AJKD
Core Curriculum in Nephrology

Biomarkers in Nephrology: Core Curriculum 2013

Gearoid M. McMahon, MB, BCh, and Sushrut S. Waikar, MD, MPH

The clinical assessment and management of patients with known or suspected kidney disease has been aided for decades by biomarkers, a term defined by a National Institutes of Health working group as "[a] characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention." The characteristics of an ideal biomarker are listed in Box 1. Historically, the first biomarker of kidney disease was the finding on physical examination of interstitial edema or ascites, a condition termed dropsy, that was not specific to what eventually became recognized as kidney failure, but that rather encompassed a number of clinical conditions, including congestive heart failure and cirrhosis. More objective biomarkers in the early days of nephrology included examination of urine sediment, followed by measurement of blood urea nitrogen and serum creatinine.

Recently, there has been an explosive growth in the search for more sensitive, specific, and prognostically accurate biomarkers to assist in the care of patients with or at risk of kidney disease. In this Core Curriculum, we aim to discuss both conventional and novel biomarkers of kidney disease in the settings of acute kidney injury (AKI), chronic kidney disease (CKD), nephrotoxin exposure, and glomerulonephritis. Anatomical localization of selected biomarkers along the nephron is shown in Fig 1.

Additional Reading

» Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther.* 2001;69(3):89-95.

Box 1. Characteristics of an Ideal Biomarker

1. Noninvasive, easily measured, inexpensive, and provides rapid results
2. From easily available sources (blood or urine)
3. High sensitivity
4. High specificity
5. Allows early detection of disease and changes in response to treatment
6. Predicts prognosis and allows stratification into categories of risk
7. Biologically plausible: provides information about the mechanisms of disease

Adapted from Edelstein CL. *Biomarkers in Kidney Disease.* 1st ed. Amsterdam, the Netherlands: Academic Press/Elsevier; 2011.

CONVENTIONAL BIOMARKERS OF KIDNEY FUNCTION AND DISEASE

Modern definitions of CKD and AKI emphasize a stage of decreased glomerular filtration rate (GFR) and a stage of kidney damage, defined as structural or functional abnormalities before a decrease in GFR. Corresponding biomarkers include filtration markers, such as creatinine and cystatin C, and markers of kidney damage, such as urine sediment abnormalities and albuminuria (Table 1).

Creatinine

The use of creatinine as a marker of GFR dates back to the 1920s. Creatinine is a 113-Da amino acid derivative that is the product of nonenzymatic breakdown of creatine in muscle. It is not protein bound, it is not metabolized in the kidney, and it is freely filtered in the glomerulus, making it an excellent marker of glomerular filtration. However, non-GFR determinants of creatinine concentration limit its utility in AKI and CKD. Proximal tubular secretion of creatinine accounts for 10%-20% of its excretion, leading to overestimation of the true GFR, particularly in patients with CKD. Gut bacteria also degrade creatinine and contribute to its clearance, the relative magnitude of which becomes more important as kidney function decreases. Creatinine can be reabsorbed after glomerular filtration in patients with very low urine and tubular flow rates. Certain medications, such as cimetidine and trimethoprim, can increase serum creatinine concentration by inhibiting tubular secretion. Creatinine is produced at a relatively constant rate, which in turn is proportional to muscle mass. Between-person variability in creatinine generation rate—related to age, sex, muscle mass, race, and perhaps other factors—limits the use of creatinine in the estimation of GFR. To account for this variability, a number of creatinine-based equations have been

From the Renal Division, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Received August 14, 2012. Accepted in revised form December 17, 2012. Originally published online March 1, 2013.

Address correspondence to Sushrut S. Waikar, MD, MPH, Brigham and Women's Hospital, MRB-4, 75 Francis St, Boston, MA 02115. E-mail: swaikar@partners.org

0272-6386/$36.00
http://dx.doi.org/10.1053/j.ajkd.2012.12.022

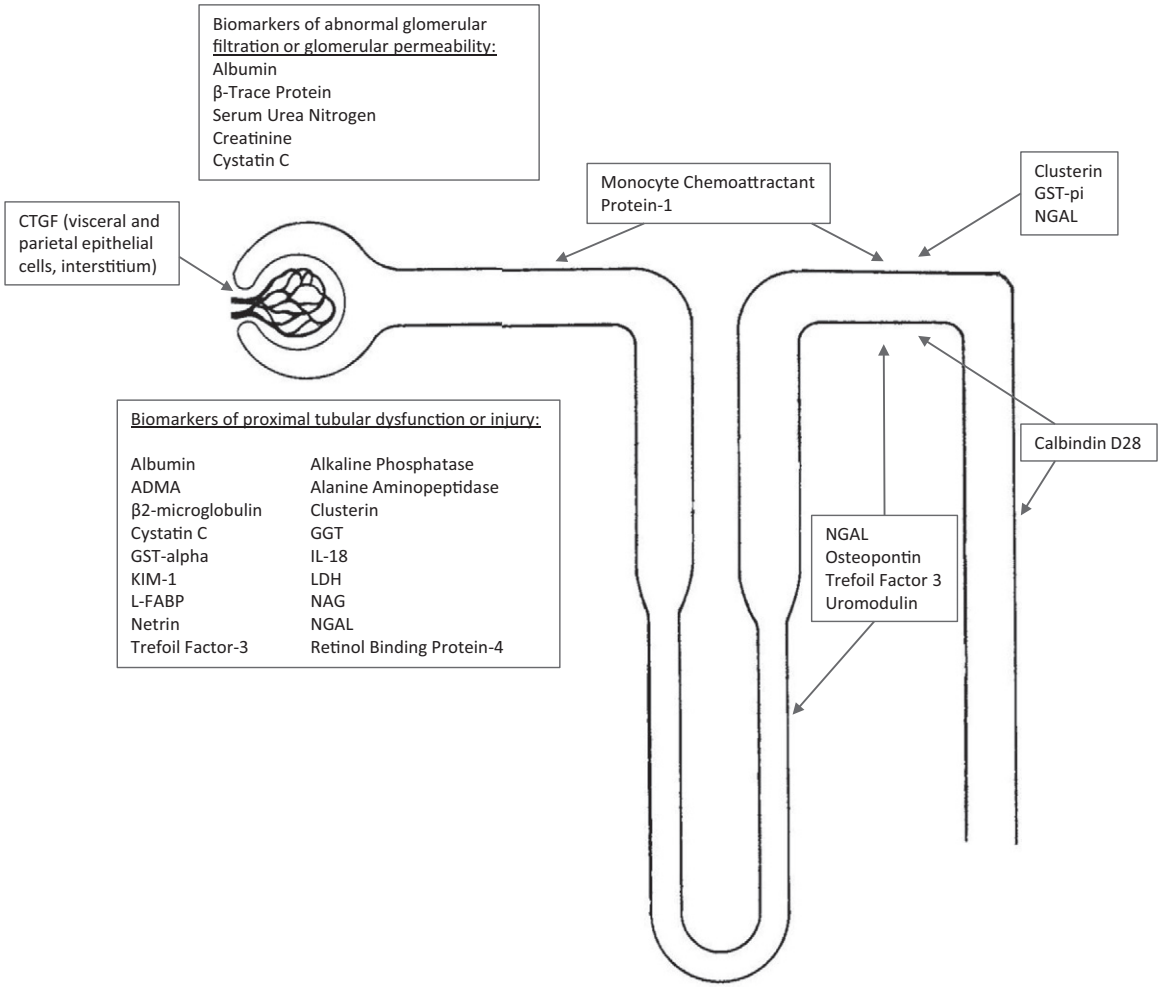

Figure 1. Anatomical localization of biomarkers along the nephron. Abbreviations: ADMA, asymmetric dimethyl arginine; CTGF, connective tissue growth factor; GGT, γ-glutamyl transpeptidase; GST, glutathione-*S*-transferase; IL-18, interleukin 18; KIM-1, kidney injury molecule 1; LDH, lactate dehydrogenase; L-FABP, liver-type fatty acid binding protein; NAG, *N*-acetyl glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin.

developed to estimate GFR, including the Cockroft-Gault, Modification of Diet in Renal Disease (MDRD) Study, and CKD-EPI (CKD Epidemiology Collaboration) equations for adults and the Schwartz equation for children. Despite improving the estimation of true GFR, all the equations have shortcomings. For example, at lower creatinine concentrations, the MDRD Study equation generally underestimates GFR, whereas the Cockroft-Gault and Schwartz equations may overestimate GFR.

