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Improving acute kidney injury diagnostic precision using biomarkers

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ABSTRACT

Acute kidney injury (AKI) is common in hospitalized patients of all ages and is associated with significant morbidity and mortality. Accurate prediction and early identification of AKI is of utmost importance because no therapy exists to mitigate AKI once it has occurred. Yet, serum creatinine lacks adequate sensitivity and specificity, and quantification of urine output is challenging in incontinent children without indwelling bladder catheters. Integration of clinically available biomarkers have the potential to delineate unique AKI phenotypes that could have important prognostic and therapeutic implications. Plasma Cystatin C, urine neutrophil gelatinase associated lipocalin (NGAL) and the urinary product of tissue inhibitor metalloproteinase (TIMP-2) and insulin growth factor binding protein-7 (IGFBP7) are clinically available. These biomarkers have been studied in heterogenous populations across the age spectrum and in a variety of clinical settings for prediction of AKI. The purpose of this review is to describe and discuss the clinically available AKI biomarkers including how they have been used to delineate AKI phenotypes.

1. Introduction

Acute kidney injury (AKI) is common in hospitalized patients of all ages and is associated with longer lengths of stay, increased hospital costs, and mortality [1–4]. Patients that survive AKI are at higher risk of chronic kidney disease (CKD) and other adverse outcomes including increased risk for infectious complications, cardiovascular dysfunction, and neurologic effects [5–8]. These systemic effects of AKI are only starting to be understood. Timely and accurate prediction of AKI is of utmost importance because no therapy exists to mitigate AKI once it has occurred, and most interventional trials for the prevention and/or treatment of AKI have failed. Recent detailed reviews and meta-analyses discussing completed and ongoing clinical trials for AKI prevention and treatment are beyond the scope of this review [9,10]. Herein, we seek to discuss clinically available AKI biomarkers and provide considerations for integration into clinical care.

1.1. AKI definition challenges

One of the challenges with improving AKI diagnostic precision lies in the definition. Multiple AKI definitions exist, but in the last decade, the Kidney Disease: Improving Global Outcomes (KDIGO) consensus AKI definition was published [11], including a neonatal (age< 30 days) modification [12], all of which standardize fixed percentage increases in serum creatinine (SCr) and decreases in urine

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output for AKI staging. Defining AKI by fixed percentage increases in SCr has the potential to overestimate AKI in patients with very low baseline SCr, which has been shown to dilute the detrimental clinical outcomes associated with AKI in adults and produce high false positive rates in patients with chronic kidney disease (CKD) and low renal reserve [13]. SCr levels are also most reliable when measured in steady state. Levels increase only after approximately 50% of nephrons are lost, making it a delayed marker of AKI. Extending beyond SCr, it is nearly impossible to precisely quantify urine output in incontinent children and adults without an indwelling catheter. The drawbacks of the current definition have led to the development of alternate definitions to better evaluate the association of AKI with outcomes. Xu and colleagues recently developed the pediatric reference change value optimized criterion for AKI in children (pROCK). This group used data from more than 150,000 children without AKI who had at least 2 SCr measures to develop a model for detecting AKI based on the reference change value of SCr using age and initial SCr level. This modeling demonstrated that a 20 μmol/L (0.23 mg/dL) or 30% increase from the initial SCr level [14] improved prediction of AKI in children, compared to the other existing definitions that generally lead to overdiagnosis [14]. pROCK seeks to improve the specificity of AKI diagnosis. However, given that SCr does not rise until 50% of kidney function is lost, a more sensitive definition allows early identification and monitoring whereas with a highly specific definition, the brief window in which to intervene might be lost.

1.2. Novel AKI biomarkers and delineating unique AKI phenotypes

Novel serum, plasma, and urinary AKI biomarkers have been discovered with the use of transcriptomic and proteomic technologies, and they have garnered excitement, although their adoption into routine clinical care has been slow. The reason for this is multifactorial, including availability of testing platforms, cost, variability in assay techniques and results, and lack of approval from national and international governance bodies. These challenges are discussed in further detail below.

Multiple AKI phenotypes exist, and more than one phenotype may exist within the same individual. AKI phenotypes may be defined by the timing, onset, duration and severity of AKI as well as nuanced patient and disease characteristics, specific exposures, or even response to specific medications. For example, quantification of urine output after an index dose of furosemide may yield actionable information on the evolution of AKI before a patient meets either SCr or urine output criteria. This may allow for measures that reduce AKI risk and alter AKI trajectory. Among adults with stage 1 and 2 AKI, 2-h urine output response to furosemide was able to predict

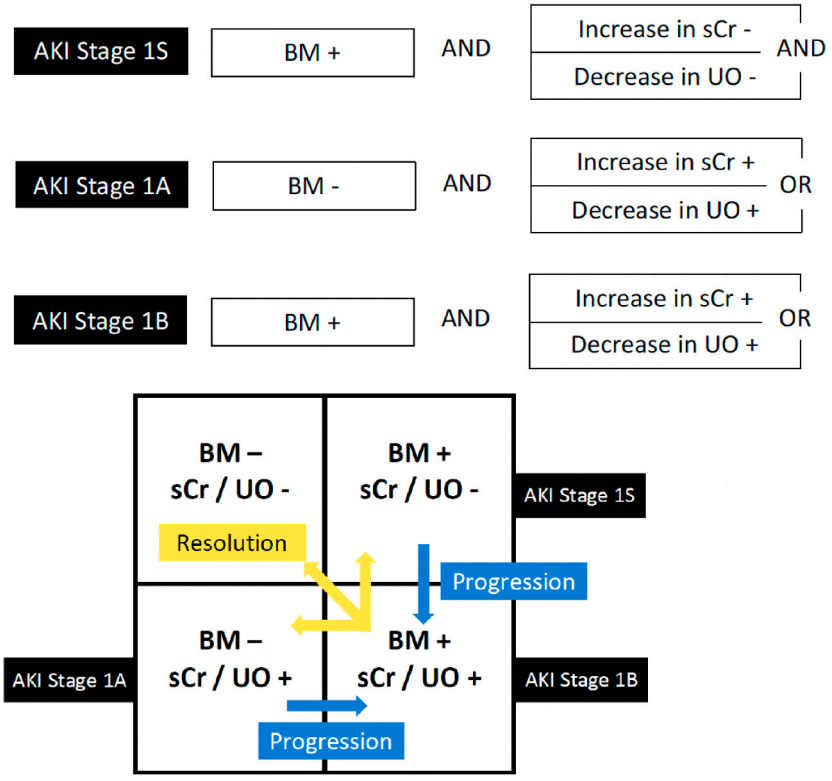

Fig. 1. Refined Staging System for the Diagnosis of Acute Kidney Injury Patients with a biomarker. Biomarker (BM) integration into the diagnosis of acute kidney injury (AKI). Biomarker positivity with or without increase or decrease in serum creatinine (sCr) level and not reaching urine output (UO) criteria should be classified as stage 1S. Reassessment should be performed according to patient clinical context and temporal trends. Patients reaching SCr and UO criteria with no increase on BM are defined as stage 1A, and those reaching SCr and UO criteria with increased BM are reclassified as stage 1B. BM positivity should be based on its mechanism and defined threshold. Reprinted from Acute Disease Quality Initiative 23 and used with permission [20].

progression to stage 3 AKI within 14 days with excellent sensitivity and specificity [15]. Among children, urine output in response to furosemide has been assessed after cardiac surgery [16,17], and in other critically ill states [18], where it was able to predict severe AKI with reasonable performance. Integration of novel urinary biomarkers into algorithms with the furosemide stress test in adults enhanced the prediction of subsequent AKI, which delineates a specific AKI phenotype in itself [19]. Whether a biomarker will delineate unique AKI phenotypes in children when evaluated with response to furosemide is unknown. Furthermore, whether the combination of testing will facilitate interventions need to be tested.

Combining an AKI biomarker with SCr or urine output was recently recommended by the 23rd Acute Disease Quality Initiative (ADQI) consensus conference [20]. Delineation of patients into separate phenotypes based on a positive or negative biomarker, and positive or negative SCr/urine output (Fig. 1) [20] may enhance diagnostic precision, allow us to prognosticate and allow for targeted interventions. Separating patients by biomarker and SCr positivity was recently described by Stanski and colleagues in a cohort of critically ill children [21]. They hypothesized that combining a novel urinary biomarker with SCr would delineate patients into 4 unique phenotypes based on pre-defined thresholds and would predict day 3 AKI severity. They found that biomarker combinations were predictive of day 3 AKI and mortality [21]. Other approaches for better delineating AKI phenotypes were recommended by the 10th and 16th ADQI conference [22,23]. The group recommended that the timing and persistence of AKI also be considered to improve diagnostic precision [22]. The remaining portion of this review will focus on 3 clinically available AKI biomarkers (Table 1) in the context of delineating unique AKI phenotypes: 1) plasma and urine cystatin C, 2) urinary neutrophil gelatinase associated lipocalin (NGAL) and 3) the urinary product of tissue inhibitor metalloproteinase-2 and insulin like growth factor binding protein-7 (TIMP-2*IGFBP7), marketed under the tradename Nephrocheck®.

