AACC ACADEMY

AACC Guidance Document on
Laboratory Investigation of Acute Kidney Injury

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INTRODUCTION

Acute kidney injury (AKI) is a significant clinical complication affecting 10%–15% of all hospitalizations and is defined as a rapid increase in blood creatinine and/or decrease in urine output (1, 2). While traditionally seen as a single disease or classified into kidney-centric categories (i.e., prerenal, intrinsic, or postrenal AKI), AKI is now more specifically described as a syndrome that includes hepatorenal, cardiorenal, nephrotoxic, perioperative, and sepsis-associated AKI, among others (3). Its co-existence with other severe syndromes such as heart failure, liver failure, and sepsis that cause significant morbidity and mortality themselves, may mask the significance of AKI on short- and long-term outcomes and make it challenging to diagnose and treat (3). Recent literature provides strong evidence that AKI is independently associated with higher risk of cardiovascular events after hospital discharge (4, 5), affects short- and long-term outcomes in liver failure patients (6), and is associated with higher 60-day mortality in patients with septic shock (7). In addition, the 72-h period immediately after AKI distinguishes the risk of important kidney-specific long-term outcomes, such as incident or progressive chronic kidney disease, long-term dialysis, or all-cause death (8). Therefore, it is essential that clinicians are aware of the clinical presentation of AKI and that laboratorians are providing them with the right tools to aid in early diagnosis and staging.

Over the previous decade, new biomarkers and electronic tools have emerged that can predict those patients at greater risk for developing AKI or identify the earliest changes seen in AKI. However, the efficacy of these new biomarkers and electronic tools have been challenged in human clinical trials. In addition, the lack of universal access to these new technologies is a significant barrier for their implementation. However, this has not prevented certain groups from adopting them into their guidelines, such as cardiac surgeons and some medical centers, from putting specific biomarker-guided management protocols in place (9, 10). This has led to considerable disparities in the identification and management of AKI care around the globe, highlighting a greater need for more uniform best practices in testing for AKI.

The purpose of this AACC Academy guidance document is to provide expert opinion from a multidisciplinary group of nephrologists and laboratory scientists based on the preponderance of available evidence to guide clinicians and laboratorians in their laboratory investigations of AKI, with the ultimate goal of promoting best practices to improve healthcare and patient outcomes. It must be remembered, however, that situations where AKI may develop are not addressed by biomarker measurements alone, but also by implementing active measures to prevent the development or limit the severity of AKI.

INITIATING CLINICAL EVALUATION OF AKI

Laboratory testing of blood creatinine and bedside monitoring of urine output are recommended routinely for the detection of AKI in the inpatient setting and are the basis of current diagnostic and staging criteria (Table 1). The use of both the blood creatinine and the urine output is more accurate than the measurement of creatinine alone because the change in creatinine is delayed after injury to the kidney and depends on both the endogenous production and the decline in excretion of creatinine. Ideally, blood creatinine and the urine output are monitored in situations when patients are at risk of either having or developing AKI. This includes a wide range of clinical presentations to the hospital such as trauma, volume depletion, sepsis, and serious infection (Table 2). Since the symptoms of AKI may be nonspecific and vary with the underlying cause, patients with unexplained edema, fatigue, shortness of breath, confusion, nausea, seizures, or coma should be investigated. Patients are also at risk when there has been a significant change in their clinical course during hospitalization, such as cardiac surgery, other significant procedures under anesthesia, or the development of hypotension. Patients should also be monitored when they are receiving medications that can be nephrotoxic or drugs that require dose adjustment with changes in kidney function. In addition, certain patient populations are at particular risk for AKI because of underlying conditions or reduced kidney function at baseline. The frequency and duration of monitoring should be individualized based on the clinical situation and degree of risk (2).

In the outpatient setting, measurement of blood creatinine is commonly undertaken and should routinely be measured in patients at high risk for developing AKI or with chronic kidney disease (CKD). This also applies to patients with a history of a disorder that can cause end organ damage such as diabetes mellitus or systemic lupus erythematosus. Finally, blood creatinine should be measured to monitor kidney function when patients are at risk of AKI from medications or an intercurrent illness (Table 2).

Once clinical diagnosis of AKI is confirmed, we recommend further classification of the prerenal and intrinsic processes within each setting. This additional classification guides early management strategies such as administration of fluids (prerenal) or volume restriction and diuretics in intrinsic AKI.

ANALYTICAL PERFORMANCE OF CREATININE ASSAYS

The two major types of creatinine assays routinely used in clinical laboratories today incorporate the Jaffe alkaline picrate or enzymatic methodologies (11). Historically, interferences affecting the accuracy of the traditional Jaffe method (as much as 15%–25% false elevation reported at physiological concentrations) led to the development of rate kinetic and rate-blank kinetic alkaline picrate methods to improve method specificity and to reduce susceptibility to interfering substances, including various proteins, glucose, and acetoacetate (12).

TABLE 1. AKI definition based on KDIGO 2012.

DIAGNOSTIC CRITERIA FOR AKI	
• Increase in blood creatinine by ≥0.3 mg/dL (26.5 mmol/L) within 48 h; *or* • Increase in blood creatinine to ≥1.5 times baseline, known or presumed to have occurred in the past 7 days; *or* • Urine volume <0.5 mL/kg/h for 6 h	
AKI STAGING	
AKI Stage I	• Increase in blood creatinine ≥0.3 mg/dL (26.5 mmol/L); or • Increase in blood creatinine to 1.5–1.9 times from baseline; or • Urine volume <0.5 mL/kg/h for 6–12 h
AKI Stage II	• Increase in blood creatinine to 2.0–2.9 times from baseline; or • Urine volume <0.5 mL/kg/h for ≥12 h
AKI Stage III	• Increase in blood creatinine to ≥3.0 times from baseline; or • Blood creatinine ≥4.0 mg/dL (354 mmol/L); or • Initiation of kidney replacement therapy; or • Decrease in eGFR to <35 mL/min/1.73m2 in patients <18 years; or • Urine volume <0.3 mL/kg/h for ≥24 h; or • Anuria for ≥12 h

TABLE 2. Clinical scenarios that would require patient monitoring for development of AKI.

CLINICAL SCENARIO	EXAMPLES
Initial presentation	Volume depletion, trauma, sepsis, rhabdomyolysis, hypotension
Change in clinical course	Surgery (especially cardiopulmonary bypass), hypotension
Nephrotoxic medications	Aminoglycosides, vancomycin, radioiodine contrast, NSAIDs, chemotherapy
Susceptibility	Advanced chronic kidney disease (stage 3 or higher), diabetes mellitus, plasma cell dyscrasia, advanced liver disease, advanced cardiac disease, use of diuretics and agents that block the renin angiotensin aldosterone system

Enzymatic methods have been shown to have fewer interferences than the Jaffe methods, but reports have also shown a number of interferences still exist, including dopamine and bilirubin, although these can often be resolved by practical solutions (13, 14). Overall, enzymatic assays demonstrate improved analytical sensitivity and specificity in comparison with Jaffe assays (15). The use of point-of-care (POC) methods for measurement of creatinine is also common, typically performing the measurement directly in whole blood.

Sources of analytical variability in creatinine methods include assay imprecision, calibration variability (both between methods and day-to-day variability within methods), and analytical interferences. As the definition and staging of AKI depends largely on changes in blood creatinine, assay imprecision and absence of interferences are the most important parameters to control. A low between-method bias is also vital if AKI is monitored using results from more than one laboratory. Therefore, the impact of creatinine assay performance on the ability to detect acute increases in blood creatinine for the identification of AKI has been assessed.

Proficiency testing (PT) data collected from the measurement of specimens commutable with fresh-frozen serum have been useful for determining the interlaboratory variability, including total imprecision and calibration consistency, of various assays to inform the total error observed across routine methods. Accuracy-based programs, those that employ real patient serum pools with supplementation of crystalline creatinine and have assigned values by way of isotope dilution mass spectrometry (IDMS) reference measurement procedures (RMP), are critical for the determination of the interlaboratory variability that is equivalent to, and informative of, what would be seen in real patient samples for clinical use. These assays have, over time, been standardized to IDMS RMPs by way of standard reference materials (SRM). As such, reduction in interlaboratory variability in blood creatinine determination has been achieved through the standardization efforts of the National Kidney Disease Education Program (NKDEP) Laboratory Working Group. This is evident through a number of external PT and calibration verification surveys. However, non-IDMS traceable methods are still commercially available and employed in clinical laboratories.

Based on the College of American Pathologists (CAP) PT C-C 2019 survey, the majority of laboratories reported using the kinetic alkaline picrate method without rate blanking (46.5%; 2332) or an enzymatic-based method (38.2%; 1918), based on the CHM-11 sample participation. Fewer laboratories employ the rate-blank kinetic alkaline picrate (12.2%; 610) or standard Jaffe (3.1%; 155) methods. The average interlaboratory coefficient-of-variation (%CV) for kinetic Jaffe, rate-blank kinetic Jaffe, and enzymatic assays ranged from 2.7%–5.0%, 3.7%-5.3%, and 2.7%–3.6%, respectively, across 5 noncommutable PT samples ranging from 1.63 to 7.15 mg/dL (144 to 632 µmol/L) creatinine,

as determined by the all-method mean. The CAP LN24-B 2019, which utilizes accuracy-based grading criteria on commutable materials, demonstrate wider distribution of results for alkaline picrate Jaffe methods (nonblanked) as compared to enzymatic assays, particularly at low creatinine concentrations with %CV ranging from 2.7%–8.0% versus 1.8%–4.5%, respectively.