Additional Readings

» Carrie BJ, Golbetz HV, Michaels AS, Myers BD. Creatinine: an inadequate filtration marker in glomerular diseases. *Am J Med.* 1980;69(2):177-182.

» Hankins DA, Babb AL, Uvelli DA, Scribner BH. Creatinine degradation I: the kinetics of creatinine removal in patients with chronic kidney disease. *Int J Artif Organs.* 1981;4(1):35-39.

» Perrone RD, Madias NE, Levey AS. Serum creatinine as an index of renal function: new insights into old concepts. *Clin Chem.* 1992;38(10):1933-1953.

» Rehberg PB. Studies on kidney function: the rate of filtration and reabsorption in the human kidney. *Biochem J.* 1926;20(3): 447-460.

Cystatin C

Cystatin C is a 13-kDa cysteine protease inhibitor that is a marker of GFR. Cystatin C is expressed by all nucleated cells and has multiple biological functions, including controlling extracellular proteolysis and modulation of the immune system. Its utility in estimating kidney function derives from the fact that after being freely filtered in the glomerulus, it then is absorbed in the kidney tubules, where it is fully degraded locally.

There is no active tubular secretion or significant extrarenal elimination, making it an excellent marker

Table 1. Uses and Limitations of Conventional Biomarkers

	Uses	Limitations
Creatinine	• Glomerular filtration marker • GFR estimation • Biomarker of acute and chronic decreased kidney function	• Variability in generation rates across individuals • Significant tubular secretion leading to overestimation of GFR • Significant extrarenal elimination • Tubular reabsorption in low urine flow states • Increases late after AKI
Cystatin C	• GFR estimation (plasma) • Biomarker of proximal tubular dysfunction (urine) • Biomarker of acute and chronic decreased kidney function	• Increases late after AKI • May increase in inflammatory states and when there is thyroid dysfunction independent of kidney function • Urinary cystatin C is altered in the presence of albuminuria
Albuminuria	• Biomarker of glomerular filtration barrier dysfunction • Biomarker of proximal tubular dysfunction • Early biomarker of AKI • Independent risk factor for cardiovascular and all-cause mortality and ESRD	• 24-hour collections unreliable • Significant intraindividual variability in albumin-creatinine ratio over short periods
Urine sediment examination	• Biomarker of AKI • Biomarker of glomerular disease • Biomarker of tubulointerstitial disease	• Poor interobserver agreement • Lack of standardization of reporting of results • Heavily dependent on experience of the reader • Uncertain correlation with histopathology

Abbreviations: AKI, acute kidney injury; ESRD, end-stage renal disease; GFR, estimated glomerular filtration rate.

of GFR. Cystatin C also may be superior to creatinine as a better predictor of cardiovascular mortality while providing a more accurate estimation of GFR.

Serum cystatin C concentration is independent of muscle mass, nutritional status, and sex, although it may be altered in patients with derangements in thyroid function, certain cancers, and glucocorticoid therapy. Although cystatin C production has been well characterized in healthy individuals, less is known about its production in disease states; for example, cystatin C levels are higher at baseline in patients with acute leukemia. There are a number of different methods for measuring cystatin C and there is significant interassay variation, with some assays performing better for the diagnosis of AKI and CKD.

A major advantage of cystatin C over creatinine level is that it is not as influenced by changes in muscle mass; therefore, estimating equations for estimated GFR are more accurate across a range of body types, including infants and the elderly. In patients at extremes of body mass, creatinine-based equations perform poorly while cystatin C is more accurate. However, the increase in accuracy is moderate at best, and it is not certain that it is clinically relevant. Some have proposed combining creatinine and cystatin C equations for more precise estimations of GFR. In this setting, agreement between the 2 estimates can be taken as a more accurate estimation of GFR. However, if there is disagreement, the clinical picture should be considered to determine the source of error (body mass, corticosteroids, etc). A recent study has suggested that the combination of creatinine and cystatin C levels for estimation of GFR is superior to creatinine-based equations and may be useful as a confirmatory test for CKD.

Cystatin C is distributed in the extracellular space, whereas creatinine is distributed in total-body water. As a result, it has a volume of distribution approximately one-third that of creatinine, meaning that it reaches a steady-state concentration 3 times faster: the half-life is 1.5 versus 4 hours. As a result, after kidney injury, cystatin C concentration increases earlier than creatinine concentration. This has been borne out in studies of patients with AKI including contrast-induced nephropathy when the increase in cystatin C level precedes that of the creatinine level by 5-24 hours. However, in a large study of patients undergoing cardiac surgery, cystatin C did not show favorable kinetic characteristics compared to serum creatinine in the diagnosis of AKI.

Urinary cystatin C also is a potential marker of acute tubular injury. Because cystatin C usually is reabsorbed in the proximal tubule, the finding of cystatin C in urine suggests some form of proximal tubular injury. However, in patients with albuminuria, there is competitive inhibition of cystatin C uptake (both are transported by megalin), and as a result, significant quantities can appear in urine even in the

absence of tubular injury. This effect has been shown in rats, and similar effects have been noted in patients with diabetes and children with nephrotic syndrome. It should be noted that cystatin C is not the only urinary biomarker that is affected by increasing levels of albuminuria: all the low-molecular-weight proteins under investigation as potential urinary biomarkers potentially could be affected by this process, including neutrophil gelatinase-associated lipocalin (NGAL), liver-type fatty-acid binding protein (L-FABP), and β_2-microglobulin.

Additional Readings

» Briguori C, Visconti G, Rivera NV, et al. Cystatin C and contrast-induced acute kidney injury. *Circulation*. 2010; 121(19):2117-2122.
» Demirtas S, Akan O, Can M, Elmali E, Akan H. Cystatin C can be affected by nonrenal factors: a preliminary study on leukemia. *Clin Biochem*. 2006;39(2):115-118.
» Edelstein CL. *Biomarkers in Kidney Disease.* 1st ed. Amsterdam, the Netherlands: Academic Press/Elsevier; 2011.
» Grubb A. Diagnostic value of analysis of cystatin C and protein HC in biological fluids. *Clin Nephrol*. 1992;38(suppl 1):S20-S27.
» Herget-Rosenthal S, Marggraf G, Husing J, et al. Early detection of acute renal failure by serum cystatin C. *Kidney Int*. 2004;66(3):1115-1122.
» Inker LA, Schmid CH, Tighiouart H, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med.* 2012;367(1):20-29.
» Manzano-Fernandez S, Januzzi JL Jr, Boronat-Garcia M, et al. Beta-trace protein and cystatin C as predictors of long-term outcomes in patients with acute heart failure. *J Am Coll Cardiol.* 2011;57(7):849-858.
» Nejat M, Hill JV, Pickering JW, Edelstein CL, Devarajan P, Endre ZH. Albuminuria increases cystatin C excretion: implications for urinary biomarkers. *Nephrol Dial Transplant.* 2011;27 Suppl 3:iii96-103.
» Nejat M, Pickering JW, Walker RJ, Endre ZH. Rapid detection of acute kidney injury by plasma cystatin C in the intensive care unit. *Nephrol Dial Transplant.* 2010;25(10): 3283-3289.
» Priem F, Althaus H, Jung K, Sinha P. Beta-trace protein is not better than cystatin C as an indicator of reduced glomerular filtration rate. *Clin Chem.* 2001;47(12):2181.
» Shlipak MG, Sarnak MJ, Katz R, et al. Cystatin C and the risk of death and cardiovascular events among elderly persons. *N Engl J Med.* 2005;352(20):2049-2060.
» Spahillari A, Parikh CR, Sint K, 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(6):922-929.