2. Cystatin C

2.1. Structure and function

Cystatin C is a 13-kDa (kDa) cysteine proteinase inhibitor that is synthesized by all nucleated cells at a constant rate and constitutively released into the bloodstream [24]. It is freely filtered (>99%) in the glomerulus, then reabsorbed and catabolized in the proximal tubule. Because it does not undergo tubular secretion, it functions mainly as a marker of glomerular filtration [24,25]. Serum cystatin C is integrated into standardized equations for the assessment of CKD in adults and children [26,27]. While measurement of serum and plasma cystatin C has become more available in the United States for the assessment of CKD and AKI, there are several important limitations that are worth mentioning. Cystatin C can be affected, although to a lesser degree than SCr, by demographic factors (age, sex, race, and ethnicity), clinical factors (smoking status, hypertension, cholesterol levels, and elevated C-reactive protein, thyroid disease), and medications (steroids) [28].

2.2. Urine cystatin C

In general, among individuals with healthy kidneys, cystatin C should not be detected in the urine. Its presence in the urine indicates proximal tubular damage. Koyner and colleagues evaluated the utility of urine cystatin C for prediction of AKI among adults

Table 1
Summary of biomarkers.

	Specimen	Biomarker Type	Intended Use	Recommended Cut-off	Limitations
Creatinine	Serum	Functional	Diagnosis of AKI and CKD	Per KDIGO criteria, >0.3 mg/dL increase in 48 h or >1.5x increase from baseline in 7 days	Affected by age, race, muscle mass, fluid status, medications. Rises 24–48 h after injury and only after >50% nephrons are lost/injured.
Cystatin C	Plasma (or serum)	Functional	Used for estimation of GFR and medication dose adjustments in pediatric and adult patients. Has been used for AKI prediction in small cohorts. In large adult cohorts can predict worse outcomes.	No universally accepted cut-offs for CKD, as used in eGFR estimating equations; in pediatric patients post-CPB, maximum sens/spec for 12-hr AKI prediction was 1.16 mg/L	Levels may be different in different age groups. Lower accuracy in hypermetabolic states such as malignancy, uncontrolled thyroid disease, with steroid use. Higher CRP, WBC, lower serum albumin associated with higher levels.
TIMP-2*IGFBP7	Urine	Stress	In conjunction with clinical evaluation, in ICU patients 21+ years with acute cardiovascular/ respiratory compromise in the past 24 h, as an aid for risk assessment for moderate/severe AKI within 12hrs of assessment.	>0.3 for high sensitivity, acceptable specificity; not official, but >2.0 for high specificity according to research studies	Per package insert, should not be used as a standalone test. Interference with urine albumin >125 mg/dL, invalidation if > 3000 mg/dL, less accurate in patients with hyperbilirubinemia
NGAL	Urine	Damage	AKI prediction in high-risk patients. Future delineation of "subclinical AKI" or a cohort of patients with normal SCr but at risk of worse clinical outcomes.	No FDA-approved assay, levels will vary by lab. >150 ng/mL has high sensitivity. >580 ng/mL has high specificity in a large adult meta-analysis.	False elevations in sepsis, malignancy, COPD exacerbations, and especially with leukocyturia. Levels impacted by hyperbilirubinemia.

undergoing cardiac surgery [29]. They compared the performance of urine and plasma cystatin C for AKI prediction. While urine cystatin C increased in all patients, the magnitude of increase was greater for increasing severity of AKI. In this study, plasma cystatin C was not useful for prediction of AKI within the first 6 h following surgery. In a separate study from the "Translational Research Investigating Biomarker Endpoints in AKI" study (TRIBE-AKI), urine cystatin C was evaluated for predicting AKI in 1200 adults and 299 children within 12 h of cardiac surgery. Urine cystatin C, when measured early in the postoperative course correlated with mild and severe cases of AKI in the entire cohort, but after covariate adjustment, the effect was attenuated, and the performance of urine cystatin C was no better than the clinical model [30]. In other clinical settings, the performance of urine cystatin C has also been variable thus limiting its use as a true predictor of AKI [31–34]. It has been postulated that urine cystatin C performance in cardiac surgery outperforms other clinical states as it relates to the timing of injury. Furthermore, its role in tubular dysfunction may be better suited for a protracted courses of AKI where presentation from the inciting event, including exposure to nephrotoxins, may be delayed. Thus, urine cystatin C may allow identification of discrete AKI phenotypes related to the timing and persistence of injury, but further investigation is needed.

2.3. Blood cystatin C

The TRIBE-AKI collaborative also evaluated the performance of serum cystatin C for detection of AKI compared to SCr [30]. Serum cystatin C was overall less sensitive than SCr, and time to AKI detection also occurred earlier with SCr than cystatin C. When predefined thresholds of both markers were used, there was no difference between markers for predicting the timing of AKI onset. In this same study, phenotypic patterns of both cystatin C and SCr were assessed using pre-defined thresholds in a 2×2 table similar to that shown in Fig. 1. Patients with both cystatin C and SCr elevation incurred the greatest morbidity and mortality compared to elevation of either alone.

In children, Hassinger and colleagues demonstrated that serum cystatin C was predictive of AKI severity as early as 8 h after cardiac surgery. Interestingly, this group measured cystatin C during surgery, where concentrations were reduced after ultrafiltration suggesting that mechanical ultrafiltration resulted in removal of this protein [35]. This is relevant because it could impact the rate of change postoperatively and vary based on the degree of ultrafiltration used. Unfortunately, there is no standardized approach to ultrafiltration during cardiopulmonary bypass, which makes interpretation of cystatin C for AKI prediction in cardiac surgery difficult.

Given the dynamic process of AKI and its progression, biomarker integration may delineate patterns of recovery, which may have management and prognostic implications, including resource utilization. Gharaibeh and colleagues evaluated whether serum cystatin C could provide clinical guidance on AKI recovery in a cohort of adult patients with non-oliguric AKI [36]. Serum cystatin C declined before SCr in 68% of the population and was more likely to decline in those with a lower baseline SCr. Although extensive data is not shown, the authors compared kinetic glomerular filtration rate (KeGFR) to cystatin C. The addition of cystatin C to KeGFR for recovery was beneficial, although the biggest challenge for integration into routine care is the cumbersome calculation of KeGFR. This study evaluating cystatin C for AKI recovery was very small, and it is unknown whether the findings will be replicated in a larger cohort with substantial disease heterogeneity.

In other critically ill patients, serial assessments of cystatin C have been used to assess for sustained AKI defined as lasting at least 24 h in a general intensive care unit (ICU) population [37]. Cystatin C may also add value in predicting persistent AKI in the context of nephrotoxic medication exposures [38]. Unfortunately, these studies are small and need larger scale validation before clinical integration can occur. In the emergency department, Soto and colleagues evaluated the performance of both urine and blood cystatin C for predicting AKI and adjudicating SCr elevation as functional loss (pre-renal AKI) or related to tubular damage. Both serum cystatin C and SCr were able to discriminate AKI, but only cystatin C was predictive of AKI. Cystatin C was able to differentiate between pre-renal AKI and true tubular damage, but similar to SCr, was not able to discriminate between AKI and CKD. Indeed, additional biomarkers are needed to improve the specificity of community acquired AKI [39].

2.4. Limitations of cystatin C

Despite the robust data on cystatin C and the fact that it is approved for use by the food and drug administration (FDA), it has not fully been integrated into routine care in the inpatient setting or in the community. This likely stems from several factors: 1) despite the standardization of 2 assays, there continues to be significant discordance with as much as 20% bias reported in 2 studies [40,41], 2) the impact of demographic and clinical factors in quantification and interpretation, and 3) the varying cutoff values across age and clinical setting. Given the substantial heterogeneity of testing and results, the American Association of Clinical Chemistry (AACC), in their recent guidance document report of Laboratory Investigation of AKI did not recommend the measurement of cystatin C due to poor standardization, general lack of availability, and high cost [42].

3. TIMP-2*IGFBP7 (Nephrocheck®)

3.1. Structure and function

TIMP-2 and IGFBP7 are proteins expressed and secreted by renal tubular cells during cell stress or injury [43]. As with most cellular/tissue injury processes, it is believed there is a "pre-injury" timepoint when insult occurs prior to permanent damage and cell loss. In the heart, this is the difference between ischemia and infarct. In the kidney, this pre-injury phase has been termed "acute kidney stress," [44] and these two cell cycle arrest biomarkers provide mechanistic plausibility for this concept. IGFBP7 directly increases

expression of p53 and p21, and TIMP-2 stimulates p27 expression. These p proteins block cyclin-dependent protein kinase complexes responsible for cell cycle promotion, inducing G1 cell cycle arrest. Physiologically, this arrest is beneficial, allowing cells to repair DNA damage and regain function prior to replication; however, if sustained, cell cycle arrest will lead to fibrosis, increasing risk not only for AKI but also for later CKD [45,46]. Thus, these markers indicate that renal stress is occurring and needs to be ameliorated.