However, the variability reported by these surveys represents interlaboratory variability (including different manufacturers of both methods) and does not necessarily reflect intralaboratory variability, which matters more for monitoring serial changes in a patient. In fact, Jaffe methods from some vendors may have comparable assay variability to enzymatic methods (%CV < 3.0%), as shown in a study performed using pooled patient samples prepared at 5 different concentrations and measured over 20 days (16). This study, however, does not take into consideration calibration variability and interlaboratory imprecision.

Whilst the mainstay in many diverse clinical care practices, POC creatinine measurements represent only a fraction of creatinine assessments performed and are under-represented in external quality control assessments as most have been granted waived status in the US, not requiring annual PT under the Clinical Laboratory Improvement Amendments (CLIA). However, the reported analytical imprecision of POC devices varies widely between 3.6% to 12.9% depending on manufacturer, which makes some unsuitable for the detection or monitoring of AKI (17–19).

Method performance specifications for total error, imprecision, and bias can be derived from inter- and intraindividual biological variation of creatinine (20). For AKI, assay imprecision is the most important variable and assays can be labeled as meeting "optimum," "desirable" or "minimum" imprecision goals based on their reported analytical variability and the analyte's biological variability. Optimum assays have intralaboratory analytical variability (CV_A) < 0.25 of the intraindividual biological variability of the analyte (CV_I), while desirable assays have CV_A < 0.50 CV_I, and minimum performance assays have CV_A < 0.75 CVI (21). Based on a recent meta-analysis, the reported intraindividual biological variability of blood creatinine in healthy adults is 4.5% (4.4%–5.7% CI) (22). This implies, to meet optimum, desirable, and minimum performance specifications, assays should have CV_A < 1.3%, 2.3%, and 3.4% for measurement of creatinine, respectively. Therefore, creatinine methods with intralaboratory variability CV_A > 3.4% are not recommended for routine use in the clinical laboratory.

BIOLOGICAL VARIABILITY AND DIAGNOSTIC THRESHOLDS

The current diagnostic criteria used for AKI are based on a rise in blood creatinine (Table 1) and/or fall in urine output, as recommended by the 2012 Kidney Disease Improving Global Outcomes guidelines (2). However, recent studies have linked these recommendations with high false-positive rates of AKI diagnoses (23). This rate was higher for patients with CKD, where 30.5% of patients with true blood creatinine ≥1.5 mg/dL were misdiagnosed with AKI (defined by 0.3 mg/dL change) vs only 2% of patients with true blood creatinine <1.5 mg/dL. In addition to high creatinine values, the effect was exacerbated with increased number of measurements and greater assay variability. Therefore, it is important to consider establishing new diagnostic criteria for creatinine-based detection of AKI that factor in the observed biological and analytical variability.

As mentioned earlier, the reported intraindividual biological variability of blood creatinine in healthy adults is ~4.5%. Intraindividual biological variability has also been shown to be minimally affected by sex, age, or time between samples, as demonstrated by a large study involving 9817 paired creatinine results from adult patients seen by general practitioners (24). In addition, studies involving CKD patients also show that CV_I is similar even with increasing creatinine concentrations (25, 26). This information can be combined with the analytical variability (CV_A) to determine the reference change value (RCV) of blood creatinine, the point at which a true change in biomarker (i.e., not due to the random variation in the result) in an individual can be detected when performing serial measurements (27). Therefore, any change in creatinine from baseline that is less than the respective RCV may not be considered significant at a given probability level. It is calculated using the formula:

$$RCV = 2^{1/2} \times Z \times (CV_A^2 + CV_I^2)^{1/2}$$

where CV_A represents intralaboratory analytical variability (varies by assay), CV_I represents intraindividual biological variability (4.5% for creatinine), and a unidirectional Z-score = 2.33 for 99% probability, regarded as a highly significant change. A unidirectional approach is recommended, as only increases in blood creatinine are relevant for the identification of reduced glomerular filtration rate (GFR). Of note, different Z-scores for different probability levels and bidirectionality may be used in this calculation (ex. bidirectional Z-score = 1.96 for 95% probability, regarded as a significant bidirectional change). Both 95% and 99% unidirectional calculations are reported in Table 3, but in this report, we decided to use a 99% probability unidirectional Z-score of 2.33, which yields higher specificity, to address the high false positive rate seen using current Kidney Disease Improving Global Outcomes (KDIGO) criteria. If clinicians prefer to have higher sensitivity for detecting AKI, lower Z-scores with lower probabilities may be used, but this comes at the expense of specificity and increases the rate of false positives. As mentioned previously, the reported intralaboratory analytical variabilities for enzymatic assays and Jaffe vary by concentration and are around 1.0% to 3.0% (16). Taken together, this yields an RCV of 15% to 18% across the range of reported creatinine

values. To simplify, for most US laboratories who use enzymatic and Jaffe methods, changes in creatinine from baseline less than ~0.20 mg/dL (~20 μmol/L) or ~20% (whichever is greater) are within analytical and biological variability, and therefore should not be considered clinically significant for AKI alerts. This may explain why the current AKI definition by KDIGO is not as specific in patients with higher baseline values of creatinine (Fig. 1). Therefore, clinicians and laboratorians should be aware of the current limitations of using current KDIGO definitions for AKI, which result in high false positive rates in patients with high creatinine concentrations. Instead, we recommend laboratories consult Table 3 to determine the RCV that is applicable to their method. We also propose using the following new RCV in developing new thresholds for diagnosing AKI: +0.20 mg/dL (~20 μmol/L) when baseline blood creatinine <1.00 mg/dL (~90 μmol/L) or +20% when baseline blood creatinine >1.00 mg/dL (~90 μmol/L). We suspect this will improve sensitivity for AKI detection in patients with low baseline creatinine values and specificity for patients with high baseline creatinine values (Fig. 1). Similar recommendations (RCV of +0.50 mg/dL [45 μmol/L] or 25%) have already been adopted by international societies for the detection of contrast-induced AKI (28). A similar RCV (+0.20 mg/dL or 30%, whichever is greater) was also established for detecting AKI in pediatric populations (29). Our proposed RCV would therefore be suitable for detection of AKI in both adult and pediatric populations, with the +0.20 mg/dL (~20 μmol/L) covering patients with lower creatinine concentrations like our pediatric populations, while the +20% being more suitable for patients and adults with higher creatinine concentrations. For ease of use, we call this the 20/20 AACC AKI criteria, referring to 0.20 mg/dL (20 μmol/L) or 20%, and coincidentally, the year it was developed. Additional strong evidence supporting the use of the 20/20 AACC AKI criteria emerged from a 14 912 adult study following patients who received 2 blood creatinine measurements within a 24 h period at a tertiary hospital and demonstrated that within-day changes of 0.20 mg/dL (20 μmol/L) or 20% are associated with all cause mortality (30). Importantly, laboratories using methods with poorer CVs will require the use of a higher clinical cutoff for detection of potential AKI (Table 3).

TABLE 3. Relationship between analytical coefficient of variation (CV) and relative change value (RCV) for creatinine. RCV was calculated using 4.5% within-subject biological variation, and for a 95% probability unidirectional change and a 99% probability bidirectional change. As the inputs to the equation and the physiology under examination are not precisely defined, the outputs should be considered as approximations only.

ANALYTICAL CV (%)	95% PROBABILITY RCV (%)*	99% PROBABILITY RCV (%)**
0.1	11	15
1.0	11	15
2.0	12	16
3.0	13	18
4.0	14	20
5.0	16	22
6.0	18	25
7.0	19	27
8.0	21	30
9.0	24	33
10.0	26	36
11.0	28	39
12.0	30	42
13.0	32	45
14.0	34	49
15.0	37	52

*Z = 1.65.
** Z = 2.33.

DEFINING "BASELINE" CREATININE

Internationally agreed definitions of AKI are predicated on comparing an index blood creatinine concentration with an earlier 'baseline' creatinine result. The Acute Kidney Injury Network (AKIN) definition of AKI defined the baseline sample as one being available within 48 h of the index sample. The 2012 KDIGO criteria extended this so that a relative increase of ≥50% compared to a sample within 7 days of the index sample could also satisfy the diagnosis (Table 1).

The baseline creatinine value is assumed to reflect an individual's premorbid, usual kidney function (homeostatic set-point) and is compared against the index ('current') sample in the detection and staging of AKI. Defining this baseline kidney function sample in the diagnosis of AKI has been an area of active debate. No consensus exists on how to determine baseline kidney function optimally when multiple preadmission creatinine measurements are available. The discussion has been thoroughly reviewed by Thomas et al. (31)

The 3 main approaches to obtaining a baseline value are:

1. Using a measured blood creatinine value within 7 days of the current value
2. Using a measured blood creatinine value between 7 and 365 days before the current value from all results within the time window.

3. By imputation when creatinine results are unavailable [e.g., by back-calculating the creatinine concentration from a standardized eGFR (e.g., 75 mL/min/1.73 m^2)] using the individual's age, sex, and race, or by using the population mean creatinine.

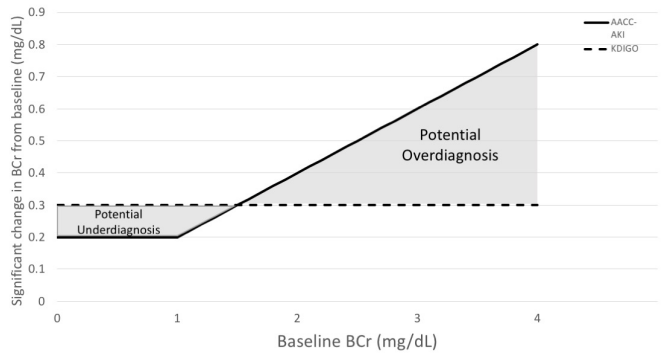
There are advantages and disadvantages to all of these approaches (31). For example, using a creatinine value from the previous 7 days might not represent the true baseline if AKI had begun to evolve prior to this, causing an under-recognition of AKI. Conversely, creatinine concentrations in acutely unwell individuals may be lower than the true baseline due to decreased production in this situation, causing an over-recognition of AKI. There has been extensive debate on which should be the preferred approach but there is little evidence on which to base a recommendation (32).