Urine Sediment Analysis

Examination of urine sediment is a time-honored test relied on by generations of nephrologists to aid in the diagnosis of kidney diseases. The actual diagnostic performance characteristics of urine sediment examination and its inter- and intraobserver variability have not been well characterized. The presence of specific findings in urine sediment can aid in the diagnosis of acute and chronic kidney disease prior to more invasive testing. Examination of urine sediment can help discriminate proliferative glomerular disease from nonproliferative diseases. Hematuria and red blood cell casts are a sensitive early sign of relapse in patients with lupus nephritis. White blood cell casts may be seen in tubulointerstitial nephritis, but also may been seen in glomerulonephritis and pyelonephritis. Muddy brown casts, granular casts, and kidney tubular epithelial cells and casts can indicate acute tubular injury, but can be seen in a variety of other acute and chronic kidney diseases.

Table 2. Scoring System for Urine Sediment Based on Numbers of Granular Casts and Kidney Tubular Epithelial Cells

	Granular Casts per HPF		
Kidney tubular epithelial cells per HPF	0 (0 points)	1-5 (1 point)	>6 (2 points)
0 (0 points)	0	1	2
1-5 (1 point)	1	2	3
>6 (2 points)	2	3	4

Abbreviation: HPF, high-power field.

Adapted from Perazella MA, et al. Urine microscopy is associated with severity and worsening of acute kidney injury in hospitalized patients. *Clin J Am Soc Nephrol.* 2010;5(3):402-408.

In the setting of AKI, recent carefully performed studies have suggested that a semiquantitative scoring system based on the number of kidney tubular epithelial cells and granular casts may provide useful information on the differential diagnosis and prognosis of AKI (Table 2). The combination of this score with novel biomarkers, including kidney injury molecule 1 (KIM-1), NGAL, and interleukin 18 (IL-18), has been shown to be more sensitive than creatinine or urine electrolyte levels alone in predicting the need for dialysis in patients with AKI.

However, the diagnostic utility of urine sediment analysis results in clinical practice is uncertain due to a lack of standardization in reporting of results and the potential for significant inter- and intraobserver variability. Most studies of the performance of urine sediment involve clinicians with many years of experience and an interest in examining urine sediment. This may contrast with the experience of the general population of practicing nephrologists, internists, and trainees. There is a need for a single standard for reporting urine sediment analysis results that correlates with results of kidney biopsies and clinical outcomes. Future studies are needed to quantify the diagnostic characteristics of urine sediment examination in a variety of kidney diseases and assess the inter- and intraobserver reliability.

Table 3. Examples of Clinical Settings in Which Novel Biomarkers of Kidney Disease Have Been Studied in Humans

Scenario	Selected Biomarkers
Cardiac surgery	B2M, CyC, GST, IL-18, KIM-1, L-FABP, NAG, netrin, NGAL
Intensive care unit	B2M, CyC, GST, IL-18, KIM-1, L-FABP, NAG, NGAL
Contrast-induced nephropathy	CyC, IL-18, KIM-1, L-FABP, NAG, NGAL
Drug-induced nephrotoxicity	AAP, AP, B2M, CyC, calbindin D, clusterin, GGT, GST, KIM-1, LDH, L-FABP, NAG, NGAL, osteopontin, RBP4
Chronic kidney disease	ADMA, BTP, CTGF, FGF-23, KIM-1, L-FABP, NGAL, RBP4, TFF3, uromodulin
Glomerular disease	IL-18, IP-10, NGAL, MCP-1, NAG, osteopontin, TGFβ

Abbreviations: AAP, alanine aminopeptidase; ADMA, asymmetric dimethyl arginine; AP, alkaline phosphatase; BTP, beta-trace protein; B2M, β_2-microglobulin; CyC, cystatin C; CTGF, connective tissue growth factor; FGF-23, fibroblast growth factor 23; GGT, γ-glutamyl transpeptidase; GST, glutathione-*S*-transferase; IL-18, interleukin 18; IP-10, interferon γ–induced protein 10; KIM-1, kidney injury molecule 1; L-FABP, Liver-type fatty acid binding protein; LDH, lactate dehydrogenase; MCP-1, monocyte chemoattractant protein 1; NAG, *N*-acetyl glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin; RBP4, retinol binding protein 4; TFF3, trefoil factor 3; TGFβ, transforming growth factor β.

Additional Readings

» Fogazzi GB, Ponticelli C, Ritz E. *The Urine Sediment: An Integrated View*, 3rd edition. Milan, Italy: Elsevier Srl; 2010.
» Hall IE, Coca SG, Perazella MA, et al. Risk of poor outcomes with novel and traditional biomarkers at clinical AKI diagnosis. *Clin J Am Soc Nephrol.* 2011;6(12):2740-2749.
» Perazella MA, Coca SG. Traditional urinary biomarkers in the assessment of hospital-acquired AKI. *Clin J Am Soc Nephrol.* 2011;7(1):167-174.
» 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(3):402-408.

Albuminuria

Proteinuria has long been recognized as a consequence of kidney damage. Measurement of urinary protein and urinary albumin is a central component of screening for and monitoring CKD. The glomerulus acts as a barrier to filtration due to pore size and charge selectivity, while most filtered albumin is reabsorbed in the proximal tubule. Thus, the presence of albuminuria usually indicates damage to the filtration barrier, but also may be the result of proximal tubular dysfunction.

Multiple studies have demonstrated that albuminuria is an independent risk factor for mortality in the general population. Albuminuria also is an independent risk factor for the progression of diabetic and nondiabetic CKD. As a result, it has been proposed that stage 3 CKD be stratified further by the presence or absence of albuminuria. Reduction in albuminuria is a commonly accepted goal of treatment in patients with CKD, although it is not as yet an acceptable surrogate end point in clinical trials. However, the US Food and Drug Administration (FDA) recently qualified its use as a biomarker of proximal tubular injury from nephrotoxin exposure in animal studies.

Additional Readings

» Chronic Kidney Disease Prognosis Consortium, Matsushita K, van der Velde M, Astor BC, et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. *Lancet*. 2010;375(9731):2073-2081.
» Levey AS, de Jong PE, Coresh J, et al. The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. *Kidney Int.* 2011; 80(1):17-28.

NOVEL BIOMARKERS

The primary rationale for the development of novel biomarkers of kidney diseases is that early and more accurate diagnosis may allow for interventions that prevent progression. Because levels of current biomarkers of kidney disease become elevated relatively late in the injury process, they do not allow for early interventions that could be successful at preventing propagation of the injury. Early identification could allow for injury-specific interventions that currently are not possible. This could be particularly useful in AKI when a defined insult, for example, after contrast administration or cardiac surgery, could allow for more appropriate risk stratification and treatment (Table 3). Thus, novel biomarkers have a number of potential roles in nephrology: (1) to more accurately diagnose AKI early in the course of the disease, (2) to correlate better with GFR in patients with CKD, (3) to determine the anatomical location of injury in patients with AKI (ie, glomerular, tubular, interstitial, or vascular), (4) to identify the cause of AKI and CKD, (5) to monitor the effectiveness of interventions, and (6) to provide information regarding the prognosis of AKI and CKD.

