Screening for Kidney Diseases: Older Measures versus Novel Biomarkers

Ian Wu* and Chirag R. Parikh*†

*Section of Nephrology, Yale University, New Haven, Connecticut, and †Clinical Epidemiology Research Center, VA Medical Center, West Haven, Connecticut

Biomarkers have been used to screen for kidney disease since creatinine was recognized to be correlated with renal function. The measurement of serum creatinine as a screening test for kidney disease falls short, however, because serum creatinine is not particularly sensitive for the diagnosis of kidney disease. Creatinine reflects renal filtering capacity, which has a lot of reserve and is therefore not sensitive to acute or chronic kidney injury unless the injury is substantial enough to compromise the filtering ability. The sensitivity of serum creatinine is further diminished in certain patient populations that are prone to kidney disease because of the physiology of creatinine. Therefore, researchers are seeking new biomarkers that can aid in the diagnosis of both acute and chronic kidney diseases. The limitations of creatinine in screening for kidney diseases in specific patient populations as well as new potential biomarkers that are actively being researched are discussed in this review.


Screening for kidney disease lends itself well to the principles of screening. First, both acute and chronic kidney diseases are prevalent. Stage 3 or worse chronic kidney disease (CKD) affects approximately 11% of adults over the age of 65 (1). Estimates of the prevalence of acute kidney injury (AKI) vary depending on the definition and the setting, with some degree of renal insufficiency noted in 7.1% of hospital admissions (2) and in 30% of patients admitted to an intensive care unit (3). Second, most patients with kidney disease are asymptomatic, underlining the need for routine screening of all patients who are at risk for developing kidney disease. Third, early detection of kidney diseases changes management. The identification of CKD leading to an earlier referral to nephrology is associated with improved outcomes (4). For acute kidney diseases that have no specific therapy, having an accurate understanding of severity of renal dysfunction leads to proper pharmacologic dosing and offers important prognostic information. More importantly, earlier detection of AKI may facilitate the development of more efficacious therapies.

We use various biomarkers to screen for kidney disease. The most common biomarker we use is serum creatinine; however, creatinine has several limitations as a biomarker for screening of kidney disease. The most predominant limitation is that serum creatinine is nonspecific and delays identification of the disease process in AKI and CKD. Often creatinine does not become elevated until the injury is well established. By relying only on serum creatinine to screen for kidney disease, we may only be seeing the tip of the iceberg of kidney diseases (Figure 1). We may be missing subclinical kidney disease in a substantial number of patients by waiting for their serum creatinine to rise before we take action. Therefore, disease-specific biomarkers are being sought out for screening and for early diagnosis of several kidney disorders (discussed below). The timeline for the development and translational research of these new biomarkers is revealed in Figure 2. A comparison of serum creatinine and a few of the newer biomarkers being used in clinical research is provided in Table 1.

AKI: Novel Biomarkers of Tubular Damage

CKD can be diagnosed by a decrease in GFR, a decrease in creatinine clearance, or an increase in serum creatinine. Acute kidney diseases are mainly diagnosed by an increase in serum creatinine, because GFR assessment is not reliable in acute nonsteady states. Thus, serum creatinine is the most common biomarker used for screening for AKI despite several important limitations. An elevation in serum creatinine is not always sensitive for the detection of AKI, because there could be substantial injury to the kidney that does not affect filtration. This phenomenon can be observed in protocol transplant kidney biopsies, in which a substantial number of patients have evidence of tubular injury without significant changes in their serum creatinine (5). Moreover, even when serum creatinine increase occurs in AKI, a detectable increase does not occur until several days after injury. One study demonstrated that serum creatinine does not increase to diagnostic levels of AKI until 48 to 72 h after an ischemic injury has occurred (6). There are many other factors that decrease the sensitivity of serum creatinine in the detection of AKI. Liver diseases and the low muscle mass seen in older patients are associated with AKI and are known to decrease creatinine production, blunting a rise in creatinine. In addition, increases in the volume of distribution...
Researchers have recognized the limitations of serum creatinine for screening of AKI and have discovered numerous novel biomarkers that have proven value in the differential diagnosis, early detection, and prognostication of AKI (8). One such marker is neutrophil-gelatinase-associated lipocalin (NGAL), a polypeptide whose production is upregulated in animal models of AKI and whose elevation can be detected in urine and serum (9). Elevated levels of NGAL have enabled detection of AKI within a few hours after cardiac catheterization (10). Plasma NGAL has also been used to predict morbidity and mortality in pediatric patients who undergo cardiac surgery (11). A recent study demonstrated that a single measurement of urinary NGAL in the emergency room could better predict clinical outcomes such as the need for nephrology consultation, intensive care unit admission, dialysis initiation, or mortality than could an elevated serum creatinine (12).

