP-7], improved diagnostic performance in assessing the risk for AKI, predicting the need for renal replacement therapy, AKI-related complications and short- and long-term prognoses. The reference intervals for these biomarkers, measured by the recently approved NephroCheck test, have been determined in apparently healthy adults and those with stable chronic morbid conditions without AKI. This review recognizes that the combination of these two cell cycle arrest markers for the early detection of AKI is promising but concludes that its clinical impact is still unproved. Clinicians should understand the utility and limitations of this test before deciding whether to make it available at their institution.

Key words: AKI, biomarkers, creatinine, glomerulosclerosis, prognosis

Introduction

During the past decade, the use of consensus definitions of acute kidney injury (AKI), including RIFLE (Risk, Injury, Failure, Loss, ESRD), AKIN (Acute Kidney Injury Network) and KDIGO (Kidney Disease: Improving Global Outcomes), has unified most of the recent epidemiological studies on AKI [1–3].

The KDIGO criteria stage patients according to changes in serum creatinine (SCr) and urine output rather than changes in glomerular filtration rate (GFR). Both SCr and urine output criteria are important predictors, and the use of the KDIGO definition without assessment of urine output underestimates the incidence and grade of AKI and can delay diagnosis [4–7]. In addition, an association between episodes of oliguria and greater risks of worsening AKI, need for renal replacement therapy (RRT) and death has been established [5, 7–10].

However, numerous studies have omitted the urine output contribution in the definition of AKI [11]. It should further be realized that SCr often lags behind the actual occurrence of renal functional alterations or structural tubular or other intrarenal cellular injuries [12, 13].

Furthermore, SCr is also limited because its concentration is affected by factors such as muscle mass, intravascular volume, assay interference and drug interactions [14, 15]. Thus, changes in SCr levels do not accurately or consistently reflect real-time changes in renal function. Similarly, damage to the renal tubular epithelia may not be reflected in SCr levels or urine output until the damage progresses to a critical threshold.

Extensive effort has been expended over the past 15 years to identify and validate novel biomarkers that are more sensitive to the onset of injury, specific for prediction and have greater discrimination for injury severity.

Essentially, three types of novel biomarkers have been identified in the field of AKI. The first group is inflammatory biomarkers, including neutrophil gelatinase-associated lipocalin (NGAL) and pro-inflammatory cytokines such as interleukin-6 (IL-6) and IL-18. The second group includes cell injury biomarkers such as kidney injury molecule-1 (KIM-1), liver fatty acid-binding protein (L-FABP), sodium/hydrogen exchanger 3 (NHE-3) and netrin 1. The third recently identified group consists of cell cycle markers, such as urinary tissue inhibitor of metalloproteinase 2 (TIMP-2) and insulin-like growth factor-binding protein 7 (IGFBP-7). Ideally, these markers could facilitate, earlier and/or more accurately than is possible with SCr and urine output, the diagnosis and differential diagnosis of AKI [12, 16, 17] and could predict the need for RRT, AKI-related complications and short- and long-term prognoses [18]. Many recent in-depth reviews have summarized the characteristics, advantages and limitations of the first two groups of biomarkers [18–20].

This review focuses on the more recently developed cell cycle markers.

Biological role of the cell cycle biomarkers

Each phase of the cell cycle has a specific function that is required for appropriate cell proliferation. Quiescent cells are normally in G0. For cells to divide and begin the process of repair, they must enter and exit each phase of the cell cycle on schedule [21–23]. Cyclins, cyclin-dependent kinases and cyclin-dependent kinase inhibitors control each phase of the cell cycle [22]. If the cell exits a phase too soon or stays in a phase too long, the normal repair and recovery process can become maladaptive [22]. Exit from the cell cycle in late G1 leads to apoptosis [24]. Failure to achieve G1 cell cycle arrest can lead to an increased proportion of renal tubular cells in the G2/M phase, producing profibrotic growth factors that are capable of stimulating fibroblast proliferation and collagen production. In the animal model, this process is correlated with lasting kidney damage, including extensive glomerulosclerosis and interstitial fibrosis [23].

On the other hand, G1 cell cycle arrest may prevent the division of cells with damaged DNA until the DNA damage is repaired [25]. Thus the cell uses cell cycle arrest as a protective mechanism whereby cell division is avoided during stress and injury. However, if the cells do not reinitiate the cell cycle and remain arrested at G1 or G2 (or possibly other phases of the cell cycle), this sustained cell cycle arrest will result in a senescent cell phenotype and lead to fibrosis [26], and a hypertrophic and fibrotic phenotype can ensue (for recent excellent reviews see [26, 27]).