One significant issue surrounding the development of biomarkers in nephrology is the frequent absence of a reliable gold standard for the diagnosis of kidney

disease. The limitations of creatinine as a biomarker were discussed, but in brief, it is insensitive, not very specific, and the level tends to increase relatively late in the course of kidney injury, when the injury may not be reversible. Currently, the commonly used definitions of AKI (RIFLE, AKIN [AKI Network], and KDIGO [Kidney Disease: Improving Global Outcomes]) incorporate creatinine concentration into the diagnostic framework, whereas equations for estimating GFR in patients with CKD (MDRD Study and CKD-EPI equations) also generally require creatinine level.

It is notable that recent studies have demonstrated the usefulness of novel biomarkers in mild AKI. Urinary levels of certain biomarkers have been found to be elevated in patients with prerenal AKI (KIM-1, cystatin C, and IL-18), whereas others (NGAL and γ-glutamyl transpeptidase [GGT]) were not. A recent meta-analysis found that elevated urinary or plasma NGAL level predicted the future need for renal replacement therapy and in-hospital mortality in critically ill patients in the intensive care unit, even in the absence of a clinically significant increase in creatinine level. This suggests that there is a spectrum of subclinical but significant AKI that is not being detected by the traditional gold-standard methods.

In animal studies, increasing levels of kidney injury biomarkers correlate with the gold standard of increasing structural damage on kidney biopsy in models of AKI. However, there have been no large studies of humans correlating structural appearance with biomarker levels. As a result, we continue to use creatinine as the gold standard in biomarker trials. However, biomarker levels may relate to the severity of kidney injury and be associated with the occurrence of clinical outcomes, including need for dialysis and death. Recently, the FDA qualified a set of urinary biomarkers of nephrotoxicity for regulatory use in certain preclinical settings (ie, animal studies). These included urinary KIM-1, albumin, total protein, albumin, β_2-microglobulin, cystatin C, clusterin, trefoil factor 3, and renal papillary antigen 1. The recent FDA qualification of novel injury biomarkers demonstrates a move away from creatinine as the gold standard for studies in nephrology toward a more nuanced approach.

Additional Readings

» Dieterle F, Sistare F, Goodsaid F, et al. Renal biomarker qualification submission: a dialog between the FDA-EMEA and predictive safety testing consortium. *Nat Biotechnol.* 2010;28(5):455-462.

» Edelstein CL. *Biomarkers in Kidney Disease.* 1st ed. Amsterdam, the Netherlands: Academic Press/Elsevier; 2011.

» Endre ZH, Pickering JW, Walker RJ. Clearance and beyond: the complementary roles of GFR measurement and injury biomarkers in acute kidney injury (AKI). *Am J Physiol Renal Physiol.* 2011;301(4):F697-F707.

» Haase M, Devarajan P, Haase-Fielitz A, et al. The outcome of neutrophil gelatinase-associated lipocalin-positive subclinical acute kidney injury: a multicenter pooled analysis of prospective studies. *J Am Coll Cardiol.* 2011;57(17):1752-1761.

» Nejat M, Pickering JW, Devarajan P, et al. Some biomarkers of acute kidney injury are increased in pre-renal acute injury. *Kidney Int.* 2012;81(12):1254-1262.

» Ozer JS, Dieterle F, Troth S, et al. A panel of urinary biomarkers to monitor reversibility of renal injury and a serum marker with improved potential to assess renal function. *Nat Biotechnol.* 2010;28(5):486-494.

» Waikar SS, Betensky RA, Emerson SC, Bonventre JV. Imperfect gold standards for kidney injury biomarker evaluation. *J Am Soc Nephrol.* 2012;23(1):13-21.

Plasma/Serum Versus Urinary Biomarkers

Urine and plasma/serum are potential sources of biomarkers in nephrology. Both are easily collected and minimally invasive. Urine has a number of advantages over serum or plasma for the evaluation of potential biomarkers. Urine is a proximal fluid, meaning that it is in close contact to the site of injury (the kidney), and as such, is a site where biomarkers can accumulate after being shed by damaged kidney tissue. In patients with AKI, urinary biomarker levels increase relatively early in the course of the disease. In contrast, for serum markers of GFR, such as creatinine and cystatin C, there is a significant lag between the time of injury and the time that their concentrations will exceed the threshold required to make a diagnosis of AKI. Urinary biomarkers can be separated into those that are present in the kidney and released in the event of damage (eg, proximal tubular enzymes) and those induced by tubular injury (eg, KIM-1, NGAL, and IL-18). Preformed biomarkers appear in urine shortly after injury, whereas there is a time lag for the appearance of the induced biomarkers.

Urine biomarkers have a number of important potential limitations. It is important that urine is handled correctly. Urinary biomarkers may not be stable for long periods and urine should be frozen promptly if it is not analyzed immediately. There is a potential for error if urine is not collected appropriately. Midstream urine samples are more informative in women, presumably because initial voids are more prone to bacterial contamination. In contrast, for males, prostate cancer markers are detectable more readily in first-void urine samples. Baseline kidney function is an important modifying influence. As detailed next, a major limitation of urinary biomarkers is that their concentration changes with hydration status, and multiple methods of dealing with this problem have been suggested, including normalizing to urinary creatinine concentration.

Serum or plasma also is a good potential source of biomarkers. It is obtainable in anuric patients and is less prone to bacterial contamination. However, changes in serum biomarker concentrations are not necessarily related to decreased kidney function alone and can be the product of a systemic response. The large number of proteins present in serum makes the discovery of specific kidney markers more complicated.

Additional Readings

» Edelstein CL. *Biomarkers in Kidney Disease.* 1st ed. Amsterdam, the Netherlands: Academic Press/Elsevier; 2011.
» Hewitt SM, Dear J, Star RA. Discovery of protein biomarkers for renal diseases. *J Am Soc Nephrol.* 2004;15(7):1677-1689.
» Wu J, Chen YD, Gu W. Urinary proteomics as a novel tool for biomarker discovery in kidney diseases. *J Zhejiang Univ Sci B.* 2010;11(4):227-237.

Normalization of Urinary Biomarkers

Because the kidney has the ability to reabsorb water over a wide range, the concentration of a biomarker in urine depends not only on its rate of production, but also on urine flow rate. Because of this, absolute values of urine biomarkers will change significantly depending on urine flow rate, thus making them difficult to interpret. Ideally, urinary biomarkers should be normalized by expressing them as a ratio to a substance that is excreted at a constant rate by the kidneys. In practice, urinary biomarkers commonly are expressed as a ratio to urinary creatinine concentration. This assumes that creatinine production is relatively constant across individuals and relative to the urinary biomarker concentrations in healthy and diseased states. However, urine creatinine concentration is affected by a number of factors, including creatinine generation rate, which varies significantly between individuals. As a result, normalizing biomarker concentrations to creatinine concentration potentially will introduce bias. There are 4 settings in particular in which normalizing to creatinine concentration could generate misleading results: (1) when there are significantly higher or lower creatinine generation rates (ie, increased or reduced muscle mass) or more extrarenal degradation of creatinine (which is substantially higher in patients with advanced CKD), (2) in the setting of an acute change in GFR in which excretion of creatinine has not reached a steady state, (3) in the setting of markedly increased or decreased tubular secretion of creatinine, and (4) in low urine flow states in which there may be reabsorption of creatinine in the proximal tubules.

As a result, alternative methods of urine normalization have been proposed. There is some interest in the use of alternative urinary peptides, although the development of these techniques is in the very early stages. The use of short timed urine collections also has been proposed, although this is not always practical in the ambulatory setting. Similarly, normalizing to the actual urine flow rate at the time of collection would lead to more unbiased results, although measuring this accurately is not without difficulty. Interestingly, one recent study suggested that absolute biomarker values were better at diagnosing AKI, whereas normalizing to creatinine level was a better predictor of overall adverse outcomes. This finding may reflect the prognostic significance of creatinine generation rate, which influences urinary creatinine concentration and therefore creatinine-normalized biomarker levels. For the moment, most studies continue to normalize to creatinine level, understanding that this is an imperfect technique. In a clinical setting, it should be recognized that results should be interpreted with reference to the factors that may influence urinary creatinine concentration.