The 2012 KDIGO guideline advocated use of the lowest creatinine concentration during the current hospitalization as the baseline value, although also allowing creatinine concentrations from a longer time period in an otherwise stable patient without progressive CKD when more recent creatinine concentrations are unavailable.

The 2012 European Renal Best Practice Guidelines recommend using the first documented blood creatinine value of the current episode as 'baseline', rather than historical creatinine values or a calculated value based on a presumed GFR of 75 mL/min. They acknowledge that this is an area of contention and indeed were concerned about the different interpretations being applied to baseline creatinine. They cite Siew et al. (33) who demonstrated that the use of the value at admission in the episode under consideration was best associated with mortality risk. However, this study evaluated the use of only various single creatinine values—use of a mean or median value from the previous 7 to 365 days results was not studied.

FIGURE 1. Depicting the difference between using criteria outlined in this document. AACC-AKI, solid line, using +0.20 mg/dL (~20 mmol/L) when creatinine less than 1.00 mg/dL (~90 mmol/L) or +20% when creatinine is greater than 1.00 mg/dL (~90 mmol/L) for detecting significant change in creatinine from baseline when compared with KDIGO 2012 criteria (using +0.30 mg/dL [26.5 mmol/L] criterion, dashed line).

BCr: Blood Creatinine in mg/dL.



In a later study from Siew et al. (34), designed to mimic clinical practice, nephrologists were asked to determine their best estimate of a patient's baseline creatinine concentration, based on careful review of clinical information and laboratory records. These values were then compared to baseline creatinine values calculated using a variety of approaches, namely; (1) the mean outpatient value, (2) the most recent outpatient value, (3) the nadir outpatient value, and (4) the most recent inpatient or outpatient value. Three time intervals were also chosen for study: 7–365, 7–730, and 1–730 days before admission. The authors concluded that the mean outpatient blood creatinine measured within a year of hospitalization most closely approximated the nephrologist-adjudicated baseline blood creatinine values. This approach has been widely used to determine the baseline creatinine value in AKI detection algorithms. It should be noted, however, that laboratory information systems may not discriminate between inpatient and outpatient results when determining the mean value. Consequently, this approach may not define the premorbid value in patients that have had acute hospital admissions.

In the UK, automated reporting of AKI alerts by National Health Service (NHS) laboratories was mandated in 2014. The algorithm uses 2 approaches to baseline assessment. In one, the current creatinine concentration (C1) is compared against the lowest creatinine result within the previous 7 days (RV1) to calculate a C1/RV1 ratio. Second, C1 is compared against the median of values from the previous 8 to 365 days (RV2) to calculate the C1/RV2 ratio. If either of these ratios exceeds 1.5 then an AKI alert is generated. If only one reference value is available (i.e., RV1 or RV2), then this is used to calculate an AKI score. If no values exist in the previous 365 days, the algorithm will not calculate an AKI score, but if the creatinine concentration on the index sample exceeds the reference range, then an alert is generated that this increased creatinine could be due to either AKI or CKD.

Introduction of the AKI alerting system in the UK was accompanied by a dramatic reduction in the repeat testing interval for blood creatinine in patients from primary care: 5 days vs 55 days (stage 1); 2 days vs 38 days (stage 2); and 1 day vs 53 days (stage 3), suggesting that the alerts act as an important prompt to clinical action (35). There was also an accompanying increase in hospitalization rates of patients receiving AKI alerts.

Although there is evidence to suggest that electronic AKI alerts increase identification of AKI, there is little evidence to suggest that they improve survival or reduce the need for kidney replacement therapy (35, 36). Given this, evidence to suggest

that any particular algorithm or approach to baseline creatinine definition is superior to another in terms of clinical outcomes is lacking.

ROLE OF TRADITIONAL MARKERS

Markers which have been used traditionally in assessing kidney injury and function include urine sodium concentration, fractional excretion of sodium, fractional excretion of urea, and the serum urea to creatinine ratio (37). These tests are not part of current definitions of AKI and so do not formally contribute to assessing the presence or severity of AKI in a patient. The roles ascribed for these tests have been to assist with identifying the underlying etiology of AKI, with greatest emphasis on separating prerenal azotemia from acute tubular necrosis (ATN). This distinction has been recognized as an important step in selecting the appropriate management, as prerenal causes are primarily treated with increased fluid intake, whereas with intrinsic kidney damage, excretion of body fluid may be impaired and administering intravenous fluids may be contraindicated (38). In most cases, the diagnosis of prerenal AKI is straightforward from the clinical history and physical examination, and volume resuscitation is appropriate. Yet, in some circumstances, the distinction between the prerenal state and ATN is less straightforward, as with patients who have already received considerable volume and have not yet improved, those with evidence of volume overload, those with pre-existing CKD, and those at risk for complications from volume expansion (e.g., a history of cardiac dysfunction or cirrhosis). In these patients, additional evidence can be useful either to support the decision for further fluid resuscitation or to help avoid excess fluid infusion.

While the traditional markers discussed in this section are commonly used, are frequently described in textbooks, and appear in many online pages and calculator websites, the evidence base for their use is rather low (39, 40). Here, we summarize the tests and assess further data available on their utility.

URINARY SODIUM (RANDOM)

A low random urine sodium concentration (below 20 mmol/L) in the setting of oliguric AKI is consistent with sodium avid state and the preserved ability to retain sodium from the urinary filtrate, as seen in prerenal AKI (38). A value above 40 mmol/L suggests that the kidney cannot normally conserve sodium, often seen in intrinsic AKI (41). Of note, a urine reference interval for spot urine sodium is not useful in making this determination and may be confusing, as a urine sodium within the reference interval may be found in patients with intrinsic AKI.

FRACTIONAL EXCRETION OF SODIUM

The fractional excretion of sodium (FENa) is designed to improve the diagnostic performance of the urine sodium test in assessing the cause of AKI by standardizing it to creatinine excretion (42). It is expressed as:

$$FENa\ (\%) = [(\text{urine sodium} \times \text{plasma creatinine}) / (\text{plasma sodium} \times \text{urine creatinine})] \times 100$$

FENa less than 1% is consistent with prerenal AKI and >2% is consistent with ATN. However, it is important to note that FENa is also <1% in healthy patients, so it is important to only use in the setting of known AKI (i.e., increased blood creatinine).

The limitations of using FENa (or urine sodium concentration) as a diagnostic tool are important to note. It has a poor area-under-the-curve (AUC) for separating prerenal from intrinsic AKI in septic AKI patients (AUC = 0.59) when used alone (43). This is not surprising considering that a finding of low urinary sodium or low FENa is not diagnostic of the prerenal state because these values can be low in individuals who do not have AKI in the setting of a low sodium diet or high urine volume. It can also be low in other causes of AKI when the kidney is sodium avid as with glomerulonephritis, hepatorenal syndrome (HRS), renal allograft rejection, and contrast-induced AKI (40, 44). Similarly, the finding of a high urine sodium or high FENa is not diagnostic of ATN because this can be present in the setting of high sodium intake without AKI, in diuretic use, and with resolution of AKI or resolution of kidney obstruction (42). Based on the varying results in different clinical contexts, the performance characteristics of these tests vary greatly in published studies and the only gold standard for the diagnosis of prerenal azotemia is the rapid resolution of AKI with restoration of volume (40). At best, taken in clinical context, these urine indices can be supportive of a diagnosis, particularly when partnered with information gleaned from other tests like urine microscopy (see section on Role of Urinary Microscopic Examination).

The diagnosis of HRS in the setting of cirrhosis is a clinical one and often one of exclusion. Recent studies support the use of FENa to assist with the diagnosis of HRS. Due to the physiology of cirrhotic circulation, virtually all patients with advanced cirrhosis have chronic kidney hypoperfusion and have a FENa <1%, even in the absence of AKI. The degree of sodium avidity in advanced cirrhosis is such that even patients with ATN typically have a FENa <1% and the test has thus historically been thought unhelpful in distinguishing HRS from ATN (45). However, in several studies, the FENa in patients diagnosed with HRS clustered tightly around 0.15%, and in each case was significantly lower than those for patients with ATN (46, 47). While the values for ATN varied across studies based on diagnostic definitions, it appears that extremely low FENa (<0.2%) may be useful for distinguishing HRS from ATN and has been suggested for incorporation into the International Club of Ascites criteria (48).

FRACTIONAL EXCRETION OF UREA

The fractional excretion of urea (FEUr) has been proposed to separate prerenal AKI from ATN in patients receiving diuretics

which can alter the urinary sodium and therefore affect both the urinary sodium and the FENa. It is expressed as:

$$FEUr\ (\%) = [(urine\ urea \times plasma\ creatinine)/(plasma\ urea \times urine\ creatinine)] \times 100$$

A FEUr <35% is consistent with prerenal AKI whereas values greater than 50% are consistent with loss of tubular function.

Studies evaluating the performance of FEUr have been variable in their findings. In patients with circulatory shock, FEUr was preferred to FENa, and also not affected by diuretics, a finding also found in the pediatric setting (49, 50). However, in another critical care setting, the FEUr was not found to be useful for separating transient from persistent AKI on the day of onset of AKI, or predicting future AKI (49, 51). More recently, the test was found to have potential in patients with cirrhosis separating prerenal AKI from intrinsic AKI from HRS (52).