The cell cycle biomarkers have been shown to be involved in a diverse array of biologic processes, including cell cycle arrest.

In the setting of cell cycle arrest, these proteins signal activation of the p-protein cascade (p53, p21 and p27), which in turn blocks the effect of the cyclin-dependent protein kinase complexes [28–32].

The complexities of these pathways have been studied in the setting of AKI/cisplatin cytotoxicity, where p21 is known to inhibit cyclin-dependent protein kinase-2 and phosphorylation of p21 alters the cellular response to this toxin [33–35].

Additionally, TIMP-2 has been shown to be an important component in the pathophysiology of ischaemia-reperfusion injury, where various subtypes of TIMP proteins may play different roles in the kidney. Wang *et al.* [36] have shown that each TIMP subtype has a distinct role in renal injury. While TIMP-3 is required for optimal response to renal injury, TIMP-2 appears to promote disease progression through the activation of matrix metalloproteinase and triggering of tubulointerstitial fibrosis and injury. Similarly, research studies in renal transplant allografts show that matrix metalloproteinase activity is important in mediating scarring in chronic allograft nephropathy [37].

IGFBP-7 is a secreted protein that is a member of the IGFBP superfamily, also known as IGFBP-related proteins. IGFs exhibit pleiotropic effects in development and disease, and IGFBP-7 regulates the bioavailability of IGFs through direct low-affinity binding [38]. Several IGF-independent effects, such as tumor suppression in melanoma and colon cancer and induction of cell senescence in breast cancer lines, are also ascribed to IGFBP-7 [31, 32].

AKI has increasingly been recognized as a major contributor to chronic and end-stage kidney disease, and the appearance of tubulointerstitial fibrosis during the repair phase after either ischaemic or toxic injury is a poor prognostic indicator and represents a common final pathway in the progression to end-stage renal disease. By detecting cell cycle arrest markers in the urine, one may actually be detecting cell stress. This stress may or may not lead to damage and functional decline, but it is the earliest possible point in the process that can be detected [39].

Upregulation of TIMP-2 and IGFBP-7 in patients with AKI has been proposed to reflect their growth inhibitory functions [40]. Although there are suggestive arguments for a direct role of both TIMP-2 and IGFBP-7 in the cell cycle arrest mechanisms and IGFBP-7 and TIMP-2 are expressed in the tubular cells as a response to DNA and possibly other forms of damage, their exact biological role in AKI beyond their utility as biomarkers is still not fully understood since both proteins are capable of inducing a wide variety of cellular responses. As stressed by Vijayan *et al.* [41], there is much that remains poorly understood regarding the biological role for these proteins in AKI beyond their utility as biomarkers. Their presumed role as inducers of G1 cell cycle arrest in the kidney remains speculative, given that these proteins are capable of inducing a wide variety of cellular responses.

Clinical studies with cell cycle biomarkers

The first major study on the role of the cell cycle markers in AKI was the international Sapphire study, in which the ability of 340 proteins, including known AKI biomarkers, to predict development of AKI was tested in a heterogeneous group of critically ill intensive care unit (ICU) patients [42]. The identified proteins represented multiple biological pathways believed to be important in the pathogenesis of AKI. The report of this study included two multicentre studies, a discovery phase and a validation phase. In the discovery phase, 522 adults were enrolled in three distinct cohorts including patients with sepsis, shock, major surgery and trauma. In the validation phase, the final analysis cohort contained 728 critically ill patients. The primary endpoint was moderate–severe AKI (KDIGO stage 2–3) [1] within 12 h of sample collection. In the Sapphire study, the product of the two urinary biomarkers, [TIMP-2]•[IGFBP-7], provided an area under the curve (AUC) of 0.80 [95% confidence interval (CI) 0.74–0.84] for severe AKI (*n* = 101; 14% of the total) in the validation cohort, with individual biomarkers providing an AUC of 0.79 and 0.76, respectively. [TIMP-2]•[IGFBP-7] was significantly superior to all previously described markers of AKI (P < 0.002), including NGAL and KIM-1, none of which achieved an AUC >0.72 [42], when also assessed in a heterogeneous population.