Another biomarker is interleukin (IL)-18 (IL-18), a cytokine that may play a role in the pathogenesis of ischemic acute tubular necrosis (ATN) through the recruiting of neutrophils in response to ischemic injury (13). Elevated levels of IL-18 in the urine have been demonstrated in patients with ATN compared with patients with other renal diseases (14). In the intensive care unit, urinary IL-18 levels have been shown to be elevated 48 h before the diagnosis of AKI using serum creatinine as a marker. It was also shown to be an independent predictor of mortality in critically ill patients (15).

Kidney Injury Molecule-1 (KIM-1) can also serve as a marker. This molecule is upregulated in postischemic injury in the proximal tubule. An elevated level in the urine has been suggestive as a specific biomarker for the diagnosis of ATN (16). An elevated urinary KIM-1 has also been shown to predict graft loss in renal transplant patients independent of other common risk factors such as creatinine clearance, proteinuria, and donor age (17).

Another biomarker is cystatin C, a cysteine protease inhibitor that is produced by all nucleated cells that appears to be less influenced by factors other than GFR (18). Serum cystatin C has been shown to be a more useful detection marker of AKI in hospitalized patients, detecting AKI 1 to 2 d earlier than serum creatinine (19). For the detection of reduced GFR, it has been shown to have a higher sensitivity and higher negative predictive value than serum creatinine, both important characteristics to consider in the evaluation of any screening test (20). One drawback is that compared with the markers of injury discussed above, cystatin C, like serum creatinine, may not be increased in situations in which injury to kidney tissues is not associated with changes in filtration. However, having a more sensitive and specific test for detecting decreased filtration may be more useful than serum creatinine in helping to predict who is vulnerable to AKI after high-risk procedures such as contrast administration.

These novel biomarkers, which may be more sensitive than serum creatinine in the early detection of AKI, are undergoing phase 4 validation in a large cohort of patients in industry- and government-sponsored studies. These new biomarkers may also add specificity above what serum creatinine offers in the diagnosis of renal diseases. An elevated serum creatinine may
alert clinicians that AKI is present, but it does not distinguish between the many potential causes of AKI. Some of these new biomarkers have been demonstrated to distinguish between prerenal azotemia, acute tubular necrosis, and other glomerular disorders (14). We hope that the results of these studies will enable U.S. Food and Drug Administration (FDA) approval of these novel biomarkers and make them available for large-scale use.

### Acute Renal Dysfunction in Patients with Cirrhosis

Patients with end-stage liver disease (ESLD) are prone to ischemic ATN secondary to hypovolemia from large-volume paracentesis, gastrointestinal bleeding, or infections (21). Diuretics to mobilize ascites may induce prerenal azotemia, and profound hypoalbuminemia and third spacing with splanchic vasodilatation may lead to intravascular depletion (22). Finally, many patients are prone to developing the hepatorenal syndrome, in which severe renal vasoconstriction leads to a decrease in GFR (23). We use serum creatinine to screen for kidney disease in this patient population, but patients with hepatic disease are particularly prone to having serum creatinine concentrations that do not correlate well with their actual GFR. Creatine, the precursor of creatinine, is synthesized in the liver. Therefore, patients with cirrhosis may have lower total creatinine pools. Patients with cirrhosis, like many chronically ill patients, frequently have low muscle mass and low protein intake. In addition, they are frequently edematous, thus increasing the total distribution of creatinine. Formulas such as the Modification of Diet in Renal Disease equation or Cockcroft-Gault, which are dependent on serum creatinine, are imprecise in estimating GFR in these patients (24). Our reliance on serum creatinine screening for kidney diseases in patients with cirrhosis prevents us from diagnosing patients until very late in their disease course.