BLOOD UREA-TO-CREATININE RATIO

Normally, urea is filtered and reabsorbed by the kidneys, whereas creatinine is filtered and actively secreted. Urea reabsorption is increased in the proximal tubule in the setting of volume depletion and the blood urea will increase out of proportion to the rise in creatinine. In fact, this is the origin of the term "prerenal azotemia" or nitrogen in the blood. Indeed, a high blood urea-to-creatinine ratio can be seen as the serum corollary of a low FEUr. When both urea nitrogen and creatinine are measured in mg/dL, a ratio of >20:1 is suggestive of the prerenal state, whereas if urea is measured in mmol/L and creatinine μmol/L, then a ratio of >0.081:1 (or rounded up to >0.1:1 for convenience) is suggestive of the prerenal state (51).

As with other traditional urine markers, this ratio is also of limited value when used in isolation and is best used in clinical context. The poor performance of this ratio has been observed in several large clinical trials (53, 54). This poor performance may be due to the wide range of other factors that may affect levels of urea, which can be increased by significant protein intake or a catabolic state, as with corticosteroid use, or lowered in the setting of severe liver disease or malnutrition. Similarly, blood creatinine can be reduced in patients with very low muscle mass or elevated from medications that block the urinary secretion of creatinine, oral creatine supplements or large protein intake.

TRADITIONAL MARKERS—SUMMARY

In summary, while there appears to be some clinical utility in the traditional markers discussed above, the markers are "not always reliable to make a clear distinction between the different forms of AKI," (2) do not provide information supporting management decisions (55), and are not part of any diagnostic criteria for AKI. The published data are inconsistent and this may reflect differences in the populations tested, the timing of sample collection in the disease, relevant co-morbidities, and the overlap between prerenal and intrinsic AKI. Additionally, all of these tests have substantial overlap in results between patients with different causes of AKI, which is demonstrated by only modest results for AUC analysis and clinical sensitivity and specificity and results near the clinical decision points are least likely to have any clinical utility. These tests can only be considered supportive rather than diagnostic with regard to identifying the cause of AKI, and then only when other factors that may affect the result are taken into consideration. With the exception of possible use in the diagnosis of HRS, these tests are not recommended for general use.

ROLE OF URINARY MICROSCOPIC EXAMINATION

Urine analysis dates back to the 17th century and is one of the oldest and most commonly utilized tests for differential diagnosis of AKI (56). In patients with prerenal azotemia, urine microscopy is usually bland or may feature an occasional hyaline cast or fine granular cast (56, 57). In patients with ATN, urine sediment analysis typically contains kidney tubular epithelial cells, granular casts, and muddy brown or cellular casts (56, 57). Therefore, urine microscopy can help differentiate these 2 entities, along with the clinical context and supporting data.

Urine microscopy can also help with the diagnosis of less common causes of AKI. The presence of significant hematuria, pyuria without bacteriuria, and cellular casts is consistent with glomerulonephritis (58). This is typically accompanied by proteinuria on the dipstick. Pyuria without bacteriuria and/or white blood cell casts is suggestive of acute interstitial nephritis (59).

Some recommend use of a urine sediment scoring system based on the number of granular casts and renal tubular epithelial cells (RTEC) visualized per high-power field in order to determine the cause of AKI (56, 58). Based on an early scoring system developed in 2008 (Table 4), a score greater than or equal to 2 is a strong predictor of ATN (56). Assessment of this scoring system using the AKI diagnosis at discharge as the gold standard indicated that urine microscopy conducted on the day of nephrology consultation was highly predictive of ATN (56). This system has been validated in other studies and is actively being taught in some academic medical centers (58, 60–65). Additionally, high scores were found to be significantly associated with increased risk of worsening AKI, as defined by worsened AKIN stages of AKI, need for dialysis, or mortality from AKI (66). This urinary sediment score may be utilized to first differentiate between ATN and prerenal azotemia and then to potentially predict the clinical course of AKI. Urine microscopy is widely and inexpensively available and its use for the differential diagnosis of AKI may assist the nephrology community clinically to provide clearer diagnoses and therapies for AKI patients. The main limitation to urine microscopy is that the automated systems are not sensitive for urinary casts and the microscopy

has to be performed manually by trained personnel or physicians (67, 68). Also, the interobserver reliability of urine microscopy is moderate and would support educational and technological advances, along with validation of methods and scoring in diverse groups of laboratory staff and clinical populations (69, 70). It should also be noted that urine microscopy is not a waived test, so physician offices or laboratories performing this testing will require a Provider-Performed Microscopy (PPM) or moderate complexity CLIA certificate.

TABLE 4. Urine microscopy scoring table for differential diagnosis of AKI. Score greater than 2 is a strong predictor of acute tubular necrosis.

SCORE	DESCRIPTION
1	RTE cells 0 and granular casts 0
2	RTE cells 0 and granular casts 1 to 5 *or* RTE cells 1 to 5 and granular casts 0
3	RTE cells 1 to 5 and granular casts 1 to 5 *or* RTE cells 0 and granular casts 6 to 10 *or* RTE cells 6 to 20 and granular casts 0

RTE: Renal tubular epithelial cells.

ROLE OF NEW BIOMARKERS

Blood creatinine has been used for the detection of changes in kidney function since the 1960s, but with a half-life of about 4 h in healthy adults (8 h when creatinine clearance is reduced by 50%), it is slow to react and can take 24 to 40 h to increase in response to kidney injury (71). Cystatin C has been proposed as a biomarker for earlier detection of changes in kidney function. However, most forms of AKI primarily involve injury in the kidney tubular epithelium, not the glomerulus, so a decrease in GFR alone (as measured by creatinine or cystatin C) is not a sensitive or early indicator (71). Over the last decade, several new AKI biomarkers have been approved for use in humans in different countries, but only measurement of urinary insulin-like growth factor binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinases 2 (TIMP2) is approved by the Food and Drug Administration (FDA) in the US for the assessment of risk for moderate or severe AKI (72). The [TIMP2].[IGFBP7] test is also available in Europe, where urinary neutrophil gelatinase-associated lipocalin (NGAL) has also been CE-Marked (Conformité Européenne) since 2009. However, FDA-approval/clearance has not yet been granted for clinical use for NGAL. While there is a plethora of other markers currently being evaluated for their potential use in the setting of AKI (73), we will focus our review and recommendations on the utility of markers that currently have FDA-approval for clinical use, namely cystatin C and [TIMP2].[IGFBP7].

BLOOD CYSTATIN C

Cystatin C is a molecule that is constantly produced by all nucleated cells in the human body and is an established marker for kidney function (74). Its utility as a marker for early detection of AKI has been proposed, but results have been mixed.

In a prospective cohort study involving 1150 high-risk adult cardiac surgery patients, called Translational Research Investigating Biomarker Endpoints for Acute Kidney Injury (TRIBE-AKI), cystatin C was less sensitive for AKI detection than creatinine (75). However, cystatin C appeared to identify a subset of patients with AKI at higher risk for adverse outcomes. The prognostic value of cystatin C was also confirmed in a separate prospective, observational study involving 412 adults admitted to the Coronary Care Unit (76). In that study, cystatin C was a strong predictor of AKI and 2-year mortality. Similar findings were reported for its use in patients admitted to the emergency department, where it did not show superior performance to creatinine in detecting AKI (77).

The combination of cystatin C with creatinine was shown to be beneficial for risk stratification and prognosis in patients after contrast media exposure (78). Cystatin C was also shown to predict kidney recovery earlier than creatinine among patients with AKI, potentially shortening hospital stays by 1–3 days and significantly reducing costs (79). This can be explained by the rapid changes in muscle mass seen in hospitalized patients, which can greatly affect creatinine but not cystatin C. However, cystatin C failed to indicate recovery prior to creatinine in certain clinical groups receiving therapy that may affect nucleated cells (like those receiving chemotherapy or with evidence of bone marrow engraftment) (79). Taken together, this information suggests that cystatin C may be more useful in detecting recovery in patients hospitalized for more than a few days, when muscle wasting is accelerated and creatinine is heavily affected.

Analytically, 2 cystatin C assays have been recently standardized, but disappointing results remain discordant between 8 different assays, with biases as high as 20% reported on some (80, 81). As a result, measurement of cystatin C cannot be universally recommended due to poor standardization, the lack of availability from most vendors, and high cost (in comparison with creatinine) worldwide (82). To laboratories with access to the assay, the reported analytical and biological variability for cystatin C are around 2.0% (83) and 4.0% (84), respectively, which yields an RCV of ~16% (see section Biological Variability and Diagnostic Thresholds for calculation). This was confirmed by a 12 month follow-up study involving 1071 patients undergoing coronary angiography where a blood cystatin C increase greater than 15% was the optimal cutoff for detection of AKI (78). Cystatin C may be useful to monitor instead of creatinine for AKI in patients with nonsteady creatinine states, like rhabdomyolysis, where creatinine production varies greatly within 24–48 h.