A primary clinical cut-off value (0.3 ng/mL^2/1000) for the combination of the two biomarkers was derived from the Sapphire study data and verified in a new cohort of 153 critically ill patients (Opal study) [43]. This cut-off was selected to have high sensitivity for the primary endpoint of moderate–severe AKI in the next 12 h, with the intent of use in routine clinical practice to identify patients at high risk for AKI. It is hoped that these patients could be candidates for so-called kidney-sparing management strategies such as those outlined in the KDIGO guideline for high-risk patients [1]. A second, high-specificity cut-off (2.0 ng/mL^2/1000) was selected and verified to identify the subgroup of patients who are at the highest risk of AKI and who therefore might be appropriate for more active interventions. The Opal study implemented cut-off values for [TIMP-2]•[IGFBP-7], with a high-sensitivity cut-off of 0.3 ng/mL^2/1000 providing a sensitivity of 95% (95% CI 85–98%) and a specificity of 46% (95% CI 41–52%), whereas a high-specificity cut-off of 2.0 ng/mL^2/1000 provided a sensitivity of 37% (95% CI 26–47%) and a specificity of 95% (95% CI 93–97%).

Both cut-offs (0.3 and 2.0) were subsequently validated in a 23-site study of 408 critically ill patients in the USA (Topaz study) using clinical adjudication to determine the primary endpoint of moderate–severe AKI. The high-sensitivity cut-off value of 0.3 ng/mL^2/1000 was tested against a clinical model containing several clinical covariates, while three clinical nephrologists who were blinded to the biomarker findings were asked to determine whether moderate to severe AKI was reached for each patient [44]. Critically ill patients with urinary [TIMP-2]•[IGFBP-7] levels >0.3 ng/mL^2/1000 had 7 times the risk for AKI (95% CI 4–22) compared with critically ill patients with a test result <0.3. In multivariate analysis, urinary [TIMP-2]•[IGFBP-7] remained a statistically significant and strong predictor of AKI when combined with the clinical model. The AUC of the combined urinary [TIMP-2]•[IGFBP-7] test and clinical model of 0.86 (95% CI 0.80–0.90) was significantly (P < 0.001) improved compared with the clinical model alone [AUC 0.70 (95% CI 0.63–0.76)]. It is striking, however, that great overlap exists in the lower-range values. Only in values >2.0 is no major overlap present.

The association of urinary TIMP-2 and IGFBP-7 with long-term outcomes was recently explored by evaluation of the 9-month incidence of a composite endpoint of all-cause mortality or the need for RRT in a secondary analysis of the Sapphire study in critically ill adults [45]. Again, the same two predefined [TIMP-2]•[IGFBP-7] cut-offs (0.3 for high sensitivity and 2.0 for high specificity) for the development of AKI were evaluated. Cox proportional hazards models were used to determine risk for the composite endpoint. Baseline [TIMP-2]•[IGFBP-7] values were available for 692 subjects, of whom 382 (55.2%) developed stage 1 AKI as defined by the KDIGO guidelines [1] within 72 h of enrolment and 217 (31.4%) met the composite endpoint. Univariate analysis showed that a [TIMP-2]•[IGFBP-7] value of 2.0 was associated with increased risk of the composite endpoint [hazard ratio (HR) 2.11 (95% CI 1.37–3.23), P < 0.001]. In a multivariate analysis adjusted for clinical variables, [TIMP-2]•[IGFBP-7] levels >0.3 were associated with death or RRT only in subjects who developed AKI. It was concluded that [TIMP-2]•[IGFBP-7] measured early in the setting of critical illness may identify patients with AKI at increased risk for mortality or receipt of RRT over the next 9 months.

Although moderate to severe AKI on ICU admission was mentioned as an exclusion criterion in the Sapphire study [42], 32 of 101 (31.7%) had already developed KDIGO stage 2 or 3 at the time of urine sampling. Moreover, baseline creatinine was significantly higher in patients who would develop AKI within 12 h.

In addition, a study of 50 patients at high risk of AKI following cardiac surgery found that maximum urinary [TIMP-2]•[IGFBP-7] concentrations in the 24 h postoperatively were a sensitive and specific predictor of AKI [46]. Of the 50 patients included in the study, 26 (52%) developed AKI. Diagnosis based on SCr and/or oliguria did not occur until 1–3 days after cardiopulmonary bypass (CPB). In contrast, the urine concentration of [TIMP-2]•[IGFBP-7] rose from a mean of 0.49 (SE 0.24) at baseline to 1.51 (SE 0.57) 4 h after CPB in patients who developed AKI. The maximum urinary [TIMP-2]•[IGFBP-7] concentration achieved in the first 24 h following surgery (composite time point) demonstrated an AUC of 0.84. Sensitivity was 0.92 and specificity was 0.81 for a cut-off value of 0.50. The decline in urinary [TIMP-2]•[IGFBP-7] values was the strongest predictor for renal recovery. This small study suggests that urinary [TIMP-2]•[IGFBP-7] may serve as a sensitive and specific biomarker to predict AKI early after cardiac surgery and to predict renal recovery.