3.2. Current clinical uses

Urine TIMP-2*IGFBP7 received FDA and Europe, Middle East and Africa (EMEA) approval for the prediction of severe stage 2 or 3 AKI within 12 h of testing in critically ill patients >21 years with cardiac or respiratory failure under the tradename Nephrocheck.® It is measured on a stand-alone Astute 140 m that requires 1 mL of urine (although the package insert suggests that at least 10 mL be obtained). A high sensitivity/high negative predictive (NPV) value (>0.3 $(ng/mL)^2/1000$) and a high specificity/high positive predictive value (>2 $(ng/mL)^2/1000$) cutoff were derived for stratification of patients at risk for imminent severe AKI from the Sapphire study [47], where the sensitivity and NPV at the 0.3 $(ng/mL)^2/1000$ cutoff was 89% and 97% respectively. For the 2 $(ng/mL)^2/1000$ cutoff, the specificity was 95% and the PPV 49%. These values were subsequently validated in the Opal and Topaz studies [48,49].

The biggest application to date has been using these biomarkers to define an at-risk population that may respond to nephroprotective interventions. "Biomarker-guided Intervention to Prevent AKI After Major Surgery" (BigPAK) was a single-center randomized controlled trial (RCT) that sought to reduce postoperative AKI in adults after major noncardiac surgery using a biomarker-triggered KDIGO care bundle [50]. Patients were randomized to usual care or the care bundle if urine TIMP-2*IGFBP7 levels exceeded 0.3 $(ng/mL)^2/1000$. AKI incidence decreased from 48% to 27% from this intervention [50]. Another single center RCT, "Prevention of cardiac surgery-associated AKI by Implementing the KDIGO Guidelines in high risk patients identified by biomarkers" (PrevAKI) study randomized adult cardiac surgery patients with a urine TIMP-2*IGFBP7 level >0.3 $(ng/mL)^2/1000$ at 4 h after cardiac surgery to usual care or a KDIGO care bundle [51]. While there was a substantial reduction in AKI incidence in the intervention group, there was no difference in AKI-associated outcomes such as mortality and length of stay [51]. Similarly, a subsequent multicenter version of PrevAKI demonstrated a reduction in moderate to severe AKI in the intervention group without effect on other secondary outcomes [52].

Several studies have been performed in children, all in the post cardiac surgery population [53,54]. The findings are similarly heterogenous with regards to their findings and identification of cutoff points, thus making standardization and incorporation into pediatric AKI prediction in the clinical setting very challenging [53,55–58]. In their recent guidance document, the AACC stated that before TIMP-2*IGFBP7 can be recommended in children, age specific reference intervals are needed [42]. Data on the performance of TIMP-2*IGFBP7 in patients outside the ICU or outside the cardiac surgery setting are limited. TIMP-2*IGFBP7 has been assessed in the emergency department for subsequent AKI prediction, reflecting community acquired AKI. In a study by Kimmel and colleagues, TIMP-2*IGFBP2 and SCr had statistically significant ($P < 0.05$) odds ratios for the development of AKI as the end point in a multivariable model [59]. Addition of TIMP-2*IGFBP7 to a clinical model significantly improved area under the receiver-operating characteristic curve from 0.67 (95% CI, 0.61 to 0.78) to 0.77 (95% CI, 0.72 to 0.86) ($P < 0.001$). Yang and colleagues reported similar findings in a multicenter international study, that integration of TIMP-2*IGFBP7 with the Emergency department score improved AKI prediction [60]. TIMP-2*IGFBP7 has not been studied in the outpatient setting in patients at risk for AKI.

3.3. Phenotypic characterization using TIMP-2*IGFBP7

TIMP-2*IGFBP7 has been studied in the context of disease-specific phenotypes rather than the onset, timing, and duration of AKI. In a secondary analysis of the "Protocol-based Care for Early Septic Shock" (ProCESS trial), TIMP-2*IGFBP7 was able to delineate patients with sepsis who had differing risks for AKI progression after initial fluid resuscitation [61]. Another study evaluated the role of TIMP-2*IGFBP7 for predicting AKI in patients exposed to platinum-based chemotherapy [62], but unfortunately, these findings were not replicated in similar study by another group [63].

Daubin and colleagues studied the performance of TIMP-2*IGFBP7 to differentiate transient (≤5 days) from persistent (>5 days) AKI in critically ill adults admitted to a medical ICU [64]. They compared the difference between the 4 h and initial measurement and the delta between the 12- and 24-h measurements as well as creatinine AKI persistence. The investigators found that the "Nephrocheck score" decreased in the first 12 h in patients with transient AKI and increased or stayed the same in those with persistent AKI. While the delta was higher at the assessed time points for transient AKI, this difference did not meet statistical significance in differentiating transient from persistent AKI. Additional studies investigating the value of serial measurements in adults and children for delineating the AKI phenotype are needed. Further studies are also needed to identify whether TIMP-2*IGFBP7 combined with other biomarkers or clinical risk tools enhance clinical decision making, including decisions on when to start kidney replacement therapy as an example.

3.4. Limitations of TIMP-2*IGFBP7

While some studies support the findings from these derivation and validation studies, unfortunately, other studies have found negative results, including the contribution of comorbidities such as diabetes that attenuated the effect of the biomarker performance on AKI prediction [65]. In addition, while some studies have found an association between TIMP-2*IGFBP7 biomarker elevation and AKI, cutoff points are inconsistent, making this biomarker nearly impossible to integrate into the clinical space as clinicians would need to be familiar with interpreting the test in the context of the underlying disease state. Specifically, although the association between diabetes and poor biomarker performance is strong, there isn't a well understood mechanism for this, apart from the interference seen

with albuminuria (PMID 25866432). The package insert states that urine albumin concentrations >125 mg/dL interferes with the results, while concentrations >3 g/dL invalidate them. Given that albuminuria is present commonly not only in diabetic renal injury but in CKD of many etiologies, these biomarkers should be interpreted with caution in the setting of AKI on CKD. Urinary bilirubin also interferes with these results at concentrations >7.2 mg/dL (NEPHROCHECK package insert). For these reasons, the American Association for Clinical Chemistry (AACC) and the National Institute for Health and Care Excellence (NICE) have both published guidance documents that do not support its routine use because of lack of evidence on outcomes in these groups [42], NICE guidelines.

4. NGAL

4.1. Structure and function

NGAL is a protein that was originally identified in activated neutrophils. There are at least 3 different types in blood and urine: a monomeric 25 kDa form, produced by neutrophils and epithelial tissues; a homodimeric 45 kDa form produced by neutrophils; and a heterodimeric 135 kDa form covalently conjugated with gelatinase (MMP-9) and specific to neutrophils and tubular cells [20,66]. The monomeric form of NGAL is secreted by kidney epithelial cells during times of stress [67]. NGAL is constitutively expressed in multiple cells at a low rate, filtered in the glomerulus, and reabsorbed in the proximal convoluted tubule, leaving little in the urine in healthy individuals [68]. Kidney, liver, and other epithelial cells increase expression and production of NGAL in response to inflammation, infection, intoxication, ischemia, AKI, and neoplastic transformation [69]. The liver plays a critical role in NGAL production, as mice with hepatocyte-specific NGAL deletion and ischemic AKI had lower hepatic NGAL mRNA as well as lower plasma and urine NGAL. This effect is thought to be mediated by interleukin-6 (IL-6), which is increased in the plasma 2 h after an AKI event [70]. NGAL is also upregulated in the thick ascending limb of the loop of Henle, distal convoluted tubule, and collecting duct following injury, thus oftentimes referred to as a "damage biomarker," but this upregulation may be because of potential nephroprotective effects. NGAL binds bacterial siderophores, acting as a potent bacteriostatic and antioxidant agent in iron-limiting conditions [71], and it may also promote mesenchymal progenitor differentiation during kidney development [67]. Thus, high NGAL levels in AKI can be from both increased renal and extra-renal expression and decreased tubular reabsorption [70].

4.2. Current clinical use

NGAL can be measured in blood and urine by Western blot, enzyme-linked immunosorbent assay, and standardized clinical platforms that use a chemiluminescent microparticle immunoassay with a noncompetitive 2-antianalyte antibody sandwich [72]. The 3 different NGAL forms mentioned above expose different epitopes, so the configuration of antibodies will impact the assay's clinical performance [67], and these tests likely measure a mixture of the forms, further decreasing their specificity for AKI [67]. Cullen et al. established a reference interval for urine NGAL of 107 μg/L, as the 95th percentile of a large healthy adult population that excluded CKD patients [73]. However, more utility will come from a higher cut-off producing greater specificity: a large meta-analysis involving over 13,000 patients from various locations (emergency department, cardiac surgery, ICU) found an urine NGAL >580 and > 589 to have 95% specificity for severe AKI and AKI requiring dialysis [74]. There is no FDA approved NGAL assay, and the research assays vary between companies and are not standardized. Therefore, each laboratory will need to establish its own cutoff. Most pediatric institutions that have implemented urine NGAL into clinical care are using the BioPorto Diagnostics assay (Hellrup, Denmark) which requires 1 mL of urine with no special handling; their cutoffs are summarized in Table 2 [75].