### Table 1. A comparison of the diagnostic accuracy of newer biomarkers with older measures

<table>
<thead>
<tr>
<th>Diagnosis/Outcome Predicted</th>
<th>Setting</th>
<th>Biomarker (Cutoff)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Accuracy by Area under ROC Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATN (14)</td>
<td>Adult inpatient</td>
<td>IL-18 (300 pg/mg)</td>
<td>95</td>
<td>82</td>
<td>0.95</td>
</tr>
<tr>
<td>Composite dialysis requirement or hospital death after AKI (48)</td>
<td>Adult inpatient</td>
<td>Serum creatinine at enrollment</td>
<td></td>
<td></td>
<td>0.60</td>
</tr>
<tr>
<td>AKI up to 48 h before the rise in serum creatinine (49)</td>
<td>Pediatric ICU</td>
<td>Urinary KIM-1 level</td>
<td></td>
<td></td>
<td>0.61</td>
</tr>
<tr>
<td>Early renal dysfunction (CrCl &lt;80 ml/min per 1.73 m²) (50)</td>
<td>Pediatric ICU</td>
<td>Urinary NGAL (0.05 mg/mg)</td>
<td>85</td>
<td>44</td>
<td>0.78</td>
</tr>
<tr>
<td>AKI 1 d prior to the diagnosis using serum creatinine (15)</td>
<td>Adult ICU</td>
<td>Serum creatinine (0.4 mg/dl)</td>
<td>42</td>
<td>54</td>
<td>0.63</td>
</tr>
<tr>
<td>GFR &lt;60 ml/min/1.73 m² determined by 51 Cr EDTA (51)</td>
<td>Adult outpatient</td>
<td>Serum cystatin C (0.6 mg/L)</td>
<td>85</td>
<td>63</td>
<td>0.85</td>
</tr>
<tr>
<td>AKI (12)</td>
<td>Adult emergency room</td>
<td>Urine IL-18 (100 pg/ml)</td>
<td>50</td>
<td>85</td>
<td>0.73</td>
</tr>
<tr>
<td>Reduced GFR (72 ml/min/1.73 m²) as measured by inulin clearance in patients with cirrhosis (27)</td>
<td>Adult outpatient</td>
<td>Cystatin C</td>
<td>95</td>
<td>77</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serum creatinine</td>
<td>70</td>
<td>92</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cockcroft-Gault formula</td>
<td>68</td>
<td>77</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MDRD formula</td>
<td>84</td>
<td>87</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NGAL 85 µg/g</td>
<td>93</td>
<td>98</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NAG 1.0 U/g</td>
<td>87</td>
<td>32</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serum creatinine 1.4 mg/dl</td>
<td>93</td>
<td>75</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serum cystatin 1.25 mg/L</td>
<td>82</td>
<td>89</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serum creatinine 1.31 mg/dl</td>
<td>23</td>
<td>100</td>
<td>0.91</td>
</tr>
</tbody>
</table>

*AKI, acute kidney injury; ATN, acute tubular necrosis; CrCl, creatinine clearance; Cr, creatinine; ICU, intensive care unit; IL-18, interleukin-18; KIM-1, Kidney Injury Molecule-1; NAG, N-acetyl-beta-glucosaminidase; NGAL, neutrophil-gelatinase-associated lipocalin; ROC, receiver operating characteristic.*
important for these biomarkers to be able to differentiate ischemic ATN from hepatorenal syndrome. This distinction is critical, because therapies vary—dialysis support is needed for the former, whereas vasoconstrictors followed by liver transplantation are necessary for the latter. Because our current therapies of hepatorenal syndrome depend on vasoconstrictive therapy to improve renal perfusion (25), one can suspect that an earlier diagnosis may lead to improved outcomes.