Gocze et al. [47] prospectively measured urinary [TIMP-2]•[IGFBP-7] excretion in 107 high-risk non-cardiac surgical patients. Forty-five of them (42%) developed AKI. A predefined cut-off value of [TIMP-2]•[IGFBP-7] >0.3 was used for assessing diagnostic accuracy. The highest median values of biomarker were detected in septic, post-transplant and post-hepatic surgery patients (1.24 versus 0.45 versus 0.47 ng/mL^2/1000). The AUC for the risk of any AKI was 0.85, for early use of RRT 0.83 and for 28-day mortality 0.77. In a multivariable model with established perioperative risk factors, the [TIMP-2]•[IGFBP-7] test was the strongest predictor of AKI and significantly improved the risk assessment.

Another recent study used the cell cycle arrest biomarkers to predict the development of moderate–severe AKI in a large cohort of heterogeneous, critically ill, postoperative surgical patients [48]. As was found in the Topaz and Opal studies, when the pre-specified cut-off [TIMP-2]•[IGFBP-7] value of 0.3 was used, the sensitivity of the test was 89% (95% CI 77–97), with an accompanying specificity of 49% (95% CI 43–54), a positive likelihood ratio of 1.72 (95% CI 1.44–2.01) and a negative likelihood ratio of 0.24 (95% CI 0.06–0.49). Patients with urinary [TIMP-2]•[IGFBP-7] test values >0.3 had >6 times the risk for AKI compared with those with a test value at or below the 0.3 cut-off. However, a rather large overlap in biomarker values between AKI and non-AKI patients was found.

Finally, the prognostic value of urinary TIMP-2 and IGFBP-7 in neonatal and paediatric AKI for adverse outcome was recently investigated [49]. The urinary concentration of [TIMP-2]•[IGFBP-7] was assessed by the NephroCheck immunoassay (see below) in a prospective cohort of 133 subjects ages 0–18 years, including 46 patients with established AKI according to the pRIFLE criteria, 27 patients without AKI (non-AKI Group I) and 60 apparently healthy neonates and children (non-AKI Group II). After classification into the pRIFLE criteria, 6/46 (13%) patients fulfilled the criteria for the category 'Risk', 13/46 (28%) for 'Injury', 26/46 (57%) for 'Failure' and 1/46 (2%) for 'Loss'. Patients in the 'Failure' stage had a median 3.7-fold higher urinary [TIMP-2]•[IGFBP-7] compared with non-AKI subjects (P < 0.001). Receiver operating characteristic curve analyses in the AKI group revealed good performance of [TIMP-2]•[IGFBP-7] in predicting 30-day [AUC 0.79 (95% CI 0.61–0.97)] and 3-month mortality [AUC 0.84 (95% CI 0.67–0.99)] and moderate performance in predicting RRT [AUC 0.67 (95% CI 0.50–0.84)].

In 2014, the US Food and Drug Administration (FDA) approved a point-of-care urinary biomarker assay, NephroCheck (Astute Medical, San Diego, CA, USA), for predicting risk of AKI. This in vitro test quantitatively measures [TIMP-2]•[IGFBP-7] in human urine on the ASTUTE140®Meter, a bench/table-top analyzer.

Recently, the reference intervals for these biomarkers, measured by the NephroCheck test, were determined in apparently healthy adults and those with stable chronic morbid conditions without AKI [50]. The reference intervals (inner 95%) for [TIMP-2]•[IGFBP-7] in all subjects ($n = 750$), apparently healthy subjects ($n = 378$) and subjects with stable chronic morbidities ($n = 372$) were 0.04–2.22, 0.04–2.25 and 0.05–2.20 ng/mL^2/1000, respectively. There was no statistical difference between reference intervals for the apparently healthy and stable chronic morbid cohorts ($P = 0.42$). It is important to mention that in the subjects with stable chronic comorbidities, patients with stable chronic kidney disease (CKD) were included. Unfortunately, the degree of CKD and the absolute number of CKD subjects are not detailed. The authors conclude that urine [TIMP-2]•[IGFBP-7] values are not elevated in patients with stable chronic morbidities who did not have AKI. However, as recognized by the authors, the reference intervals in apparently healthy and stable chronic morbid subjects overlap with the values obtained from AKI patients [50]. Reference interval data show that overall 50% of the population has values >0.33 and the rest of the [TIMP-2]•[IGFBP-7] values are ≤0.3. As such, the [TIMP-2]•[IGFBP-7] values obtained in critically ill patients should only be used in conjunction with patient condition and clinical signs and symptoms. The authors stress that this test was developed to assess the risk of AKI and was not intended as a sole indicator for the diagnosis of AKI. Furthermore, in the stable chronic disease patients, a number of patients suffering from active cancer were included; also, the test population in the Sapphire and Topaz studies included 25 and 27% cancer patients, respectively. As already outlined above, TIMP-2 can, in some instances, promote cellular proliferation [30] and is strongly expressed in renal cell carcinoma, indicating that renal epithelia are capable of expressing it [51]. It could be of interest to analyse separately the results of the cell cycle markers in cancer patients with and without AKI.