NGAL has been evaluated in hundreds of studies across heterogenous cohorts of adults and children and disease types. One of the first large studies took place in the pediatric cardiac surgery population. NGAL predicted AKI on multivariable analysis with an AUC-ROC of 0.998, increasing just 2 h after CPB initiation and rising nearly 100-fold up to 48 h before AKI was detected by serum creatinine [76]. In 2011, NGAL outperformed other candidate biomarkers including interleukin-18, liver fatty acid binding protein (L-FABP) and kidney injury molecule-1 for AKI prediction, and the 2 and 6 h NGAL level improved the AUC from 0.74 to 0.85 and 0.72 to 0.91 in comparison to a clinical model on multivariable logistic regression [77]. The TRIBE-AKI consortium also found the highest quintile of urine NGAL levels associated with a >4x higher odds of AKI compared with the lowest quintiles in children post-cardiac surgery [78].

4.3. Using urine NGAL to phenotype and risk stratify

Similar to the other biomarkers, NGAL has been used to improve diagnostic precision through enhanced risk stratification and phenotyping. Varnell and colleagues recently described a series of cases in which urine NGAL was used for prediction of tubular injury

Table 2
NGAL Cutoff values.

Urine NGAL Level (ng/mL)	AKI risk	Interpretation
<50	Low	Does not exclude subsequent AKI development Repeat if indicated
50–149	Equivocal	Repeat measures if indicated
150–300	Moderate	High sensitivity/moderate specificity
>300	High	High specificity

and anuria, prediction of tubular recovery and urine production, prediction of response to therapy (theragnosis), renal function recovery in the setting of extracorporeal kidney support therapy, and clinical decision support (management) [75]. Each of the cases described in this report enrich the AKI phenotype using serial monitoring of NGAL in a dynamic real time process that added to clinical care.

Basu and colleagues evaluated the performance of cystatin C in combination with a tubular damage biomarker (NGAL) to predict SCr based discrete outcomes. In this study, positivity of both biomarkers was predictive of AKI severity and AKI persistence. Additionally, those who only had elevation of cystatin C but not NGAL experienced transient AKI [21]. Stanski and colleagues utilized the 2 $\times$ 2 table for delineating unique AKI phenotypes based on SCr and NGAL elevation for predicting AKI severity on hospital day 3. In this study, the only patients to have all stage day 3 AKI and mortality were those who were NGAL+. The authors concluded that unique biomarker combinations may enable a more personalized approach to the management of AKI, although this needs prospective testing.

NGAL has also been combined with risk tools. The concept of renal angina was proposed to optimize pre-test probability by combining patient risk factors and early signs of kidney injury (fluid overload and changes in creatinine) for prediction of severe (stage 2 or 3) ICU Day 3 AKI [79]. The RAI is assessed 12 h after ICU admission, and on initial assessment performed well, with high sensitivity and negative predictive value [79]. These results were recapitulated in the prospective, multi-national "Assessment of Worldwide AKI, Renal Angina, and Epidemiology" (AWARE) study that included 1590 patients across 32 sites. In this study, the RAI outperformed serum creatinine elevation, with an almost 5x increased odds of predicting day 3 severe AKI after multivariate regression [80]. In a single center study, incorporation of urine NGAL into the RAI improved the AUC-ROC for severe ICU Day 3 AKI from 0.8 to 0.97, making it a nearly perfect test [81]. Others have modified the RAI for use in the acute care setting [82]. Patients who were acute RAI positive and NGAL positive had a 60% greater probability of developing inpatient AKI. Modifications and calibrations to the RAI for other cohorts including sepsis [83] and cardiac surgery [84] have recently been reported. Whether NGAL integration improves risk stratification is unknown.

In recent years, the Nephrotoxic Injury Negated by Just-In-Time Action (NINJA) program, a multicenter, quality improvement initiative aimed at reducing nephrotoxic medication-associated AKI through daily systematic medication surveillance and creatinine monitoring, has demonstrated sustained reductions in nephrotoxic medication exposures and AKI in both pediatric and neonatal populations [85,86]. One of the limitations of this program is daily measurement of serum creatinine which requires a blood draw. Goldstein and colleagues recently reported on the utility of daily urine NGAL monitoring in nephrotoxin-exposed children [87]. In this 2-center study, the authors hypothesized that daily urine NGAL could be used to screen for AKI in patients exposed to nephrotoxins and that previous cutoffs predictive of AKI in other populations would be highly specific to detect severe, persistent AKI (stage 2/3 AKI that lasts more than 2 days). At the standard threshold of 150 ng/mL, the specificity and negative predictive values were 92% and 93%, respectively, demonstrating that NGAL could be used to rule out the development of almost every severe AKI episode. In the study, the authors estimated that 204 venipunctures and creatinine measures were saved in 113 patients by obtaining daily urine NGAL and every other day creatinine samples. This improves the utility of incorporating NGAL in patient care, as long as there is reasonable pre-test probability [87].

In the ongoing clinical trial "Use of NGAL for Fluid Dosing and CRRT Initiation in Pediatric AKI" (Taking FOCUS 2, NCT03541785), clinical decision support tools and biomarkers are integrated into a standardized care pathway to guide management of patients at high risk for AKI. There are several care pathways based on risk stratification using the RAI, NGAL, and response to furosemide. RAI $\geq$8 12 h after ICU admission results in an automated NGAL measurement. Elevated NGAL $\geq$500 ng/mL places patients into bundles targeting fluid restriction and early fluid removal. With NGAL concentrations between 150 and 500 ng/mL, response to furosemide is evaluated and there is further delineation into specific care pathways targeting fluid management and potentially even early initiation of kidney replacement therapy. The results of this study are forthcoming and have the potential to change the way we manage critically ill children with real time integrated clinical decision support tools.

Urine NGAL expression occurs within 2–4 h of injury and peaks at 6–12 h, which is advantageous when the timing of an insult is known but makes levels difficult to interpret when it isn't. This limitation may possibly be overcome by comparing percent change in urine NGAL or in trending levels over time. Slagle and colleagues found a nearly 200% increase from pre-to post-operative urine NGAL levels in neonates who developed AKI after abdominal/thoracic surgeries compared to a <1% increase in those who did not [88]. In a small study of adults with AKI, every other day urine NGAL analysis through day 8 of AKI could independently predict recovery from AKI. These studies highlight other ways to integrate urine biomarkers, specifically NGAL, into clinical care. The obvious limitation to trending any urinary biomarker is that the sickest patients, those who are most likely to benefit from the prognostic information afforded by these tests, often are or become anuric.

4.4. Limitations of NGAL

As with the other 2 biomarkers, NGAL also has limitations, specifically with poor sensitivity and moderate diagnostic accuracy [74,89]. As stated previously, IL-6 alone can increase both plasma and urine NGAL without AKI, thus making it less specific during any inflammatory process [70]. Leukocyturia will increase urine NGAL compared to controls and thus should not be used when patients have urinary tract infections [73,90]. Even in the CPB population, extracorporeal circuit time alone can positively correlate with NGAL levels [91]. Similar to other tests, its measure is also impacted by elevated bilirubin. In a study in neonates, urine NGAL by

enzyme-linked immunosorbent assay was higher in severe hyperbilirubinemia (cut-off 1.36 μg/L) compared to mild/moderate ranges of bilirubin [92].

Finally, NGAL is not FDA approved for use in the United States. Most centers that are able to measure NGAL have validated it through laboratory developed tests, and thus analytic performance specifications are established by the clinical laboratory itself. At the time of writing this, the "Establishment of the BioPorto Diagnostics NGAL Test Clinical Cut-off Value for Risk Assessment of Moderate to Severe Acute Kidney Injury in a Pediatric Population" (EARNEST) study for NGAL prediction of day 3 AKI in critically ill children is complete and the "NGAL Usage In Determining AKI Risk in Critically Ill Children" (GUIDANCE) study, that will validate the cutoff, is underway. Both EARNEST and GUIDANCE are being used for a future FDA submission.

5. Conclusions

In summary, differentiating AKI phenotypes using combination biomarkers may improve performance for predicting adverse outcomes. However, there needs to be better understanding of the AKI phenotypes as they are likely to each have important prognostic and therapeutic implications. The differing outcomes dependent on the elevation of functional and/or damage markers needs additional investigation before integration into routine clinical care can occur. Furthermore, delineation of these phenotypes may only be helpful when the course of AKI and its adverse outcomes can be modified. Future trials evaluating novel therapeutics should focus on incorporating risk stratification tools with biomarkers.