Serum cystatin C has been shown to be a more sensitive test for renal dysfunction than serum creatinine in patients with cirrhosis (26). In one study, serum creatinine was blunted by the degree of liver disease, whereas cystatin C appeared to be more sensitive in detecting renal dysfunction (27). A serum creatinine cutoff of 1.3 mg/dl, the cutoff value for an “abnormal” creatinine level in many laboratories, was only 23% sensitive in diagnosing patients with a reduced GFR as measured by inulin clearance, as compared with a sensitivity of 82% when using cystatin, with only an 11% decrease in specificity compared with serum creatinine, which was 100% specific at that level.

Some biomarkers have demonstrated potential in the differential diagnosis of such disorders such as hepatorenal syndrome. Endothelin-1 appears to be elevated in patients with hepatorenal syndrome but not elevated in patients with other types of renal disease (28). It is also likely that the novel biomarkers of AKI such as IL-18, NGAL, or KIM-1 may assist in excluding ATN in patients with cirrhosis and thereby facilitate the diagnosis of hepatorenal syndrome.

Nephrotic Syndrome

Patients with nephrotic syndrome are also at high risk for renal dysfunction. Creatinine clearances and protein excretion are frequently measured by 24-h timed urine collection in this condition. The amount of protein excretion signifies the severity of nephrotic syndrome and creatinine clearance screens for a loss in GFR. However, the creatinine clearance method has another limitation in assessing GFR decrease in patients with nephrotic syndrome, because the tubular secretion of creatinine can increase significantly with heavy protein excretion in urine. Measured creatinine clearance can be almost double compared with true GFR, measured with inulin clearance, in nephrotic patients with low albumin (29). This implies that over 25% of GFR may be lost without a change in serum creatinine or measured creatinine clearance in patients with nephrotic syndrome. Thus, our ability to monitor kidney dysfunction in patients with nephrotic syndrome by using creatinine clearance alone remains limited. This can severely constrain our ability to monitor responses to therapy and to offer prognostic information.

N-acetyl-β-glucosaminidase (NAG) is a lysosomal enzyme whose excretion is known to increase in glomerular disorders (30). It is thought that in patients with nephrotic syndrome, heavy proteinuria may lead to tubule-interstitial damage, which portends a poorer prognosis (31). This tubulo-interstitial damage can be detected by the presence of biomarkers such as NAG. One study demonstrated the utility of measuring levels of NAG in predicting treatment outcomes and response to therapy (32). Through the measurement of NAG and other biomarkers, we may be able to determine which nephrotic patients have tubular damage and would respond to therapy.

Diabetic Nephropathy

Serum creatinine and creatinine clearance are known to be late biomarkers in diabetic nephropathy. The presence of microalbuminuria is known to precede an elevation in serum creatinine by several years (33). The presence of microalbuminuria has proven to be a powerful screening tool and biomarker to detect diabetic patients at risk for diabetic nephropathy (34). Its identification can lead physicians to intervene earlier with therapies such as angiotensin-converting enzyme inhibitors and angiotensin receptor blockers with proven efficacy (35,36). It is now a standard of care to screen annually for the presence of microalbuminuria in all patients with diabetes mellitus (37). However, the onset of microalbuminuria may be considered a late finding in patients with type 2 diabetes, given its strong association with cardiovascular disease and increased risk of mortality (38). This suggests a need to screen patients many years before the onset of microalbuminuria so that interventions can be undertaken to prevent cardiovascular disease. Urine proteomic profiling has enabled researchers to detect potential biomarkers in patients with type 2 diabetes up to 10 yr before the onset of microalbuminuria (39). As new biomarkers are discovered, clinicians will be able to screen for diabetic nephropathy many years earlier than they now can. Earlier diagnosis may make it possible to identify new targets of therapy that may forestall the onset of microalbuminuria and with it, the associated morbidity and mortality.