Finally, a recent study from Bell *et al.* [52] found that in general ICU patients, the NephroCheck point-of-care analyzer readings of [TIMP-2]•[IGFBP-7] or measurement of the previously suggested urinary biomarkers NGAL and cystatin C did not predict AKI within 12–48 h. Biomarker values were significantly affected by comorbidities even in the absence of AKI. These findings challenge the robustness and utility of cell cycle arrest biomarkers for the prediction of AKI in general ICU patients and also suggest that their performance may decrease markedly in general ICU patients with heterogeneous diagnoses, differing comorbidities and multiple sources of inflammation [52].

Recommendations of the American Society of Nephrology AKI Advisory Group

Very recently, members of the American Society of Nephrology the AKI Advisory Group, some of whom participated in the clinical studies with cell cycle biomarkers, published interesting and carefully worded recommendations on the use of these biomarkers in clinical practice [41]. The advisory group emphasizes that the NephroCheck test should only be used in ICU patients >21 years of age with cardiovascular and/or respiratory compromise within the prior 24 h. The test is 'an aid in the risk assessment for moderate or severe AKI'. The group also reminded that the FDA letter states that 'test results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods'. The FDA decision summary clarifies that [TIMP-2]•[IGFBP-7] is not a standalone test and should not be used as point-of-care testing.

It is further suggested that the combination of urine sediment score and a positive [TIMP-2]•[IGFBP-7] result could potentially have higher sensitivity and specificity compared with the individual biomarker results, as was previously shown, as well as with urine NGAL, KIM-1 and IL-18 levels [53–55].

Finally, Vijayan *et al.* [41] correctly stated that the cost benefit of [TIMP-2]•[IGFBP-7] testing is unknown. If early recognition and treatment leads to reduced severity of AKI, a favourable cost–benefit ratio of [TIMP-2]•[IGFBP-7] might be anticipated, but currently there are no data to support the premise that early recognition of kidney injury with [TIMP-2]•[IGFBP-7] or other AKI biomarkers will prevent the progression of AKI or be associated with a cost benefit to the patient or institution. The relatively low specificity of the biomarker tests leading to false-positive results may lead to unnecessary and expensive diagnostic and therapeutic evaluations.

Conclusions

The combination of two cell cycle arrest markers (TIMP-2 and IGFBP-7) for the early detection of AKI is promising, but its clinical impact is still unproven. We agree with Prowle *et al.* [56] that finding a statistical association of any biomarker with the development of AKI is relatively easy, but demonstrating that such a biomarker meaningfully alters practice, let alone clinical outcomes, is much trickier [57]. AKI complicating critical illness is highly heterogeneous in severity, aetiology and timing [58], all of which may variably affect biomarker results. Thus, defining the appropriate timing and frequency of biomarker measurement and interpreting these results in individual patients are extremely difficult.

We also agree with the conclusions and recommendations of the American Society of Nephrology AKI Advisory Group that approval of the novel biomarker combination of [TIMP-2]•[IGFBP-7] is a positive step in the search for robust and accurate means of early diagnosis of AKI. However, clinicians should understand the utility and limitations of this test before deciding whether to make it available at their institution [41].

To translate this advancement in AKI biomarkers to meaningful improvement in clinical outcomes, standardization of care and early nephrology involvement will be important. We believe that for now careful clinical appraisal using close clinical haemodynamic monitoring of the patient, avoiding nephrotoxic exposures and regular follow-up of standard parameters such as SCr and diuresis remain the cornerstones in the approach to a patient at risk of AKI. Additional trials to study the role of cell cycle and other biomarkers in preventive strategies and interventional trials are needed to assess its effectiveness in improving outcomes in AKI.

Conflict of interest statement

None declared.

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