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References

[1] A. Kaddourah, R.K. Basu, S.M. Bagshaw, S.L. Goldstein, A. Investigators, Epidemiology of acute kidney injury in critically ill children and young adults, N. Engl. J. Med. 376 (1) (2017) 11–20.
[2] O. Rewa, S.M. Bagshaw, Acute kidney injury-epidemiology, outcomes and economics, Nat. Rev. Nephrol. 10 (4) (2014) 193–207.
[3] J.G. Jetton, L.J. Boohaker, S.K. Sethi, S. Wazir, S. Rohatgi, D.E. Soranno, A.S. Chishti, R. Woroniecki, C. Mammen, J.R. Swanson, S. Sridhar, C.S. Wong, J. C. Kupferman, R.L. Griffin, D.J. Askenazi, C. Neonatal Kidney, Incidence and outcomes of neonatal acute kidney injury (AWAKEN): a multicentre, multinational, observational cohort study, Lancet Child Adolesc. Health 1 (3) (2017) 184–194.
[4] J.A. Alten, D.S. Cooper, J.J. Blinder, D.T. Selewski, S. Tabbutt, J. Sasaki, M.G. Gaies, R.A. Bertrandt, A.H. Smith, G. Reichle, K.M. Gist, M. Banerjee, W. Zhang, K. M. Hock, S. Borasino, Neonatal, H. Pediatric, I. Renal Outcomes Network, Epidemiology of acute kidney injury after neonatal cardiac surgery: a report from the multicenter neonatal and pediatric heart and renal outcomes network, Crit. Care Med. 49 (10) (2021) e941–e951.
[5] B.R. Griffin, Z. You, J. Holmen, M. SooHoo, K.M. Gist, J.F. Colbert, M. Chonchol, S. Faubel, A. Jovanovich, Incident infection following acute kidney injury with recovery to baseline creatinine: a propensity score matched analysis, PLoS One 14 (6) (2019), e0217935.
[6] S. Menon, E.S. Kirkendall, H. Nguyen, S.L. Goldstein, Acute kidney injury associated with high nephrotoxic medication exposure leads to chronic kidney disease after 6 months, J. Pediatr. 165 (3) (2014) 522–527 e2.
[7] J. Gameiro, F. Marques, J.A. Lopes, Long-term consequences of acute kidney injury: a narrative review, Clin. Kidney J. 14 (3) (2021) 789–804.
[8] S. Tanaka, M.D. Okusa, Crosstalk between the nervous system and the kidney, Kidney Int. 97 (3) (2020) 466–476.
[9] S.W. Benoit, P. Devarajan, Acute kidney injury: emerging pharmacotherapies in current clinical trials, Pediatr. Nephrol. 33 (5) (2018) 779–787.
[10] S. Faubel, L.S. Chawla, G.M. Chertow, S.L. Goldstein, B.L. Jaber, K.D. Liu, N. Acute kidney injury advisory group of the American society of, ongoing clinical trials in AKI, Clin. J. Am. Soc. Nephrol. : CJASN 7 (5) (2012) 861–873.
[11] J.A. Kellum, N. Lameire, K.A.G.W. Group, Diagnosis, evaluation, and management of acute kidney injury: a KDIGO summary (Part 1), Crit. Care 17 (1) (2013) 204.
[12] D.T. Selewski, J.R. Charlton, J.G. Jetton, R. Guillet, M.J. Mhanna, D.J. Askenazi, A.L. Kent, Neonatal acute kidney injury, Pediatrics 136 (2) (2015) e463–e473.
[13] J. Lin, H. Fernandez, M.G. Shashaty, D. Negoianu, J.M. Testani, J.S. Berns, C.R. Parikh, F.P. Wilson, False-positive rate of AKI using consensus creatinine-based criteria, Clin. J. Am. Soc. Nephrol. 10 (10) (2015) 1723–1731.
[14] X. Xu, S. Nie, A. Zhang, M. Jianhua, H.P. Liu, H. Xia, H. Xu, Z. Liu, S. Feng, W. Zhou, X. Liu, Y. Yang, Y. Tao, Y. Feng, C. Chen, M. Wang, Y. Zha, J.H. Feng, Q. Li, S. Ge, J. Chen, Y. He, S. Teng, C. Hao, B.C. Liu, Y. Tang, L.J. Wang, J.L. Qi, W. He, P. He, Y. Liu, F.F. Hou, A new criterion for pediatric AKI based on the reference change value of serum creatinine, J. Am. Soc. Nephrol. 29 (9) (2018) 2432–2442.
[15] L.S. Chawla, D.L. Davison, E. Brasha-Mitchell, J.L. Koyner, J.M. Arthur, A.D. Shaw, J.A. Tumlin, S.A. Trevino, P.L. Kimmel, M.G. Seneff, Development and standardization of a furosemide stress test to predict the severity of acute kidney injury, Crit. Care 17 (5) (2013) R207.
[16] J. Penk, K.M. Gist, E.L. Wald, L. Kitzmiller, T.N. Webb, Y. Li, D.S. Cooper, S.L. Goldstein, R.K. Basu, Furosemide response predicts acute kidney injury in children after cardiac surgery, J. Thorac. Cardiovasc. Surg. 157 (6) (2019) 2444–2451, https://doi.org/10.1016/j.jtcvs.2018.12.076.
[17] A. Kakajiwala, J.Y. Kim, J.Z. Hughes, A. Costarino, J. Ferguson, J.W. Gaynor, S.L. Furth, J.J. Blinder, Lack of furosemide responsiveness predicts acute kidney injury in infants after cardiac surgery, Ann. Thorac. Surg. 104 (4) (2017) 1388–1394.
[18] K.M. Gist, J. Penk, E. Wald, L. Kitzmiller, T.N. Webb, J.T. Brinton, D.E. Soranno, S.L. Goldstein, R.J. Basu, Urine quantification following furosemide for severe acute kidney injury prediction in critically ill children, J. Pediatr. Intensive Care (03) (2021), https://doi.org/10.1055/s-0041-1732447.
[19] J.L. Koyner, D.L. Davison, E. Brasha-Mitchell, D.M. Chalikonda, J.M. Arthur, A.D. Shaw, J.A. Tumlin, S.A. Trevino, M.R. Bennett, P.L. Kimmel, M.G. Seneff, L. S. Chawla, Furosemide stress test and biomarkers for the prediction of AKI severity, J. Am. Soc. Nephrol. 26 (8) (2015) 2023–2031.
[20] M. Ostermann, A. Zarbock, S. Goldstein, K. Kashani, E. Macedo, R. Murugan, M. Bell, L. Forni, L. Guzzi, M. Joannidis, S.L. Kane-Gill, M. Legrand, R. Mehta, P. T. Murray, P. Pickkers, M. Plebani, J. Prowle, Z. Ricci, T. Rimmele, M. Rosner, A.D. Shaw, J.A. Kellum, C. Ronco, Recommendations on acute kidney injury biomarkers from the acute disease quality initiative consensus conference: a consensus statement, JAMA Netw. Open 3 (10) (2020), e2019209.
[21] N. Stanski, S. Menon, S.L. Goldstein, R.K. Basu, Integration of urinary neutrophil gelatinase-associated lipocalin with serum creatinine delineates acute kidney injury phenotypes in critically ill children, J. Crit. Care 53 (2019) 1–7.
[22] L.S. Chawla, R. Bellomo, A. Bihorac, S.L. Goldstein, E.D. Siew, S.M. Bagshaw, D. Bittleman, D. Cruz, Z. Endre, R.L. Fitzgerald, L. Forni, S.L. Kane-Gill, E. Hoste, J. Koyner, K.D. Liu, E. Macedo, R. Mehta, P. Murray, M. Nadim, M. Ostermann, P.M. Palevsky, N. Pannu, M. Rosner, R. Wald, A. Zarbock, C. Ronco, J.A. Kellum, W. Acute Disease Quality Initiative, Acute kidney disease and renal recovery: consensus report of the Acute Disease Quality Initiative (ADQI) 16 Workgroup, Nat. Rev. Nephrol. 13 (4) (2017) 241–257.
[23] P.T. Murray, R.L. Mehta, A. Shaw, C. Ronco, Z. Endre, J.A. Kellum, L.S. Chawla, D. Cruz, C. Ince, M.D. Okusa, A. workgroup, Potential use of biomarkers in acute kidney injury: report and summary of recommendations from the 10th Acute Dialysis Quality Initiative consensus conference, Kidney Int. 85 (3) (2014) 513–521.

[24] M. Abrahamson, H. Dalboge, I. Olafsson, S. Carlsen, A. Grubb, Efficient production of native, biologically active human cystatin C by Escherichia coli, FEBS Lett. 236 (1) (1988) 14–18.