Additional Readings

» Conti M, Moutereau S, Esmilaire L, et al. Should kidney tubular markers be adjusted for urine creatinine? The example of urinary cystatin C. *Clin Chem Lab Med.* 2009;47(12):1553-1556.
» Jantos-Siwy J, Schiffer E, Brand K, et al. Quantitative urinary proteome analysis for biomarker evaluation in chronic kidney disease. *J Proteome Res.* 2009;8(1):268-281.
» Ralib AM, Pickering JW, Shaw GM, et al. Test characteristics of urinary biomarkers depend on quantitation method in acute kidney injury. *J Am Soc Nephrol.* 2012;23(2):322-333.
» Waikar SS, Sabbisetti VS, Bonventre JV. Normalization of urinary biomarkers to creatinine during changes in glomerular filtration rate. *Kidney Int.* 2010;78(5):486-494.

NOVEL BIOMARKERS IN CLINICAL CONDITIONS

Novel Biomarkers of AKI

AKI is a syndrome characterized by a rapid decrease in GFR associated with the accumulation of waste products usually excreted by the kidney. It is relatively common, occurring in up to 9% of all hospitalized patients, and is particularly prevalent in patients admitted to the intensive care unit. The mortality of patients requiring dialysis in the intensive care unit is 40%-60%, and this has remained high during the past 20 years. Current definitions of AKI include a change in serum creatinine level over a defined period as one of the major criteria. As mentioned, this leads to a delay in diagnosis because of the lag time between the occurrence of the injury and an increase in serum creatinine level (Fig 2). As a result, much effort is being focused on biomarkers of AKI that can make the diagnosis earlier and more accurately and possibly enable the use of strategies to prevent progression of this syndrome.

One of the best-studied new biomarkers of AKI is NGAL, a 21-kDa protein. It is involved in innate

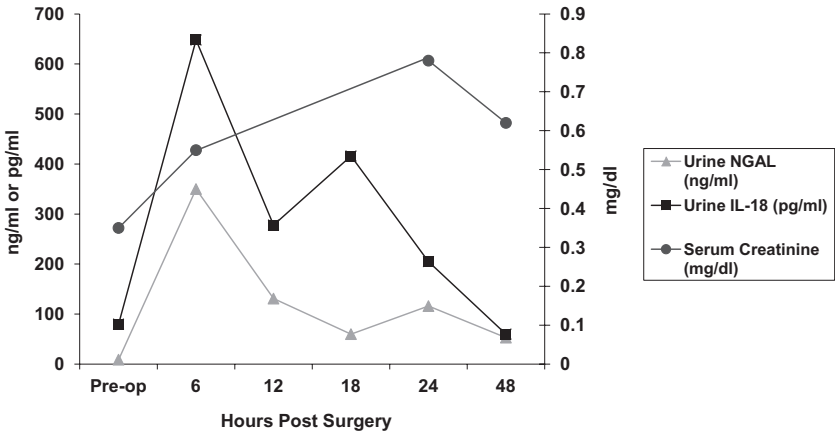

Figure 2. Pattern of change in biomarkers over time in patients with acute kidney injury following cardiac surgery. Abbreviation: NGAL, neutrophil gelatinase-associated lipocalin. Conversion factor for units: serum creatinine in mg/dL to μmol/L, $\times$88.4. Data from Parikh CR, et al. Postoperative biomarkers predict acute kidney injury and poor outcomes after pediatric cardiac surgery. *J Am Soc Nephrol.* 2011;22:1737-1747.

immunity and is expressed primarily by immune cells, but also by hepatocytes and kidney tubular cells. It originally was identified through transcriptome analysis in a mouse model of ischemia-reperfusion injury in which its production in kidney tubules was noted to increase rapidly after kidney injury. It is readily detectable in urine and resistant to degradation by proteases, making it a potentially ideal biomarker of AKI. NGAL appears in urine early after injury, and studies of cultured human tubular cells confirmed that it is produced in response to hypoxic injury. Urinary and plasma NGAL levels have been shown to correlate with degree of kidney injury, and levels return to baseline on resolution of AKI. Interestingly, the source of plasma and urinary NGAL appears to be different. Most urinary NGAL is produced in tubules in response to injury, whereas most plasma NGAL originates in distant organs, where its production is upregulated in the setting of AKI. Commercial assays are available for NGAL measurement.

Urinary NGAL has been shown to be a possible early biomarker of AKI in adults and children after cardiac surgery and after administration of radiocontrast. Similarly, elevations of NGAL levels predict AKI in patients presenting to the emergency department. A large meta-analysis of studies of NGAL in AKI found that there was no advantage in using urinary NGAL as opposed to serum NGAL. NGAL performed better in children, although it remained useful in adults, suggesting that there may be other factors that influence NGAL levels in this population. A wide range of cutoff values for urinary NGAL were used in the various studies, but a level $>$150 ng/mL appeared to be the most appropriate, particularly when commercial assays were used. Interestingly, normalization of results to urinary creatinine level did not appear to affect the test accuracy. Elevated NGAL level in the absence of elevated serum creatinine level may be prognostically significant and suggests that creatinine level may misclassify individuals with currently subclinical kidney disease. This was borne out by a meta-analysis that found that elevations in NGAL level even in the absence of an elevated creatinine level predicted worse outcomes. This finding suggests that there may need to be further changes in the way that we define AKI to incorporate these biomarkers.

IL-18 is an 18-kDa proinflammatory cytokine that is expressed primarily by macrophages, but also by monocytes, dendritic cells, and kidney epithelial cells. It has a role in the innate and adaptive immune response and is upregulated in inflammatory states. IL-18 is thought to be one of the mediators of injury in ischemic AKI. Inhibition of IL-18 has been shown to be effective in treating inflammatory disorders in mice, and interstitial IL-18 expression is increased in mouse models of AKI. Mice deficient in caspase, an IL-18–activating enzyme, develop less acute tubular necrosis than wild-type mice in models of ischemia. Urinary IL-18 originates in the kidney tubular epithelium and thus was proposed as a potential marker of AKI.

IL-18 level has been shown to increase early in patients with sepsis in the intensive care unit and was a good predictor of AKI, particularly when combined with NGAL level. IL-18 levels increased in patients with acute tubular necrosis, but not those with prerenal AKI or CKD and healthy controls. Similarly, increasing IL-18 levels predicted AKI in pediatric patients undergoing cardiac surgery. Higher postoperative urinary IL-18 excretion also has been shown to identify AKI and predict progression of AKI after cardiac surgery in adults. Potential limitations of IL-18 derive from the fact that it may be a more generalized marker of inflammation rather than a specific marker of AKI, particularly in older age groups, for whom there may be underlying baseline decreased kidney function.

Another biomarker that has attracted considerable interest is KIM-1. KIM-1 initially was found to be highly upregulated in a rat model of ischemic kidney

injury. It is a transmembrane protein that is expressed at very low levels in normal kidney, but its production increases in dedifferentiated proximal tubular cells in the presence of ischemic or nephrotoxic AKI. The extracellular domain of KIM-1 appears in urine shortly after ischemic injury and can be detected readily by a new KIM-1 urinary dipstick, potentially making it a convenient and readily measured marker of AKI.

Early studies of humans found that, in common with the rat, KIM-1 expression was increased in kidney biopsy specimens from patients with acute tubular necrosis. Elevated urinary KIM-1 level predicted increased risk of mortality or need for dialysis in hospitalized patients with AKI and was a sensitive predictor of AKI in children undergoing cardiac surgery. Its performance as a biomarker of AKI may prove to be better in patients with normal baseline kidney function, as is the case for many novel biomarkers. This may be because although expression is very limited in normal kidney tissue, there is upregulation of expression in various CKDs, with the degree of expression correlating with tubulointerstitial fibrosis. Its role in CKD currently is under investigation.