URINARY [TIMP2].[IGFBP7]

The first FDA-approved test for the assessment of risk for AKI is [TIMP2].[IGFBP7], currently marketed as Nephrocheck® (Astute Medical, San Diego, CA, now part of bioMérieux, Lyon, France). Both TIMP2 and IGFBP7 are cell-cycle regulators that can induce cell-cycle arrest, and are mainly produced by the distal and proximal tubules, respectively (85). They were discovered in 2013 as part of a prospective, multicenter investigation using a cohort of critically ill adult patients, and subsequently validated in an independent cohort (Sapphire study) using a clinical assay and in comparison with existing markers of AKI (86). The Sapphire validation study reported superior performance of urinary [TIMP2].[IGFBP7] (also referred to as AKIRiskTM) with an AUC of 0.80 for the development of AKI (stage 2 or 3) within 12 h. It also demonstrated that urine [TIMP2].[IGFBP7] outperformed urine NGAL (AUC: 0.72), plasma cystatin C (AUC: 0.71), urine KIM-1 (AUC: 0.70), plasma NGAL (AUC: 0.69), urine IL-18 (AUC: 0.69), urine pi-GST (AUC: 0.61) and urine L-FABP (AUC: 0.61). In addition, the risk of AKI (stage 2 or 3 within 12 h) and major adverse kidney events occurring within 30 days increased when urinary [TIMP2].[IGFBP7] was above 0.3 $(ng/mL)^2/1000$, and drastically increased when value was above 2.0 $(ng/mL)^2/1000$. However, it is important to note the significant overlap between measured urinary [TIMP2].[IGFBP7] in healthy urine donors and the 0.3 threshold. This was also separately demonstrated by a large multicenter study that recruited 750 healthy subjects and chronic comorbid subjects without AKI, and where a reference interval of 0.04–2.22 $(ng/mL)^2/1000$ was established for urinary [TIMP2].[IGFBP7] (87). This overlap explains why the reported sensitivity and specificity for this test using a > 0.3 $(ng/mL)^2/1000$ threshold is 92% and 46%, respectively, while using the higher threshold of >2.0 $(ng/mL)^2/1000$ yields 46% and 95%, respectively (88). As a result, using a 0.3 $(ng/mL)^2/1000$ threshold provides better sensitivity but can yield a significantly high number of false positives (~50% of healthy patients tested). It is possible that normalizing to urine creatinine or urine osmolality may improve the performance of this test, as demonstrated by a recent report that recruited healthy volunteers and measured urinary [TIMP2].[IGFBP7] before and after hydration, and showed a significant drop in their score (89, 90). However, data on biological variability of urinary [TIMP2].[IGFBP7] is lacking in the literature, and the effects of normalizing to urine creatinine or osmolality should be checked in critically ill patients at risk of AKI as well before it can be recommended for implementation.

The clinical performance of urinary [TIMP2].[IGFBP7] was also validated in the Opal (91) and Topaz (88) studies and tested in critically-ill patients with different etiologies (92). So far, urinary [TIMP2].[IGFBP7] has been shown to provide early detection and risk stratification for imminent stage 2/3 AKI in over 1800 critically-ill adult patients with different etiologies (92). It is important to note the variable performance of the marker in studies using different cutoffs and timepoints. In several of the studies listed, there is significant deterioration in the performance of the marker when measured beyond 12 h from an AKI event. In addition, it is not surprising that the AUC is lower in studies that attempted to use [TIMP2].[IGFBP7] to also detect AKI Stage 1, which it does not distinguish from healthy individuals as well and which has not received FDA-approval. However, clinical outcomes studies conducted by Meersch et al. (93) and Gocze et al. (94) both showed no significant difference between the intervention (i.e., use of [TIMP2].[IGFBP7]) and the control arms for the need of kidney replacement therapy and mortality, and major adverse kidney events by 30 days.

In pediatric populations, fewer studies have been conducted but the markers are also showing promise (Table 5). However, a comprehensive approach that uses age-specific reference intervals derived from pediatric patients is needed before [TIMP2].[IGFBP7] can be recommended in this population (100).

Based on the current body of literature, urinary [TIMP2].[IGFBP7] is not yet recommended for routine risk assessment of AKI due to the lack of evidence of benefit shown in outcome studies, its suboptimal specificity at the recommended 0.3 $(ng/mL)^2/1000$ cutoff (causing a 50% false positive rate), and limited performance studies outside of the ICU or perioperative setting. This recommendation is consistent with the National Institute of Health and Care Excellence, based on the evidence reviewed as of June 17, 2020 (101). Urinary [TIMP2].[IGFBP7] may play a role in specific populations, such as perioperative care in cardiac surgery, when combined with other clinical and diagnostic findings, as an aid in the risk assessment for the development of moderate or severe (KDIGO Stage 2 or 3) AKI in patients ≥ 21 years who are at high risk for AKI. However, positive outcome studies and further optimization of different cutoffs and collection times for these specific populations are also needed prior to implementation.

For laboratories implementing this test for translational research or ultimately clinical purposes, they should verify that the reported reference interval of 0.04–2.22 $(ng/mL)^2/1000$ applies to their own population (using n = 20 urine samples with 90% of samples within proposed range for acceptance) or otherwise should consider validating their own reference intervals using samples from healthy individuals (n = 120), notwithstanding the substantial cost of such a validation. In addition, we recommend that the result report for [TIMP2].[IGFBP7] includes a clarifying statement to aid in interpreting results, like "Risk for developing moderate to severe AKI within 12 h is low (AKIRisk ≤ 0.30 $(ng/mL)^2/1000$), moderate (AKIRisk = 0.31–2.00 $(ng/mL)^2/1000$), or high (AKIRisk > 2.00 $(ng/mL)^2/1000$)." We do not currently recommend testing urinary [TIMP2].[IGFBP7] on patients < 21 years old, on those who are low risk for AKI such as ambulatory patients or those who had minor surgery, or performing daily or serial measurements of the markers. Finally, there is currently only one assay (Nephrocheck®) on which

TABLE 5. Urine microscopy scoring table for differential diagnosis of AKI. Score greater than 2 is a strong predictor of acute tubular necrosis.

CAUSE OF AKI	STUDY	PATIENT POPULATION	AKI DIAGNOSTIC CRITERIA	AKI THRESHOLD	NO. OF PATIENTS ENROLLED/NO. OF PATIENTS DEVELOPED AKI	[TIMP2].[IGFBP7] DETECTION TIME	AUC	CUT OFF
Liver transplantation	Fuhrman et al. (95)	Patients (<18 years) undergoing liver transplantation	KDIGO	AKI within 48–96 h	16/6	At 6 h after liver transplant	0.93	NR
Cardiopulmonary bypass surgery	Dong et al. (96)	Patients (<18 years) undergoing cardiopulmonary bypass surgery	KDIGO	AKI within 72 h from surgery	150/50	At 2, 6, 12, and 24 h after cardiopulmonary bypass	0.83 (12 h)	NR
	Meersch et al. (97)	Patients (<18 years) undergoing cardiopulmonary bypass surgery	pRIFLE	AKI within 72 h from surgery	51/12	4 h after cardiopulmonary bypass	0.85	0.7
	Gist et al. (98)	Patients (<18 years) undergoing cardiopulmonary bypass surgery	KDIGO	AKI stage ≥ 1 within 72 h from surgery	94/31	At 2, 6, 12, 24, 48 and 72 h after cardiopulmonary bypass	0.71 (12 h alone) 0.79 (12 h with clinical model)	0.78
General	Westhoff et al. (99)	Patients (<18 years) referred to clinic with established AKI	pRIFLE	NR (30 d and 3mo mortality)	133/46	At admission	0.84 (30 d mortality) 0.88 (3mo mortality) 0.77 (renal replacement therapy)	0.3

all of these studies have been conducted, therefore the derived thresholds and recommendations may not be applicable to a new assay for urinary [TIMP2].[IGFBP7], unless concordance with Nephrocheck® is clearly demonstrated.

ELIMINATING WASTEFUL TESTING

Urine Eosinophils in Acute Interstitial Nephritis

The test for urine eosinophils is not useful to confirm or exclude acute interstitial nephritis and should no longer be considered in the evaluation of AKI (102).

UTILITY OF AUTOMATED AKI ALERTS

The international consensus definition of AKI as defined by the KDIGO consortium is relatively straightforward. AKI is diagnosed when there is a 0.30 mg/dL (26.5 μmol/L) increase in creatinine within a 48 h period or a 50% increase over 7 days (2). There are urine output criteria as well, but detecting AKI based on urine output is beyond the reach of most clinical laboratories. The seemingly simple AKI definition requires only time- and individual-stamped creatinine values to be evaluated, but there are several complexities that need to be considered by the clinical laboratory.

First, the definition of AKI depends exquisitely on the creatinine value taken to be "baseline," which is not straightforward to define (see section Defining "Baseline" Creatinine). Second, the 0.30 mg/dL (26.5 μmol/L) increase criterion increases the risk of false-positive AKI diagnoses in patients with CKD, while increasing the risk of false-negative AKI diagnoses in non-CKD patients with low creatinine values (Fig. 1). Therefore, it is no surprise that the data on notification of providers have been relatively mixed. To date, the only published randomized trial was a single-center study of 2393 patients with AKI detected by an automated sniffer algorithm (103). Randomization, at the patient level, to the alert group was not associated with clinical improvement (change in creatinine, dialysis, or death). However, there is some evidence to suggest that an AKI alert system, coupled to an educational program about AKI management, may have beneficial results. In a 5-center, stepped-wedge trial, an AKI alert coupled to an educational program decreased hospital length of stay and improved the rate of certain key best practice metrics (104).

However, there was no difference in 30-day mortality.

Recently, researchers leveraged the US Veterans Affairs clinical database to create a data set of more than 700 000 individuals across 1239 health care facilities and implemented a machine learning (ML)-based approach to predict AKI with great success (AUC = 0.92) (105). This AUC significantly exceeded that of other studies using novel blood and urine biomarkers to predict AKI, which rarely exceeds 0.75–0.80 (see section Role of New Biomarkers). However, there are major barriers to implementing ML. Most notably, the inclusion of a high number of variables as inputs (620 000 in this study), which can easily "break" if any single variable is changed (as when a lab information system is updated) (106).