[25] L.A. Stevens, C.H. Schmid, T. Greene, L. Li, G.J. Beck, M.M. Joffe, M. Froissart, J.W. Kusek, Y.L. Zhang, J. Coresh, A.S. Levey, Factors other than glomerular filtration rate affect serum cystatin C levels, Kidney Int. 75 (6) (2009) 652–660.

[26] C. Delgado, M. Baweja, D.C. Crews, N.D. Eneanya, C.A. Gadegbeku, L.A. Inker, M.L. Mendu, W.G. Miller, M.M. Moxey-Mims, G.V. Roberts, W.L. St Peter, C. Warfield, N.R. Powe, A unifying approach for GFR estimation: recommendations of the NKF-ASN task force on reassessing the inclusion of race in diagnosing kidney disease, Am. J. Kidney Dis. 79 (2) (2022) 268–288 e1.

[27] G.J. Schwartz, M.F. Schneider, P.S. Maier, M. Moxey-Mims, V.R. Dharnidharka, B.A. Warady, S.L. Furth, A. Munoz, Improved equations estimating GFR in children with chronic kidney disease using an immunonephelometric determination of cystatin C, Kidney Int. 82 (4) (2012) 445–453.

[28] A. Kottgen, E. Selvin, L.A. Stevens, A.S. Levey, F. Van Lente, J. Coresh, Serum cystatin C in the United States: the third national Health and nutrition examination survey (NHANES III), Am. J. Kidney Dis. 51 (3) (2008) 385–394.

[29] J.L. Koyner, M.R. Bennett, E.M. Worcester, Q. Ma, J. Raman, V. Jeevanandam, K.E. Kasza, M.F. O'Connor, D.J. Konczal, S. Trevino, P. Devarajan, P.T. Murray, Urinary cystatin C as an early biomarker of acute kidney injury following adult cardiothoracic surgery, Kidney Int. 74 (8) (2008) 1059–1069.

[30] J.L. Koyner, A.X. Garg, M.G. Shlipak, U.D. Patel, K. Sint, K. Hong, P. Devarajan, C.L. Edelstein, M. Zappitelli, H. Thiessen-Philbrook, C.R. Parikh, A.K.I.C. Translational Research Investigating Biomarker Endpoints in, Urinary cystatin C and acute kidney injury after cardiac surgery, Am. J. Kidney Dis. 61 (5) (2013) 730–738.

[31] R. Bellomo, C. Ronco, J.A. Kellum, R.L. Mehta, P. Palevsky, w. Acute Dialysis Quality Initiative, Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group, Crit. Care 8 (4) (2004) R204–R212.

[32] S. Herget-Rosenthal, D. Poppen, J. Husing, G. Marggraf, F. Pietruck, H.G. Jakob, T. Philipp, A. Kribben, Prognostic value of tubular proteinuria and enzymuria in nonoliguric acute tubular necrosis, Clin. Chem. 50 (3) (2004) 552–558.

[33] S. Herget-Rosenthal, J.A. van Wijk, M. Brocker-Preuss, A. Bokenkamp, Increased urinary cystatin C reflects structural and functional renal tubular impairment independent of glomerular filtration rate, Clin. Biochem. 40 (13–14) (2007) 946–951.

[34] M. Nejat, J.W. Pickering, R.J. Walker, J. Westhuyzen, G.M. Shaw, C.M. Frampton, Z.H. Endre, Urinary cystatin C is diagnostic of acute kidney injury and sepsis, and predicts mortality in the intensive care unit, Crit. Care 14 (3) (2010) R85.

[35] A.B. Hassinger, C.L. Backer, J.C. Lane, S. Haymond, D. Wang, E.L. Wald, Predictive power of serum cystatin C to detect acute kidney injury and pediatric-modified RIFLE class in children undergoing cardiac surgery, Pediatr. Crit. Care Med. 13 (4) (2012) 435–440.

[36] K.A. Gharaibeh, A.M. Hamadah, Z.M. El-Zoghby, J.C. Lieske, T.S. Larson, N. Leung, Cystatin C predicts renal recovery earlier than creatinine among patients with acute kidney injury, Kidney Int. Rep. 3 (2) (2018) 337–342.

[37] M. Nejat, J.W. Pickering, R.J. Walker, Z.H. Endre, Rapid detection of acute kidney injury by plasma cystatin C in the intensive care unit, Nephrol. Dial. Transplant. 25 (10) (2010) 3283–3289.

[38] R.A. Larki, B. Jamali, M. Meidani, S. Mousavi, Serum cystatin C for evaluation of acute kidney injury in adults treated with colistin, J. Res. Pharm. Pract. 7 (4) (2018) 178–181.

[39] K. Soto, S. Coelho, B. Rodrigues, H. Martins, F. Frade, S. Lopes, L. Cunha, A.L. Papoila, P. Devarajan, Cystatin C as a marker of acute kidney injury in the emergency department, Clin. J. Am. Soc. Nephrol. 5 (10) (2010) 1745–1754.

[40] N. Ebert, P. Delanaye, M. Shlipak, O. Jakob, P. Martus, J. Bartel, J. Gaedeke, M. van der Giet, M. Schuchardt, E. Cavalier, E. Schaeffner, Cystatin C standardization decreases assay variation and improves assessment of glomerular filtration rate, Clin. Chim. Acta 456 (2016) 115–121.

[41] A.S. Bargnoux, L. Pieroni, J.P. Cristol, N. Kuster, P. Delanaye, M.C. Carlier, S. Fellahi, A. Boutten, C. Lombard, A. Gonzalez-Antuna, V. Delatour, E. Cavalier, C. Societe Francaise de Biologie, Multicenter evaluation of cystatin C measurement after assay standardization, Clin. Chem. 63 (4) (2017) 833–841.

[42] J.M. El-Khoury, M.P. Hoenig, G.R.D. Jones, E.J. Lamb, C.R. Parikh, N.V. Tolan, F.P. Wilson, AACC guidance document on laboratory investigation of acute kidney injury, J. Appl. Lab. Med. 6 (5) (2021) 1316–1337.

[43] A. Vijayan, S. Faubel, D.J. Askenazi, J. Cerda, W.H. Fissell, M. Heung, B.D. Humphreys, J.L. Koyner, K.D. Liu, G. Mour, T.D. Nolin, A. Bihorac, G. American Society of Nephrology, Acute kidney injury advisory, clinical use of the urine biomarker [TIMP-2] x [IGFBP7] for acute kidney injury risk assessment, Am. J. Kidney Dis. : Off. J. Natl. Kidney Found. 68 (1) (2016) 19–28.

[44] N.M. Katz, J.A. Kellum, C. Ronco, Acute kidney stress and prevention of acute kidney injury, Crit. Care Med. 47 (7) (2019) 993–996.

[45] S.J. Shankland, M. Al'Douahji, Cell cycle regulatory proteins in glomerular disease, Exp. Nephrol. 7 (3) (1999) 207–211.

[46] Q.H. Yang, D.W. Liu, Y. Long, H.Z. Liu, W.Z. Chai, X.T. Wang, Acute renal failure during sepsis: potential role of cell cycle regulation, J. Infect. 58 (6) (2009) 459–464.

[47] K. Kashani, A. Al-Khafaji, T. Ardiles, A. Artigas, S.M. Bagshaw, M. Bell, A. Bihorac, R. Birkhahn, C.M. Cely, L.S. Chawla, D.L. Davison, T. Feldkamp, L.G. Forni, M.N. Gong, K.J. Gunnerson, M. Haase, J. Hackett, P.M. Honore, E.A. Hoste, O. Joannes-Boyau, M. Joannidis, P. Kim, J.L. Koyner, D.T. Laskowitz, M.E. Lissauer, G. Marx, P.A. McCullough, S. Mullaney, M. Ostermann, T. Rimmele, N.I. Shapiro, A.D. Shaw, J. Shi, A.M. Sprague, J.L. Vincent, C. Vinsonneau, L. Wagner, M. G. Walker, R.G. Wilkerson, K. Zacharowski, J.A. Kellum, Discovery and validation of cell cycle arrest biomarkers in human acute kidney injury, Crit. Care 17 (1) (2013) R25.

[48] A. Bihorac, L.S. Chawla, A.D. Shaw, A. Al-Khafaji, D.L. Davison, G.E. Demuth, R. Fitzgerald, M.N. Gong, D.D. Graham, K. Gunnerson, M. Heung, S. Jortani, E. Kleerup, J.L. Koyner, K. Krell, J. Letourneau, M. Lissauer, J. Miner, H.B. Nguyen, L.M. Ortega, W.H. Self, R. Sellman, J. Shi, J. Straseski, J.E. Szalados, S. T. Wilber, M.G. Walker, J. Wilson, R. Wunderink, J. Zimmerman, J.A. Kellum, Validation of cell-cycle arrest biomarkers for acute kidney injury using clinical adjudication, Am. J. Respir. Crit. Care Med. 189 (8) (2014) 932–939.