The kidneys contain large amounts of enzymes that perform specialized functions in tubular cells. These enzymes can be released into urine in the event of kidney injury both as a result of leakage from cells (preformed enzymes) or by upregulation of their production in response to injury. Because they are localized to specific cells along the nephron, the presence of certain enzymes in urine can give a clue to the specific location of the kidney injury. *N*-Acetylglucosaminidase (NAG) is a lysosomal enzyme primarily localized to the proximal tubule. Glutathione-S-transferase (GST) is a family of enzymes with 8 different classes found throughout the nephron. However, the α isoform is present in proximal tubular cells alone, whereas the π isoform is found in only distal tubular cells. Alkaline phosphatase, GGT, alanine aminopeptidase, and lactate dehydrogenase are brush-border enzymes originating in the proximal tubule that normally are present in urine in small quantities and increase significantly in the setting of AKI. Tubular enzymuria has been noted in patients with a broad range of kidney diagnoses, including acute tubular necrosis, interstitial nephritis, nephrotoxicity, and acute transplant rejection. No individual enzyme has been shown to be a consistent predictor of AKI or need for dialysis across all studies, and although some have shown promise in certain homogenous populations, these results have not been replicated across heterogeneous groups. This may be due in part to the fact that enzymuria is increased in many settings and is not specific for AKI. There also may be settings in which activity of the enzymes is decreased by exogenous factors, for example, although NAG has been shown to be a sensitive marker of AKI in certain situations, the presence of heavy metals and other nephrotoxins in urine inhibits its activity, reducing the sensitivity of the assay. There is a suggestion that panels of enzymes may be more useful than individual enzymes in diagnosing kidney disorders, although their primary use may lie in the diagnosis of drug nephrotoxicity, in which levels of these enzymes increase prior to any increase in creatinine level and may help guide drug dosing.

Apart from cystatin, there are a number of other proteins that normally are filtered at the glomerulus, but do not appear in urine because of uptake and metabolism in the proximal tubule. The presence of these biomarkers in urine suggests tubular dysfunction (although, as mentioned previously, in the presence of sufficient albuminuria, the normal uptake mechanism can be overwhelmed and they can become present even with normal tubular function). The most important of these are β_2-microglobulin and retinol binding protein 4 (RBP-4). β_2-Microglobulin is an 11.8-kDa protein component of the major histocompatibility complex class I molecule that is filtered freely in the glomerulus and reabsorbed and metabolized by the tubules, with $<1\%$ appearing in urine. Urinary β_2-microglobulin excretion increases in the setting of nonselective proteinuria and also as a result of tubular damage, particularly as a result of exposure to tubular toxins. Urinary β_2-microglobulin has been studied in a wide range of clinical contexts and appears to be useful in distinguishing prerenal azotemia from acute tubular necrosis, but is of uncertain utility in patients with sepsis, in whom its levels can increase in the absence of AKI. RBP-4 is synthesized primarily by hepatocytes and is involved in the transport of retinol in blood. The C-terminally processed form is excreted in urine and accumulates in patients with decreased GFR. RBP-4 is reabsorbed in the proximal tubule and appears in large quantities when there is significant proximal tubular damage. It is a particularly sensitive marker of nephrotoxicity and levels are elevated in decreased kidney function caused by heavy metals and in patients with diabetic nephropathy. One advantage that it carries over β_2-microglobulin is that it is more stable at low pH.

There are a large number of other biomarkers that are under investigation to determine their utility in the early diagnosis of kidney diseases, including AKI. Most are proteins expressed in tubules for which urinary concentrations change in the event of tubular injury. Trefoil factor 3 is a protein involved in maintenance of the integrity of mucosal surfaces. It normally is found in urine, and decreasing levels are a sensitive marker of proximal tubular/collecting duct dysfunc-

tion, particularly due to nephrotoxicity. L-FABP is a cytoplasmic protein expressed in proximal tubules that binds free fatty acids and transports them to the mitochondria for metabolism. Increased urinary L-FABP excretion has been noted in patients with AKI (particularly ischemic), nephrotoxicity, and severe sepsis in the absence of AKI.

Osteopontin is a 44-kDa protein synthesized primarily in bone, but also in macrophages, activated T cells, and endothelial cells. In the kidney, it is expressed in the loop of Henle and distal tubules and is involved in kidney protection against oxidative stress and ischemia. Increased urinary osteopontin levels have been noted in some chronic kidney diseases, including immunoglobulin A (IgA) nephropathy, membranous nephropathy, and glomerulonephritis, as well as in patients with AKI. Netrin-1 is an axonal-guidance molecule that is not expressed in normal tubular cells but is highly expressed after kidney injury, with levels increasing within 2 hours of an acute insult. Levels correlate with degree of injury and return to normal as the injury resolves. Clusterin is an 80-kDa protein that is expressed constitutively during normal kidney development. Its production is upregulated in response to kidney injury and it is expressed throughout the nephron. It has shown promise as a biomarker of tubular injury and regeneration in animal models, but has not yet been investigated in large studies in humans.

Additional Readings

» Amin AP, Salisbury AC, McCullough PA, et al. Trends in the incidence of acute kidney injury in patients hospitalized with acute myocardial infarction. *Arch Intern Med.* 2012;172(3):246-253.

» Bagshaw SM, Langenberg C, Haase M, Wan L, May CN, Bellomo R. Urinary biomarkers in septic acute kidney injury. *Intensive Care Med.* 2007;33(7):1285-1296.

» Bellomo R, Kellum JA, Ronco C. Acute kidney injury. *Lancet*. 2012;380(9843):756-766.

» Bonventre JV. Kidney injury molecule-1 (KIM-1): a urinary biomarker and much more. *Nephrol Dial Transplant.* 2009;24(11):3265-3268.

» Edelstein CL. *Biomarkers in Kidney Disease.* 1st ed. Amsterdam, the Netherlands: Academic Press/Elsevier; 2011.

» Koyner JL, Garg AX, Coca SG, et al. Biomarkers predict progression of acute kidney injury after cardiac surgery. *J Am Soc Nephrol.* 2012;23(5):905-914.

» Haase M, Bellomo R, Devarajan P, Schlattmann P, Haase-Fielitz A; NGAL Meta-analysis Investigator Group. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. *Am J Kidney Dis.* 2009;54(6):1012-1024.

» Haase M, Bellomo R, Story D, Davenport P, Haase-Fielitz A. Urinary interleukin-18 does not predict acute kidney injury after adult cardiac surgery: a prospective observational cohort study. *Crit Care.* 2008;12(4):R96.

» Haase M, Devarajan P, Haase-Fielitz A, et al. The outcome of neutrophil gelatinase-associated lipocalin-positive subclinical acute kidney injury: a multicenter pooled analysis of prospective studies. *J Am Coll Cardiol.* 2011;57(17):1752-1761.

» Han WK, Bailly V, Abichandani R, Thadhani R, Bonventre JV. Kidney injury molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury. *Kidney Int.* 2002;62(1):237-244.

» Liangos O, Perianayagam MC, Vaidya VS, et al. Urinary *N*-acetyl-beta-(D)-glucosaminidase activity and kidney injury molecule-1 level are associated with adverse outcomes in acute renal failure. *J Am Soc Nephrol.* 2007;18(3):904-912.

» Lisowska-Myjak B. Serum and urinary biomarkers of acute kidney injury. *Blood Purif.* 2010;9(4):357-365.

» Mishra J, Ma Q, Prada A, et al. Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. *J Am Soc Nephrol.* 2003;14(10):2534-2543.

» Parikh CR, Coca SG, Thiessen-Philbrook H, et al. Postoperative biomarkers predict acute kidney injury and poor outcomes after adult cardiac surgery. *J Am Soc Nephrol.* 2011;22(9):1748-1757.