Taken together, the value of communicating results regarding the presence of AKI is unclear. Whilst there is evidence of improved clinical practice, as yet this has not been linked to improved outcomes. If providers are to be informed, it is likely important to include a robust educational program to aid in their decision-making. Future studies to determine which subsets of patients and providers may benefit from alerts are necessary. Current definitions of AKI and "baseline" creatinine may also be contributing factors that should be investigated further. Machine learning may hold a greater promise for accurate prediction of AKI but robust validation and continuous monitoring of these models will be essential to their success (107).

TABLE 6. Summary of findings and recommendations to laboratories and clinicians.

#	FINDING(S) AND/OR RECOMMENDATION(S)	TARGET GROUP	
		Laboratory	Clinician
1	Monitor blood creatinine and/or urine output routinely for patients at risk of having or developing AKI. Frequency of length of monitoring should be individualized based on the clinical situation and degree of risk.		X
2	Once clinical diagnosis of AKI is confirmed, we recommend further classification of the prerenal and intrinsic processes within each setting.		X
3	Only employ creatinine assays with intra-laboratory analytical variability ≤ 3.4% for detection of AKI.	X	
4	Implement the use of +0.20 mg/dL (~20 μmol/L) or +20% (whichever is greater), as new thresholds for diagnosing AKI.	X	X
5	Laboratories measuring creatinine with analytical methods with poor precision (CVA > 3.4%), including point-of-care technologies, require the use of a higher clinical cut-off for the diagnosis of AKI (refer to Table 3).	X	X
6	There is currently no evidence to suggest that any particular algorithm or approach to baseline creatinine definition is superior to another in terms of clinical outcomes.	X	X
7	The fractional excretion of sodium (FENa) is used to improve the diagnostic performance of the urine sodium test in assessing the cause of AKI by standardizing it to creatinine excretion: Values < 1% are suggestive of prerenal, and values < 0.2% are suggestive of hepatorenal syndrome (HRS) in the appropriate clinical setting.		X
8	Urine microscopy can help differentiate prerenal azotemia from acute tubular necrosis. It can also help with the diagnosis of less common causes of AKI, such as glomerulonephritis and acute interstitial nephritis.		X
9	The use of a urine sediment scoring system based on the number of granular casts and renal tubular epithelial cells (RTEC) per high-power field in order to differentially diagnose AKI is recommended (see Table 4).		X
10	Cystatin C may be helpful in predicting renal recovery earlier than creatinine among hospitalized patients with AKI. However, the assay cannot be universally recommended due to poor standardization, the lack of availability from most vendors and high cost (in comparison with creatinine) worldwide.	X	X

TABLE 6. Summary of findings and recommendations to laboratories and clinicians. (cont'd.)			
#	FINDING(S) AND/OR RECOMMENDATION(S)	TARGET GROUP	
		Laboratory	Clinician
11	Urinary [TIMP2].[IGFBP7] is not yet recommended for routine risk assessment of AKI due to the lack of evidence of benefit shown in outcome studies, high false positive rate, and limited performance studies outside of the intensive care unit or perioperative setting.	X	X
12	The test for urine eosinophils is not useful to confirm or exclude acute interstitial nephritis and should no longer be considered in the evaluation of AKI	X	X
13	The value of automated alerts and communicating results regarding the presence of AKI is unclear. Whilst there is evidence of improved clinical practice, as yet this has not been linked to improved outcomes.	X	X

SUMMARY

Our understanding of and tools used for detecting AKI have both evolved since KDIGO was published in 2012. The information and opinions provided within this document are intended to shed light on the current status of the field and to generate discussions among clinical organizations leading to a much-needed update to our current practice of investigating AKI. A summary of our findings and recommendations to laboratories and clinicians can be found in Table 6. Clinicians and laboratorians should work together to implement them, and researchers are needed to fill in the remaining gaps in our understanding of these testing strategies.

Nonstandard Abbreviations

AKI, acute kidney injury; CKD, chronic kidney disease; POC, point-of-care; PT, proficiency testing; RMP, reference measurement procedures; IDMS, isotope dilution mass spectrometry; SRM, standard reference materials; NKDED, National Kidney Diseases Education Program; CAP, College of American Pathologists; %CV, coefficient-of-variation; CLIA, Clinical Laboratory Improvement Amendments; CVA, intralaboratory analytical variability; RCV, reference change volume; GFR, glomerular filtration rate; AKIN, Acute Kidney Injury Network; NHS, National Health Service; C1, current creatinine concentration; RV1, lowest creatinine result in previous 7 days; RV2, median creatinine result in previous 8–365 days; ATN, acute tubular necrosis; FENa, fractional excretion of sodium; AUC, area under curve; HRS, hepatorenal syndrome; FEUr, fractional excretion of urea; RTEC, renal tubular epithelial cells; PPM, provider-performed microscopy;IGFBP7, insulin-like growth factor binding protein 7; TIMP2, tissue inhibitor of metalloproteinases 2; FDA, Federal Drug Administration; NGAL, neutrophil gelatinase-associated lipocalin, TRIBE-AKI, Translational Research Investigating Biomarker Endpoints for Acute Kidney Injury; KDIGO, Kidney Disease Improving Global Outcomes; ML, machine learning.

Author Contributions

All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.

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REFERENCES

1. Al-Jaghbeer M, Dealmeida D, Bilderback A, Ambrosino R, Kellum JA. Clinical decision support for In-Hospital AKI. JASN 2018;29:654–60.
2. KDIGO Clinical Practice Guideline for Acute Kidney Injury. Kidney Int 2012;2:1–141.

3. Ronco C, Bellomo R, Kellum JA. Acute kidney injury. Lancet 2019;394:1949–64.

4. Go AS, Hsu C-Y, Yang J, Tan TC, Zheng S, Ordonez JD, et al. Acute kidney injury and risk of heart failure and atherosclerotic events. Clin J Am Soc Nephrol 2018;13:833–41.

5. Jotwani V, Katz R, Ix JH, Gutiérrez OM, Bennett M, Parikh CR, et al. Urinary biomarkers of kidney tubular damage and risk of cardiovascular disease and mortality in elders. Am J Kidney Dis 2018;72:205–13.

6. Tujios SR, Hynan LS, Vazquez MA, Larson AM, Seremba E, Sanders CM, et al. Risk factors and outcomes of acute kidney injury in patients with acute liver failure. Clin Gastroenterol Hepatol 2015;13:352–9.

7. Kellum JA, Chawla LS, Keener C, Singbartl K, Palevsky PM, Pike FL, et al.; ProCESS and ProGReSS-AKI Investigators. The effects of alternative resuscitation strategies on acute kidney injury in patients with septic shock. Am J Respir Crit Care Med 2016;193:281–7.

8. Bhatraju PK, Zelnick LR, Chinchilli VM, Moledina DG, Coca SG, Parikh CR, et al. Association between early recovery of kidney function after acute kidney injury and long-term clinical outcomes. JAMA Netw Open 2020;3:e202682.

9. Lieske JC, Kashani K, Kellum J, Koyner J, Mehta R, Parikh CR. Use of biomarkers to detect and manage acute kidney injury: has progress stalled? Clin Chem 2020;66:271–6.

10. Engelman DT, Ben Ali W, Williams JB, Perrault LP, Reddy VS, Arora RC, et al. Guidelines for perioperative care in cardiac surgery: enhanced recovery after surgery society recommendations. JAMA Surg 2019;154:755–66.

11. Lamb EJ, Jones GRD. Diagnostics kidney function tests. In: Rifai N, Horvath AR, Wittwer CT, editors. Tietz textbook of clinical chemistry and molecular. 6th Ed. St. Louis (MI): Elsevier; 2018:479–517.

12. Myers GL, Miller WG, Coresh J, et al. Recommendations for improving serum creatinine measurement: a report from the Laboratory Working Group of the National Kidney Disease Education Program. Clin Chem 2006;52:5–18.

13. Charifa A, Bunch DR, El-Khoury JM. Practical approach to eliminate bilirubin interference in icteric samples for creatinine measurement. J Appl Lab Med 2019;4:477–9.

14. Charifa A, Bunch DR, El-Khoury JM. Erratic serum creatinine concentrations in a cardiac patient. Clin Chem 2018;64:1543–4.

15. Delanaye P, Cavalier E, Pottel H. Serum creatinine: not so simple!. Nephron 2017;136:302–8.

16. Schmidt RL, Straseski JA, Raphael KL, Adams AH, Lehman CM. A risk assessment of the Jaffe vs enzymatic method for creatinine measurement in an outpatient population. PLoS One 2015;10:e0143205.

17. Bargnoux A-S, Beaufils O, Oguike M, Lopasso A, Dupuy A-M, Sebbane M, et al. Point-of-care creatinine testing in patients receiving contrast-enhanced computed tomography scan. Clin Chim Acta 2018;478:111–3.

18. Kosack CS, de Kieviet W, Bayrak K, Milovic A, Page AL. Evaluation of the Nova StatSensor(R) Xpress(TM) Creatinine point-of-care handheld analyzer. PLoS One 2015;10:e0122433.

19. Straseski JA, Lyon ME, Clarke W, Dubois JA, Phelan LA, Lyon AW. Investigating interferences of a whole-blood point-of-care creatinine analyzer: comparison to plasma enzymatic and definitive creatinine methods in an acute-care setting. Clin Chem 2011;57:1566–73.

20. Topic E, Nikolac N, Panteghini M, Theodorsson E, Salvagno GL, Miler M, et al. How to assess the quality of your analytical method? Clin Chem Lab Med 2015;53:1707–18.