[49] E.A. Hoste, P.A. McCullough, K. Kashani, L.S. Chawla, M. Joannidis, A.D. Shaw, T. Feldkamp, D.L. Uettwiller-Geiger, P. McCarthy, J. Shi, M.G. Walker, J. A. Kellum, I. Sapphire, Derivation and validation of cutoffs for clinical use of cell cycle arrest biomarkers, Nephrol. Dial. Transplant. 29 (11) (2014) 2054–2061.

[50] I. Gocze, D. Jauch, M. Gotz, P. Kennedy, B. Jung, F. Zeman, C. Gnewuch, B.M. Graf, W. Gnann, B. Banas, T. Bein, H.J. Schlitt, T. Bergler, Biomarker-guided intervention to prevent acute kidney injury after major surgery: the prospective randomized BigpAK study, Ann. Surg. 267 (6) (2018) 1013–1020.

[51] M. Meersch, C. Schmidt, A. Hoffmeier, H. Van Aken, C. Wempe, J. Gerss, A. Zarbock, Prevention of cardiac surgery-associated AKI by implementing the KDIGO guidelines in high risk patients identified by biomarkers: the PrevAKI randomized controlled trial, Intensive Care Med. 43 (11) (2017) 1551–1561, https://doi.org/10.1007/s00134-016-4670-3.

[52] A. Zarbock, M. Kullmar, M. Ostermann, G. Lucchese, K. Baig, A. Cennamo, R. Rajani, S. McCorkell, C. Arndt, H. Wulf, M. Irqsusi, F. Monaco, A.L. Di Prima, M. Garcia Alvarez, S. Italiano, J. Miralles Bagan, G. Kunst, S. Nair, C. L'Acqua, E. Hoste, W. Vandenberghe, P.M. Honore, J.A. Kellum, L.G. Forni, P. Grieshaber, C. Massoth, R. Weiss, J. Gerss, C. Wempe, M. Meersch, Prevention of cardiac surgery-associated acute kidney injury by implementing the KDIGO guidelines in high-risk patients identified by biomarkers: the PrevAKI-multicenter randomized controlled trial, Anesth. Analg. 133 (2) (2021) 292–302.

[53] K.M. Gist, S.L. Goldstein, J. Wrona, J.A. Alten, R.K. Basu, D.S. Cooper, D.E. Soranno, J. Duplantis, C. Altmann, Z. Gao, S. Faubel, Kinetics of the cell cycle arrest biomarkers (TIMP-2*IGFBP-7) for prediction of acute kidney injury in infants after cardiac surgery, Pediatr. Nephrol. 32 (9) (2017) 1611–1619, https://doi.org/10.1007/s00467-017-3655-y.

[54] M. Meersch, C. Schmidt, H. Van Aken, J. Rossaint, D. Gorlich, D. Stege, E. Malec, K. Januszewska, A. Zarbock, Validation of cell-cycle arrest biomarkers for acute kidney injury after pediatric cardiac surgery, PLoS One 9 (10) (2014), e110865.

[55] M. Meersch, C. Schmidt, H. Van Aken, S. Martens, J. Rossaint, K. Singbartl, D. Gorlich, J.A. Kellum, A. Zarbock, Urinary TIMP-2 and IGFBP7 as early biomarkers of acute kidney injury and renal recovery following cardiac surgery, PLoS One 9 (3) (2014), e93460.

[56] J. Chen, Y. Sun, S. Wang, X. Dai, H. Huang, Z. Bai, X. Li, J. Wang, Y. Li, The effectiveness of urinary TIMP-2 and IGFBP-7 in predicting acute kidney injury in critically ill neonates, Pediatr. Res. 87 (6) (2020) 1052–1059.

[57] H. Chui, J. Caldwell, M. Yordanova, V. Cockovski, D. Fredric, M. Harel-Sterling, M. Haasz, Z. Al-Ismaili, M. Pizzi, Q. Ma, P. Devarajan, S.L. Goldstein, M. Zappitelli, Tubular injury and cell-cycle arrest biomarkers to predict acute kidney injury in noncritically ill children receiving aminoglycosides, Biomarkers Med. 14 (10) (2020) 879–894.

[58] J.H. Westhoff, B. Tonshoff, S. Waldherr, J. Poschl, U. Teufel, T.H. Westhoff, A. Fichtner, Urinary tissue inhibitor of metalloproteinase-2 (TIMP-2) * insulin-like growth factor-binding protein 7 (IGFBP7) predicts adverse outcome in pediatric acute kidney injury, PLoS One 10 (11) (2015), e0143628.

[59] M. Kimmel, J. Shi, J. Latus, C. Wasser, D. Kitterer, N. Braun, M.D. Alscher, Association of renal stress/damage and filtration biomarkers with subsequent AKI during hospitalization among patients presenting to the emergency department, Clin. J. Am. Soc. Nephrol. 11 (6) (2016) 938–946.

[60] H.S. Yang, M. Hur, K.R. Lee, H. Kim, H.Y. Kim, J.W. Kim, M.T. Chua, W.S. Kuan, H.R. Chua, C. Kitiyakara, P. Phattharapornjaroen, A. Chittamma, T. Werayachankul, U. Anandh, S. Herath, Z. Endre, A.R. Horvath, P. Antonini, S. Di Somma, G. Network, Biomarker rule-in or rule-out in patients with acute diseases for validation of acute kidney injury in the emergency department (BRAVA): a multicenter study evaluating urinary TIMP-2/IGFBP7, Ann. Lab. Med. 42 (2) (2022) 178–187.

[61] M. Fiorentino, Z. Xu, A. Smith, K. Singbartl, P.M. Palevsky, L.S. Chawla, D.T. Huang, D.M. Yealy, D.C. Angus, J.A. Kellum, ProCess, G.-A.K.I.I. Pro, Serial measurement of cell-cycle arrest biomarkers [TIMP-2]*[IGFBP7] and risk for progression to death, dialysis or severe acute kidney injury in patients with septic Shock, Am. J. Respir. Crit. Care Med. 202 (9) (2020) 1262–1270, https://doi.org/10.1164/rccm.201906-1197OC.

[62] M. Schanz, A. Hoferer, J. Shi, M.D. Alscher, M. Kimmel, Urinary TIMP2IGFBP7 for the prediction of platinum-induced acute renal injury, Int. J. Nephrol. Renovascular Dis. 10 (2017) 175–181.

[63] Z. Toprak, E. Cebeci, S.A. Helvaci, I.D. Toprak, Y. Kutlu, A. Sakin, T. Tukek, Cisplatin nephrotoxicity is not detected by urinary cell-cycle arrest biomarkers in lung cancer patients, Int. Urol. Nephrol. 49 (6) (2017) 1041–1047.

[64] D. Daubin, J.P. Cristol, A.M. Dupuy, N. Kuster, N. Besnard, L. Platon, A. Buzancais, V. Brunot, F. Garnier, O. Jonquet, K. Klouche, Urinary biomarkers IGFBP7 and TIMP-2 for the diagnostic assessment of transient and persistent acute kidney injury in critically ill patients, PLoS One 12 (1) (2017), e0169674.

[65] M. Bell, A. Larsson, P. Venge, R. Bellomo, J. Martensson, Assessment of cell-cycle arrest biomarkers to predict early and delayed acute kidney injury, Dis. Markers 2015 (2015) 158658.

[66] K. Kashani, W. Cheungpasitporn, C. Ronco, Biomarkers of acute kidney injury: the pathway from discovery to clinical adoption, Clin. Chem. Lab. Med.: CCLM / FESCC 55 (8) (2017) 1074–1089.

[67] J. Martensson, R. Bellomo, The rise and fall of NGAL in acute kidney injury, Blood Purif. 37 (4) (2014) 304–310.

[68] J.R. Charlton, D. Portilla, M.D. Okusa, A basic science view of acute kidney injury biomarkers, Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association, Eur. Ren. Assoc. 29 (7) (2014) 1301–1311.

[69] E. Singer, L. Marko, N. Paragas, J. Barasch, D. Dragun, D.N. Muller, K. Budde, K.M. Schmidt-Ott, Neutrophil gelatinase-associated lipocalin: pathophysiology and clinical applications, Acta Physiol. 207 (4) (2013) 663–672.

[70] N.I. Skrypnyk, K.M. Gist, K. Okamura, J.R. Montford, Z. You, H. Yang, R. Moldovan, E. Bodoni, J.T. Blaine, C.L. Edelstein, D.E. Soranno, L.A. Kirkbride-Romeo, B.R. Griffin, C. Altmann, S. Faubel, IL-6-mediated hepatocyte production is the primary source of plasma and urine neutrophil gelatinase-associated lipocalin during acute kidney injury, Kidney Int. 97 (5) (2020) 966–979.

[71] D.H. Goetz, M.A. Holmes, N. Borregaard, M.E. Bluhm, K.N. Raymond, R.K. Strong, The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition, Mol. Cell 10 (5) (2002) 1033–1043.