» Parikh CR, Jani A, Melnikov VY, Faubel S, Edelstein CL. Urinary interleukin-18 is a marker of human acute tubular necrosis. *Am J Kidney Dis.* 2004;43(3):405-414.

» Parikh CR, Mishra J, Thiessen-Philbrook H, et al. Urinary IL-18 is an early predictive biomarker of acute kidney injury after cardiac surgery. *Kidney Int.* 2006;70(1):199-203.

» Urbschat A, Obermuller N, Haferkamp A. Biomarkers of kidney injury. *Biomarkers*. 2011;16(suppl 1):S22-S30.

» Waikar SS, Betensky RA, Bonventre JV. Creatinine as the gold standard for kidney injury biomarker studies? *Nephrol Dial Transplant*. 2009;24(11):3263-3265.

» Waring WS, Moonie A. Earlier recognition of nephrotoxicity using novel biomarkers of acute kidney injury. *Clin Toxicol (Phila)*. 2011;49(8):720-728.

» Yu Y, Jin H, Holder D, et al. Urinary biomarkers trefoil factor 3 and albumin enable early detection of kidney tubular injury. *Nat Biotechnol.* 2010;28(5):470-477.

Novel Biomarkers of CKD

CKD affects ~11% of the US population and is a major contributor to morbidity and mortality. Early identification of those at risk of CKD is important and has been facilitated by the routine use of estimating equations for GFR (MDRD Study and CKD-EPI equations). Classification of CKD into stages and stratification by degree of proteinuria has helped identify patients at risk of progression to end-stage renal disease. However, current techniques for estimating GFR rely on serum creatinine level, with all its attendant deficiencies, and newer biomarkers are being sought that may better stratify patients by risk category. In common with AKI, no single biomarker has been identified that can reliably predict progression of CKD.

As discussed, creatinine and cystatin C are the biomarkers used most often to determine GFR. Another promising marker of GFR is beta-trace protein (BTP). BTP is a low-molecular-mass protein belonging to the lipocalin protein family that is produced at a constant rate by glial cells in the central nervous

system. It is filtered freely at the glomerulus and reabsorbed and metabolized in the proximal tubule. There is almost no nonrenal elimination. It has been shown to be as good as cystatin C and creatinine levels at detecting mild abnormalities in kidney function and also may be better than creatinine level at predicting adverse outcomes in patients with acute heart failure. Estimating equations are being derived that include serum BTP but they have not been validated in large studies. BTP can aid in the prediction of progression of CKD, but similar to cystatin C, corticosteroids can influence levels independent of GFR.

As well as its potential role in the diagnosis of AKI, NGAL may be a useful biomarker in patients with CKD, particularly for identifying patients at risk of a significant decrease in GFR, because of its ability to detect subtle changes in tubular function. Urinary and serum NGAL levels are elevated in a wide range of kidney diseases, including IgA nephropathy, autosomal dominant polycystic kidney disease, and diabetic nephropathy. Urinary NGAL level has been shown to differentiate HIV (human immunodeficiency virus) nephropathy from other forms of kidney disease, whereas higher levels were associated with increased risk of progression in a diverse group of patients with CKD.

AKI itself is a risk factor for future CKD, but there is no reliable means of determining who will recover entirely and who will be left with some kidney impairment after an episode of AKI. NGAL is a potential marker of future progression. Similarly, KIM-1 may have a role in predicting the transition from AKI to CKD. Urinary KIM-1 levels have been shown to correlate with proteinuria, decreasing in response to treatment with angiotensin-converting enzyme inhibitors or a low-sodium diet, suggesting a potential role as a measure of therapeutic efficacy.

Urinary L-FABP level correlates with degree of proteinuria in patients with CKD. In patients with diabetes, it has been shown to correlate with GFR and predict progression to end-stage renal disease. Baseline levels predicted the future development of albuminuria, suggesting a potential role in stratifying patients who might benefit from early preventative therapies. Other potential urinary biomarkers of CKD include connective tissue growth factor, L-FABP, and trefoil factor 3.

Asymmetric dimethylarginine (ADMA) is an inhibitor of nitric oxide synthase and a marker of endothelial function. Increasing plasma levels predict progression of CKD and mortality in patients with chronic kidney failure in diabetic and nondiabetic kidney disease. Increased plasma levels also are predictive of future cardiovascular mortality in patients with known CKD and cardiovascular disease.

Fibroblast growth factor 23 (FGF-23) is a phosphaturic hormone that is elevated in patients with CKD. The degree of elevation correlates with stage of CKD, and elevations in FGF-23 levels are noted prior to elevation in phosphate levels.

In order to be useful for clinical practice, new biomarkers of CKD progression will have to prove superior to the impressive prognostic ability of estimated GFR and albuminuria. New biomarkers of GFR estimation will have to improve precision, bias, and the prognostic ability of creatinine and cystatin C levels.

Studies from the National Institutes of Health CKD Biomarkers Consortium and other investigators are underway to identify and validate novel biomarkers of CKD.

Additional Readings

» Bolignano D, Lacquaniti A, Coppolino G, et al. Neutrophil gelatinase-associated lipocalin (NGAL) and progression of chronic kidney disease. *Clin J Am Soc Nephrol.* 2009;4(2):337-344.

» Fassett RG, Venuthurupalli SK, Gobe GC, Coombes JS, Cooper MA, Hoy WE. Biomarkers in chronic kidney disease: a review. *Kidney Int.* 2011;80(8):806-821.

» Kamijo-Ikemori A, Sugaya T, Yasuda T, et al. Clinical significance of urinary liver-type fatty acid-binding protein in diabetic nephropathy of type 2 diabetic patients. *Diabetes Care.* 2011;34(3):691-696.

» Lu TM, Chung MY, Lin CC, Hsu CP, Lin SJ. Asymmetric dimethylarginine and clinical outcomes in chronic kidney disease. *Clin J Am Soc Nephrol*. 2011;6(7):1566-1572.

» Manzano-Fernandez S, Januzzi JL, Boronat-Garcia M, et al. Impact of kidney dysfunction on plasma and urinary N-terminal pro-B-type natriuretic peptide in patients with acute heart failure. *Congest Heart Fail.* 2010;16(5):214-220.

» Priem F, Althaus H, Jung K, Sinha P. Beta-trace protein is not better than cystatin C as an indicator of reduced glomerular filtration rate. *Clin Chem.* 2001;47(12):2181.

» Ravani P, Tripepi G, Malberti F, Testa S, Mallamaci F, Zoccali C. Asymmetrical dimethylarginine predicts progression to dialysis and death in patients with chronic kidney disease: a competing risks modeling approach. *J Am Soc Nephrol.* 2005;16(8):2449-2455.

» Spanaus KS, Kollerits B, Ritz E, et al. Serum creatinine, cystatin C, and beta-trace protein in diagnostic staging and predicting progression of primary nondiabetic chronic kidney disease. *Clin Chem*. 2010;56(5):740-749.

» Waanders F, Vaidya VS, van Goor H, et al. Effect of renin-angiotensin-aldosterone system inhibition, dietary sodium restriction, and/or diuretics on urinary kidney injury molecule 1 excretion in nondiabetic proteinuric kidney disease: a post hoc analysis of a randomized controlled trial. *Am J Kidney Dis.* 2009;53(1):16-25.