21. Fraser CG, Hyltoft Petersen P, Libeer JC, Ricos C. Proposals for setting generally applicable quality goals solely based on biology. Ann Clin Biochem 1997;34: 8–12.

22. EFLM Biological Variation Database. https://biologicalvariation.eu/search?q=creatinine (Accessed April 30, 2020)

23. Lin J, Fernandez H, Shashaty MGS, Negoianu D, Testani JM, Berns JS, et al. False-positive rate of AKI using consensus creatinine-based criteria. CJASN 2015;10:1723–31.

24. Jones GRD. Estimates of within-subject biological variation derived from pathology databases: an approach to allow assessment of the effects of age, sex, time between sample collections, and analyte concentration on reference change values. Clin Chem 2019;65:579–88.

25. Rowe C, Sitch AJ, Barratt J, Brettell EA, Cockwell P, Dalton RN, et al.; eGFR-C Study Group. Biological variation of measured and estimated glomerular filtration rate in patients with chronic kidney disease. Kidney Int 2019;96:429–35.

26. Carter JL, Parker CT, Stevens PE, Eaglestone G, Knight S, Farmer CKT, et al. Biological variation of plasma and urinary markers of acute kidney injury in patients with chronic kidney disease. Clin Chem 2016;62:876–83.

27. Fraser CG. Reference change values. Clin Chem Lab Med 2011;50:807–12.

28. Chalikias G, Drosos I, Tziakas DN. Contrast-induced acute kidney injury: an update. Cardiovasc Drugs Ther 2016;30:215–28.

29. Xu X, Nie S, Zhang A, Jianhua M, Liu H-P, Xia H, et al. A new criterion for pediatric AKI based on the reference change value of serum creatinine. J Am Soc Nephrol 2018;29:2432–42.

30. Yeh H-C, Lo Y-C, Ting I-W, Chu P-L, Chang S-N, Chiang H-Y, et al. 24-hour serum creatinine variation associates with short- and long-term all-cause mortality: a real-world insight into early detection of acute kidney injury. Sci Rep 2020;10:6552.

31. Thomas ME, Blaine C, Dawnay A, Devonald MAJ, Ftouh S, Laing C, et al. The definition of acute kidney injury and its use in practice. Kidney Int 2015;87:62–73.

32. Doi K, Nishida O, Shigematsu T, Sadahiro T, Itami N, Iseki K, et al.; Japanese Clinical Practice Guideline for Acute Kidney Injury 2016 Committee. The Japanese clinical practice guideline for acute kidney injury 2016. Clin Exp Nephrol 2018;22:985–1045.

33. Siew ED, Matheny ME, Ikizler TA, et al. Commonly used surrogates for baseline renal function affect the classification and prognosis of acute kidney injury. Kidney Int. 2010;77:536-42.

34. Siew ED, Ikizler TA, Matheny ME, Shi Y, Schildcrout JS, Danciu I, et al. Estimating baseline kidney function in hospitalized

patients with impaired kidney function. Clin J Am Soc Nephrol 2012;7:712–9.

35. Aiyegbusi O, Witham MD, Lim M, Gauld G, Bell S. Impact of introducing electronic acute kidney injury alerts in primary care. Clin Kidney J 2019;12:253–7.
36. Lachance P, Villeneuve P-M, Rewa OG, Wilson FP, Selby NM, Featherstone RM, et al. Association between e-alert implementation for detection of acute kidney injury and outcomes: a systematic review. Nephrol Dial Transplant 2017;32:265–72.
37. Palmer BF, Clegg DJ. The use of selected urine chemistries in the diagnosis of kidney disorders. Clin J Am Soc Nephrol 2019;14:306–16.
38. Schrier RW. Diagnostic value of urinary sodium, chloride, urea, and flow. J Am Soc Nephrol 2011;22:1610–3.
39. Bagshaw SM, Langenberg C, Bellomo R. Urinary biochemistry and microscopy in septic acute renal failure: a systematic review. Am J Kidney Dis 2006;48:695–705.
40. Perazella MA, Coca SG. Traditional urinary biomarkers in the assessment of hospital-acquired AKI. Clin J Am Soc Nephrol 2012;7:167–74.
41. Yang L, Bonventre JV. Diagnosis and clinical evaluation of acute kidney injury. In: Floege J, Johnson RJ, Feehally J, editors. Comprehensive clinical nephrology. 4th Ed. Maryland Heights (MO): Mosby; 2010:821–9.
42. Espinel CH. The FENa test. Use in the differential diagnosis of acute renal failure. Jama 1976;236:579–81.
43. Vanmassenhove J, Glorieux G, Hoste E, Dhondt A, Vanholder R, Van Biesen W. Urinary output and fractional excretion of sodium and urea as indicators of transient versus intrinsic acute kidney injury during early sepsis. Crit Care 2013;17:R234.
44. Zarich S, Fang LS, Diamond JR. Fractional excretion of sodium. Exceptions to its diagnostic value. Arch Intern Med 1985;145:108–12.
45. Alsaad AA, Wadei HM. Fractional excretion of sodium in hepatorenal syndrome: Clinical and pathological correlation. World J Hepatol 2016;8:1497–501.
46. Belcher JM, Sanyal AJ, Peixoto AJ, Perazella MA, Lim J, Thiessen-Philbrook H, et al.; TRIBE-AKI Consortium. Kidney biomarkers and differential diagnosis of patients with cirrhosis and acute kidney injury. Hepatology 2014;60:622–32.
47. Ohashi N, Tsuji N, Naito Y, Iwakura T, Isobe S, Ono M, et al. Relationship between urinary fractional excretion of sodium and life prognosis in liver cirrhosis patients. Hepatol Res 2013;43:1156–62.
48. Angeli P, Garcia-Tsao G, Nadim MK, Parikh CR. News in pathophysiology, definition and classification of hepatorenal syndrome: a step beyond the International Club of Ascites (ICA) consensus document. J Hepatol 2019;71:811–22.
49. Yassin AR, Sherif HM, Mousa AY, Esmat A. Comparison between fractional excretion of sodium and fractional excretion of urea in differentiating prerenal from renal azotemia in circulatory shock. Egypt J Crit Care Med 2013;1:69–77.
50. Fujita H, Shinjoh M, Ishii T, Awazu M. Utility of fractional excretion of urea in the differential diagnosis of acute kidney injury in children. Pediatr Nephrol 2016;31:1349–53.
51. Wlodzimirow KA, Abu-Hanna A, Royakkers AANM, Spronk PE, Hofstra LS, Kuiper MA, et al. Transient versus persistent acute kidney injury and the diagnostic performance of fractional excretion of urea in critically ill patients. Nephron Clin Pract 2014;126:8–13.
52. Patidar KR, Kang L, Bajaj JS, Carl D, Sanyal AJ. Fractional excretion of urea: a simple tool for the differential diagnosis of acute kidney injury in cirrhosis. Hepatology 2018;68:224–33.
53. Manoeuvrier G, Bach-Ngohou K, Batard E, Masson D, Trewick D. Diagnostic performance of serum blood urea nitrogen to creatinine ratio for distinguishing prerenal from intrinsic acute kidney injury in the emergency department. BMC Nephrol 2017;18:173.
54. Uchino S, Bellomo R, Goldsmith D. The meaning of the blood urea nitrogen/creatinine ratio in acute kidney injury. Clin Kidney J 2012;5:187–91.
55. Pahwa AK, Sperati CJ. Urinary fractional excretion indices in the evaluation of acute kidney injury. J Hosp Med 2016;11:77–80.
56. Perazella MA, Coca SG, Kanbay M, Brewster UC, Parikh CR. Diagnostic value of urine microscopy for differential diagnosis of acute kidney injury in hospitalized patients. Clin J Am Soc Nephrol 2008;3:1615–9.
57. Perazella MA, Parikh CR. How can urine microscopy influence the differential diagnosis of AKI? Clin J Am Soc Nephrol 2009;4:691–3.
58. Cavanaugh C, Perazella MA. Urine sediment examination in the diagnosis and management of kidney disease: core curriculum 2019. Am J Kidney Dis 2019;73:258–72.
59. Nussbaum EZ, Perazella MA. Diagnosing acute interstitial nephritis: considerations for clinicians. Clin Kidney J 2019;12:808–13.
60. Bagshaw SM, Haase M, Haase-Fielitz A, Bennett M, Devarajan P, Bellomo R. A prospective evaluation of urine microscopy in septic and non-septic acute kidney injury. Nephrol Dial Transplant 2012;27:582–8.
61. Hall IE, Coca SG, Perazella MA, Eko UU, Luciano RL, Peter PR, et al. Risk of poor outcomes with novel and traditional biomarkers at clinical AKI diagnosis. Clin J Am Soc Nephrol 2011;6:2740–9.
62. Mansour SG, Verma G, Pata RW, Martin TG, Perazella MA, Parikh CR. Kidney injury and repair biomarkers in marathon runners. Am J Kidney Dis 2017;70:252–61.
63. Goldani JC, Poloni JA, Klaus F, Kist R, Pacheco LS, Keitel E. Urine microscopy as a biomarker of acute kidney injury following cardiac surgery with cardiopulmonary bypass. J Bras Nefrol 2020;42:18–23.
64. Schinstock CA, Semret MH, Wagner SJ, Borland TM, Bryant SC, Kashani KB, et al. Urinalysis is more specific and urinary neutrophil gelatinase-associated lipocalin is more sensitive for early detection of acute kidney injury. Nephrol Dial Transplant 2013;28:1175–85.
65. Chawla LS, Dommu A, Berger A, Shih S, Patel SS. Urinary sediment cast scoring index for acute kidney injury: a pilot

study. Nephron Clin Pract 2008;110:c145–50.