[72] F.C. Grenier, S. Ali, H. Syed, R. Workman, F. Martens, M. Liao, Y. Wang, P.Y. Wong, Evaluation of the ARCHITECT urine NGAL assay: assay performance, specimen handling requirements and biological variability, Clin. Biochem. 43 (6) (2010) 615–620.

[73] M.R. Cullen, P.T. Murray, M.C. Fitzgibbon, Establishment of a reference interval for urinary neutrophil gelatinase-associated lipocalin, Ann. Clin. Biochem. 49 (Pt 2) (2012) 190–193.

[74] C. Albert, A. Zapf, M. Haase, C. Rover, J.W. Pickering, A. Albert, R. Bellomo, T. Breidthardt, F. Camou, Z. Chen, S. Chocron, D. Cruz, H.R.H. de Geus, P. Devarajan, S. Di Somma, K. Doi, Z.H. Endre, M. Garcia-Alvarez, P.B. Hjortrup, M. Hur, G. Karaolanis, C. Kavalci, H. Kim, P. Lentini, C. Liebetrau, M. Lipcsey, J. Martensson, C. Muller, S. Nanas, T.L. Nickolas, C. Pipili, C. Ronco, G.J. Rosa-Diez, A. Ralib, K. Soto, R.C. Braun-Dullaeus, J. Heinz, A. Haase-Fielitz, Neutrophil gelatinase-associated lipocalin measured on clinical laboratory platforms for the prediction of acute kidney injury and the associated need for dialysis therapy: a systematic review and meta-analysis, Am. J. Kidney Dis. : Off. J. Natl. Kidney Found. 76 (6) (2020) 826–841, e1.

[75] C.D. Varnell Jr., S.L. Goldstein, P. Devarajan, R.K. Basu, Impact of near real-time urine neutrophil gelatinase-associated lipocalin assessment on clinical practice, Kidney Int. Rep. 2 (6) (2017) 1243–1249.

[76] J. Mishra, C. Dent, R. Tarabishi, M.M. Mitsnefes, Q. Ma, C. Kelly, S.M. Ruff, K. Zahedi, M. Shao, J. Bean, K. Mori, J. Barasch, P. Devarajan, Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery, Lancet 365 (9466) (2005) 1231–1238.

[77] C.D. Krawczeski, S.L. Goldstein, J.G. Woo, Y. Wang, N. Piyaphanee, Q. Ma, M. Bennett, P. Devarajan, Temporal relationship and predictive value of urinary acute kidney injury biomarkers after pediatric cardiopulmonary bypass, J. Am. Coll. Cardiol. 58 (22) (2011) 2301–2309.

[78] C.R. Parikh, P. Devarajan, M. Zappitelli, K. Sint, H. Thiessen-Philbrook, S. Li, R.W. Kim, J.L. Koyner, S.G. Coca, C.L. Edelstein, M.G. Shlipak, A.X. Garg, C. D. Krawczeski, Postoperative biomarkers predict acute kidney injury and poor outcomes after pediatric cardiac surgery, J. Am. Soc. Nephrol. : JASN (J. Am. Soc. Nephrol.) 22 (9) (2011) 1737–1747.

[79] R.K. Basu, M. Zappitelli, L. Brunner, Y. Wang, H.R. Wong, L.S. Chawla, D.S. Wheeler, S.L. Goldstein, Derivation and validation of the renal angina index to improve the prediction of acute kidney injury in critically ill children, Kidney Int. 85 (3) (2014) 659–667.

[80] R.K. Basu, A. Kaddourah, S.L. Goldstein, A.S. Investigators, Assessment of a renal angina index for prediction of severe acute kidney injury in critically ill children: a multicentre, multinational, prospective observational study, Lancet Child Adolesc. Health 2 (2) (2018) 112–120.

[81] S. Menon, S.L. Goldstein, T. Mottes, L. Fei, A. Kaddourah, T. Terrell, P. Arnold, M.R. Bennett, R.K. Basu, Urinary biomarker incorporation into the renal angina index early in intensive care unit admission optimizes acute kidney injury prediction in critically ill children: a prospective cohort study, Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association, Eur. Ren. Assoc. 31 (4) (2016) 586–594.

[82] H.R. Hanson, M.A. Carlisle, R.S. Bensman, T. Byczkowski, H. Depinet, T.C. Terrell, H. Pitner, R. Knox, S.L. Goldstein, R.K. Basu, Early Prediction of Pediatric Acute Kidney Injury from the Emergency Department: A Pilot Study, The American journal of emergency medicine, 2020.

[83] N.L. Stanski, H.R. Wong, R.K. Basu, N.Z. Cvijanovich, J.C. Fitzgerald, S.L. Weiss, M.T. Bigham, P.N. Jain, A. Schwarz, R. Lutfi, J. Nowak, G.L. Allen, N.J. Thomas, J.R. Grunwell, M. Quasney, B. Haileselassie, L.S. Chawla, S.L. Goldstein, Recalibration of the renal angina index for pediatric septic Shock, Kidney Int. Rep. 6 (7) (2021) 1858–1867.

[84] K.M. Gist, M. SooHoo, E. Mack, Z. Ricci, D.M. Kwiatkowski, D.S. Cooper, C.D. Krawczeski, J.A. Alten, S.L. Goldstein, R.K. Basu, Modifying the renal angina index for predicting AKI and related adverse outcomes in pediatric heart surgery, World J. Pediatr. Congenital. Heart Surg. 13 (2) (2022) 196–202.

[85] C. Stoops, S. Stone, E. Evans, L. Dill, T. Henderson, R. Griffin, S.L. Goldstein, C. Coghill, D.J. Askenazi, Baby NINJA (nephrotoxic injury negated by just-in-time action): reduction of nephrotoxic medication-associated acute kidney injury in the neonatal intensive care unit, J. Pediatr. 215 (2019) 223–228 e6.

[86] S.L. Goldstein, D. Dahale, E.S. Kirkendall, T. Mottes, H. Kaplan, S. Muething, D.J. Askenazi, T. Henderson, L. Dill, M.J.G. Somers, J. Kerr, J. Gilarde, J. Zaritsky, V. Bica, P.D. Brophy, J. Misurac, R. Hackbarth, J. Steinke, J. Mooney, S. Ogrin, V. Chadha, B. Warady, R. Ogden, W. Hoebing, J. Symons, K. Yonekawa, S. Menon, L. Abrams, S. Sutherland, P. Weng, F. Zhang, K. Walsh, A prospective multi-center quality improvement initiative (NINJA) indicates a reduction in nephrotoxic acute kidney injury in hospitalized children, Kidney Int. 97 (3) (2020) 580–588.

[87] S.L. Goldstein, K.A. Krallman, A. Schmerge, L. Dill, B. Gerhardt, P. Chodaparavu, A. Radomsky, C. Kirby, D.J. Askenazi, Urinary neutrophil gelatinase-associated lipocalin rules out nephrotoxic acute kidney injury in children, Pediatr. Nephrol. 36 (7) (2021) 1915–1921.

[88] C.L. Slagle, S.L. Goldstein, H.W. Gavigan, J.A. Rowe, K.A. Krallman, H.C. Kaplan, C. Liu, S.R. Ehrlich, M. Kotagal, A.J. Bondoc, B.B. Poindexter, Association between elevated urine neutrophil gelatinase-associated lipocalin and postoperative acute kidney injury in neonates, J. Pediatr. 238 (2021) 193–201 e2.

[89] C.R. Parikh, P. Devarajan, M. Zappitelli, K. Sint, H. Thiessen-Philbrook, S. Li, R.W. Kim, J.L. Koyner, S.G. Coca, C.L. Edelstein, M.G. Shlipak, A.X. Garg, C. D. Krawczeski, T.-A. Consortium, Postoperative biomarkers predict acute kidney injury and poor outcomes after pediatric cardiac surgery, J. Am. Soc. Nephrol. : JASN (J. Am. Soc. Nephrol.) 22 (9) (2011) 1737–1747.

[90] A. Yilmaz, E. Sevketoglu, A. Gedikbasi, S. Karyagar, A. Kiyak, M. Mulazimoglu, G. Aydogan, T. Ozpacaci, S. Hatipoglu, Early prediction of urinary tract infection with urinary neutrophil gelatinase associated lipocalin, Pediatr. Nephrol. 24 (12) (2009) 2387–2392.
[91] L. Cai, J. Borowiec, S. Xu, W. Han, P. Venge, Assays of urine levels of HNL/NGAL in patients undergoing cardiac surgery and the impact of antibody configuration on their clinical performances, Clin. Chim. Acta 403 (1–2) (2009) 121–125.
[92] H. Wang, M. Su, B. Yang, Y. Ren, L. Li, D. Zhao, D. Huang, X. Gao, The influence of hyperbilirubinemia on indexes of kidney function in neonates, Pediatr. Nephrol. 36 (11) (2021) 3711–3716.