Polycystic Kidney Disease

It has long been recognized that measurement of serum creatinine or creatinine clearance was a very insensitive tool for diagnosing polycystic kidney disease (PKD). Imaging is currently recommended for screening for the disease in at-risk patients (40). Nonetheless, physicians routinely use serum creatinine, probably because of cost and reimbursement issues, to monitor progression in patients with PKD. The trouble with this approach is that by the time that serum creatinine is elevated, renal volume has already substantially expanded because renal cysts have already destroyed much of the renal parenchyma (41). Our reliance on serum creatinine as an outcome that is measured and the trials are limited in duration. Magnetic resonance imaging has been shown to be a more sensitive biomarker of disease progression than either serum creatinine or estimated GFR. Magnetic resonance imaging is already being used as a surrogate outcome to increases in creatinine in clinical trials (42,43). It is thought that if we can intervene before the rise in serum creatinine, therapies can have a greater chance of success.
Development of Biomarkers as Screening Tools

A systematic phased approach and multidisciplinary involvement are essential for translating urinary biomarkers into screening tests. We have discussed the five phases of the development process as they would pertain to kidney diseases (44). Phase 5 studies evaluate the effect of the new test on the health of a population as a “screening test.” If biomarkers are used as a screening tool, then it should be shown in randomized controlled trials that application of interventions earlier in the process of AKI is indeed beneficial (45). The common design involves randomizing patients, and for one group, measuring the biomarker and providing the intervention for anyone in the cohort with an elevated level. Patients in the other arm, not tested for the biomarker, receive the standard of care. In an alternate design, all patients have the biomarker measured, and those with elevated levels are randomized to be treated with therapy or provided with the standard of care (46). Clinically important outcomes are the basis for comparing the study arms in both of the scenarios. Cost and compliance outcomes are equally important for evaluation in phase 5. It is also possible, although unlikely, that a randomized trial may not be necessary to adapt the new biomarker test for screening purposes. If the diagnosis is accurate, treatment is convenient and not harmful, and the consequences of the disease in the absence of the treatment are severe, then a trial may not be justified. This might occur in a situation such as that of a high-risk group of patients with AKI requiring dialysis in which the mortality is high and for whom an intervention such as sodium bicarbonate (probably very safe) would be beneficial to patients with elevated biomarkers.

Industry, academia, and the National Institutes of Health (NIH) have a responsibility to pursue development of biomarkers to find their roles as screening tools or as surrogate endpoints in clinical trials. NIH can assist with increasing support for translational research, building clinical research networks, and supporting educational programs. Partnerships between industry and academia are also important: Industry will move toward FDA and other regulatory approvals for the newer tests and then disseminate the tests in the health care system, improving care for individual patients. Thus, academicians would work with NIH to translate discoveries from laboratory to clinical use (developing “diagnostic tests”) and would then collaborate with industry to further translate the tests from validated diagnostic tests to tools for large-scale clinical use.

We foresee a time when newer biomarkers are used as screening tools that will complement the routine measuring of serum creatinine after procedures such as contrast administration or high-risk surgery or at admission into the critical care unit, and that these biomarkers would be used to monitor outpatients at risk for CKD. The use of these biomarkers will enable clinicians to diagnose AKI and CKD at a much earlier stage. Just as clinicians now use multiple cardiac enzymes and electrocardiograms to diagnose an acute myocardial infarction, it may require a bundle of newer biomarkers to improve the sensitivity and specificity of diagnosis of AKI and of underly-


32. Bazzi C, Petrini C, Rizza V, Arrigo G, Napodano P, Papa-