66. Perazella MA, Coca SG, Hall IE, Iyanam U, Koraishy M, Parikh CR. Urine microscopy is associated with severity and worsening of acute kidney injury in hospitalized patients. Clin J Am Soc Nephrol 2010;5:402–8.
67. Sharda N, Bakhtar O, Thajudeen B, Meister E, Szerlip H. Manual urine microscopy versus automated urine analyzer microscopy in patients with acute kidney injury. Lab Med 2014;45:e152-5–e155.
68. Cho J, Oh KJ, Jeon BC, Lee SG, Kim JH. Comparison of five automated urine sediment analyzers with manual microscopy for accurate identification of urine sediment. Clin Chem Lab Med 2019;57:1744–53.
69. Palsson R, Colona MR, Hoenig MP, Lundquist AL, Novak JE, Perazella MA, et al. Assessment of interobserver reliability of nephrologist examination of urine sediment. JAMA Netw Open 2020;3:e2013959.
70. Hernandez AM, Bilbrough GEA, DeNicola DB, Myrick C, Edwards S, Hammond JM, et al. Comparison of the performance of the IDEXX SediVue Dx(R) with manual microscopy for the detection of cells and 2 crystal types in canine and feline urine. J Vet Intern Med 2019;33:167–77.
71. Srisawat N, Kellum JA. The role of biomarkers in acute kidney injury. Crit Care Clin 2020;36:125–40.
72. Vijayan A, Faubel S, Askenazi DJ, Cerda J, Fissell WH, Heung M, et al.; American Society of Nephrology Acute Kidney Injury Advisory Group. Clinical use of the urine biomarker [TIMP-2] x [IGFBP7] for acute kidney injury risk assessment. Am J Kidney Dis 2016;68:19–28.
73. Zhang WR, Parikh CR. Biomarkers of acute and chronic kidney disease. Annu Rev Physiol 2019;81:309–33.
74. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. N Engl J Med 2012;367:20–9.
75. Spahillari A, Parikh CR, Sint K, Koyner JL, Patel UD, Edelstein CL, et al. Serum cystatin C- versus creatinine-based definitions of acute kidney injury following cardiac surgery: a prospective cohort study. Am J Kidney Dis 2012;60:922–9.
76. Hu Y, Liu H, Du L, Wan J, Li X. Serum cystatin C predicts AKI and the prognosis of patients in coronary care unit: a prospective, observational study. Kidney Blood Press Res 2017;42:961–73.
77. Bongiovanni C, Magrini L, Salerno G, Gori CS, Cardelli P, Hur M, et al. Serum cystatin C for the diagnosis of acute kidney injury in patients admitted in the emergency department. Dis Markers 2015;2015:1–7.
78. Zhang WF, Zhang T, Ding D, et al. Use of both serum cystatin C and creatinine as diagnostic criteria for contrast-induced acute kidney injury and its clinical implications. J Am Heart Assoc 2017;6:e004747.
79. Gharaibeh KA, Hamadah AM, El-Zoghby ZM, Lieske JC, Larson TS, Leung N. Cystatin C predicts renal recovery earlier than creatinine among patients with acute kidney injury. Kidney Int Rep 2018;3:337–42.
80. Ebert N, Delanaye P, Shlipak M, Jakob O, Martus P, Bartel J, et al. Cystatin C standardization decreases assay variation and improves assessment of glomerular filtration rate. Clin Chim Acta 2016;456:115–21.
81. Bargnoux A-S, Piéroni L, Cristol J-P, Kuster N, Delanaye P, Carlier M-C, et al.; Société Française de Biologie Clinique (SFBC). Multicenter evaluation of cystatin C measurement after assay standardization. Clin Chem 2017;63:833–41.
82. Shlipak MG, Mattes MD, Peralta CA. Update on cystatin C: incorporation into clinical practice. Am J Kidney Dis 2013;62:595–603.
83. Keevil BG, Kilpatrick ES, Nichols SP, Maylor PW. Biological variation of cystatin C: implications for the assessment of glomerular filtration rate. Clin Chem 1998;44:1535–9.
84. EFLM Biological Variation Database. https://biologicalvariation.eu/search?q=cystatin+c (Accessed May 7, 2020).
85. Emlet DR, Pastor-Soler N, Marciszyn A, Wen X, Gomez H, Humphries WH, et al. Insulin-like growth factor binding protein 7 and tissue inhibitor of metalloproteinases-2: differential expression and secretion in human kidney tubule cells. Am J Physiol Renal Physiol 2017;312:F284–F96.
86. Kashani K, Al-Khafaji A, Ardiles T, Artigas A, Bagshaw SM, Bell M, et al. Discovery and validation of cell cycle arrest biomarkers in human acute kidney injury. Crit Care 2013;17:R25.
87. Chindarkar NS, Chawla LS, Straseski JA, Jortani SA, Uettwiller-Geiger D, Orr RR, et al. Reference intervals of urinary acute kidney injury (AKI) markers [IGFBP7][TIMP2] in apparently healthy subjects and chronic comorbid subjects without AKI. Clin Chim Acta 2016;452:32–7.
88. Bihorac A, Chawla LS, Shaw AD, Al-Khafaji A, Davison DL, Demuth GE, et al. Validation of cell-cycle arrest biomarkers for acute kidney injury using clinical adjudication. Am J Respir Crit Care Med 2014;189:932–9.
89. L'Acqua C, Sisillo E, Salvi L, Introcaso G, Biondi ML. Nephrocheck after cardiac surgery: Does it play a role in daily practice? A sequel of "Nephrocheck results should be corrected for dilution.". Int J Artif Organs 2019;42:665–7.
90. Noto A, Cortegiani A, David A. NephroCheck: should we consider urine osmolality? Crit Care 2019;23:48.
91. Hoste EAJ, McCullough PA, Kashani K, Chawla LS, Joannidis M, Shaw AD, et al.; Sapphire Investigators. Derivation and validation of cutoffs for clinical use of cell cycle arrest biomarkers. Nephrol Dial Transplant 2014;29:2054–61.
92. Fan W, Ankawi G, Zhang J, Digvijay K, Giavarina D, Yin Y, et al. Current understanding and future directions in the application of TIMP-2 and IGFBP7 in AKI clinical practice. Clin Chem Lab Med 2019;57:567–76.
93. Meersch M, Schmidt C, Hoffmeier A, Van Aken H, Wempe C, Gerss J, et al. 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 2017;43:1551–61.
94. Göcze I, Jauch D, Götz M, Kennedy P, Jung B, Zeman F, et al. Biomarker-guided intervention to prevent acute kidney injury after major surgery: The Prospective Randomized BigpAK Study. Ann Surg 2018;267:1013–20.
95. Fuhrman DY, Kellum JA, Joyce EL, Miyashita Y, Mazariegos GV,

Ganoza A, et al. The use of urinary biomarkers to predict acute kidney injury in children after liver transplant. Pediatr Transplant 2020;24:e13608.

96. Dong L, Ma Q, Bennett M, Devarajan P. Urinary biomarkers of cell cycle arrest are delayed predictors of acute kidney injury after pediatric cardiopulmonary bypass. Pediatr Nephrol 2017;32:2351–60.

97. Meersch M, Schmidt C, Van Aken H, Rossaint J, Görlich D, Stege D, et al. Validation of cell-cycle arrest biomarkers for acute kidney injury after pediatric cardiac surgery. PLoS One 2014;9:e110865.

98. Gist KM, Goldstein SL, Wrona J, Alten JA, Basu RK, Cooper DS, et al. Kinetics of the cell cycle arrest biomarkers (TIMP-2IGFBP-7) for prediction of acute kidney injury in infants after cardiac surgery. Pediatr Nephrol 2017;32:1611–9.

99. Westhoff JH, Tönshoff B, Waldherr S, Pöschl J, Teufel U, Westhoff TH, et al. 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 2015;10:e0143628.

100. Greenberg JH, Parikh CR. Biomarkers for diagnosis and prognosis of AKI in children: one size does not fit all. Clin J Am Soc Nephrol 2017;12:1551–7.

101. Tests to help assess risk of acute kidney injury for people being considered for critical care admission (ARCHITECT and Alinity i Urine NGAL assays, BioPorto NGAL test and NephroCheck test). National Institute for Health and Care Excellence. https://www.nice.org.uk/guidance/dg39/chapter/1-Recommendations (Accessed June 17th, 2020).

102. Perazella MA, Bomback AS. Urinary eosinophils in AIN: farewell to an old biomarker? Clin J Am Soc Nephrol 2013;8:1841–3.

103. Wilson FP, Shashaty M, Testani J, Aqeel I, Borovskiy Y, Ellenberg SS, et al. Automated, electronic alerts for acute kidney injury: a single-blind, parallel-group, randomised controlled trial. Lancet 2015;385:1966–74.

104. Selby NM, Casula A, Lamming L, Stoves J, Samarasinghe Y, Lewington AJ, et al. An organizational-level program of intervention for AKI: a pragmatic stepped wedge cluster randomized trial. J Am Soc Nephrol 2019;30:505–15.

105. Tomašev N, Glorot X, Rae JW, Zielinski M, Askham H, Saraiva A, et al. A clinically applicable approach to continuous prediction of future acute kidney injury. Nature 2019;572:116–9.

106. Wilson FP. Machine learning to predict acute kidney injury. Am J Kidney Dis 2020;75:965-967.

107. Schulz WL, Durant TJS, Krumholz HM. Validation and regulation of clinical artificial intelligence. Clin Chem 2019;65:1336–7.