Biomarkers of Nephrotoxicity

Nephrotoxicity is an unfortunate side effect of many drugs and a relatively common cause of acute and chronic kidney injury. Currently, clinical nephrotoxicity is diagnosed when there is an increase in serum creatinine concentration or a decrease in urine

output. These are relatively late complications of drug-induced kidney injury and also are nonspecific. Often, nephrotoxicity is a diagnosis of exclusion when other causes are ruled out. Nephrotoxicity can be localized to a specific part of the nephron, which serum creatinine cannot distinguish. Proteinuria (eg, due to vascular endothelial growth factor inhibitors) and subtle signs of defects in proximal tubular function (eg, related to the use of antiretroviral medications) can suggest specific forms of nephrotoxicity, but these generally arise late in the process. Specific biomarkers that have increased levels earlier in the course of nephrotoxicity and more accurately localize the site of the lesion would be of great benefit in both the clinical management of nephrotoxicity (by identifying patients at risk and allowing the withdrawal of an offending agent) and drug development. Because it is a defined insult at a specific time, drug nephrotoxicity is a good model for the study of AKI and there is much ongoing research in this field.

NAG is produced by proximal tubular cells in response to ischemic and oxidative stress. In animal studies, increased urinary NAG excretion is a sensitive marker of gentamicin and cisplatin toxicity, with decreasing levels after antioxidant therapy suggesting a potential role for this class of drugs in some cases. Other tubular enzymes also are potential markers of nephrotoxicity. Increased GST-α levels suggest proximal tubular damage in models of methotrexate-induced kidney damage, and GST-π is a marker of distal tubular dysfunction. Levels of GGT, alanine aminopeptidase, and lactate dehydrogenase all increase in the setting of gentamicin and vancomycin use, in the absence of a decrease in GFR, suggesting a potential role in monitoring for potential toxicity. Urinary calbindin-D may be a marker of distal renal tubular injury caused by cisplatin-based chemotherapy.

One study evaluated the performance of a panel of biomarkers in a model of acute and subacute gentamicin toxicity. The panel included urinary cystatin C, NGAL, KIM-1, β_2-microglobulin, clusterin, GST-α, and osteopontin. Urinary cystatin C and NGAL were the most sensitive markers of gentamicin toxicity, with level changes appearing within 1 day, whereas KIM-1 levels best correlated with histologic appearance in the subacute model. In another model of gentamicin nephrotoxicity, urinary KIM-1 levels increased within 24 hours of administration, while urinary NAG and serum urea nitrogen and creatinine levels remained in the normal range. This was despite histologic evidence of necrosis in half the kidney tubules, thus elegantly illustrating the need for more sensitive biomarkers than the traditional panel.

As noted earlier, the FDA has qualified the following biomarkers—urinary KIM-1, albumin, total protein, β_2-microglobulin, cystatin C, clusterin, trefoil factor-3, and renal papillary antigen 1—along with traditional clinical chemistry markers and histopathology for the detection of acute drug-induced nephrotoxicity in rat toxicology studies.

Additional Readings

» Ali BH, Al-Salam S, Al-Husseini I, Nemmar A. Comparative protective effect of *N*-acetyl cysteine and tetramethylpyrazine in rats with gentamicin nephrotoxicity. *J Appl Toxicol.* 2009;29(4):302-307.

» Hoffmann D, Fuchs TC, Henzler T, et al. Evaluation of a urinary kidney biomarker panel in rat models of acute and subchronic nephrotoxicity. *Toxicology*. 2010;277(1-3):49-58.

» Waring WS, Moonie A. Earlier recognition of nephrotoxicity using novel biomarkers of acute kidney injury. *Clin Toxicol (Phila).* 2011;49(8):720-728.

» Zhou Y, Vaidya VS, Brown RP, et al. Comparison of kidney injury molecule-1 and other nephrotoxicity biomarkers in urine and kidney following acute exposure to gentamicin, mercury, and chromium. *Toxicol Sci*. 2008;101(1):159-170.

Biomarkers of Glomerular Disease

The clinical course of glomerular diseases is variable and difficult to predict without serial kidney biopsies. Treatments generally are toxic, and repeated courses of immunosuppression sometimes are indicated in relapsing cases. An ideal biomarker in patients with glomerular diseases would be one that gives information about disease activity, prognosis, and likelihood of clinical relapse.

This is particularly relevant in patients with lupus nephritis. Lupus nephritis is a highly variable disease with multiple classes requiring different treatments. Patients can move from one class to another, and rates of relapse are high. Biomarkers that could noninvasively identify the class of lupus, predict response to therapy, and alert to relapse could revolutionize treatment. Urinary NGAL has been investigated as a potential biomarker of disease activity in patients with lupus. It has been shown to predict kidney flares better than traditional methods without any correlation with extrarenal disease. Urinary NGAL excretion also was higher in patients with lupus nephritis than in those with clinical lupus without kidney involvement. A recent study examined a panel of biomarkers in a diverse group of patients with lupus nephritis and found that the combination of urinary NGAL and monocyte chemotactic peptide 1 (MCP-1) was an excellent test of lupus nephritis chronicity, whereas the combination of MCP-1, α_1-acid glycoprotein, and ceruloplasmin predicted disease activity. MCP-1 is a chemotactic chemokine that is specific for monocytes. Increased urinary levels have been noted in patients at the time of lupus flares, and the increase can be seen

up to 4 months before the clinical flare. A recent pilot study has suggested that a combination of urinary proteins and clinical variables could be used to derive a potentially useful composite biomarker panel that reflects specific pathologic lesions, such as tubulointerstitial inflammation in lupus nephritis. Currently, no single biomarker can definitively predict a lupus flare. Because the treatment is relatively toxic, the specificity of any predictive test would have to be very high to justify its use. It is possible that a suitable panel of biomarkers may be developed that will allow for this degree of certainty.

Apart from predicting relapses, another potential benefit of biomarkers would be to noninvasively determine which class of lupus is present in patients with known lupus nephritis. Currently, this is done by examining the urine sediment and quantifying proteinuria. The presence of red blood cell casts suggests class III or IV nephritis, whereas heavy proteinuria is more suggestive of membranous lupus nephritis. This is not an exact science and it is not unusual for a kidney biopsy specimen to be unexpectedly abnormal in a patient with relatively benign urine sediment. A number of biomarkers are being investigated for their potential in identifying lupus class. A recent report examined urine metabolomic profiles of patients with different classes of lupus and found that patients with class III/IV lupus had significantly lower taurine levels than controls and patients with other classes of lupus. Patients with class V lupus had significantly lower urinary citrate levels. Urinary CXCR3, interferon-producing protein 10, transforming growth factor β, and vascular endothelial growth factor also were able to differentiate class IV lupus from other classes, with urinary interferon-producing protein 10 performing the best. None of these tests are reliable enough currently to take the place of kidney biopsy at present.

IgA nephropathy is the most common glomerulonephritis and a frequent cause of end-stage renal disease. The clinical course of IgA nephropathy is very variable; the presence of crescents on a kidney biopsy specimen suggests a more aggressive disease with a poorer prognosis and mandates the use of aggressive immunosuppression. Currently, patients are stratified according to the presence of specific histologic features on kidney biopsy specimens and degree of proteinuria at presentation. Many biomarkers are being investigated to determine their utility in predicting which patients will progress to chronic kidney failure and earlier stages of CKD and the overall response to therapy without resorting to follow-up biopsies. One study of patients with crescentic IgA found that combining the number of preserved glomeruli on biopsy with serum creatinine level at baseline and fractional excretion of IgG was able to classify patients as responders or nonresponders to therapy in all cases. One limitation of this study of course was the requirement for a kidney biopsy. More recently, urinary IL-18 has been shown to discriminate patients who experience progression from those who remain stable. NGAL and NAG levels are elevated in patients with IgA nephropathy and significant tubulointerstitial disease. IL-6 is a cytokine expressed by antigen-presenting cells that has been shown to have elevated levels in urine of patients with active lupus nephritis. Similarly, elevated urinary IL-6 levels have been noted in patients with progressive IgA nephropathy relative to controls, suggesting ongoing intrarenal inflammation. Cytokines including MCP-1 also have been shown to have increased levels in patients with progressive disease.

Antineutrophil cytoplasmic antibody (ANCA) disease is a pauci-immune crescentic vasculitis that commonly affects the kidneys and is associated with a high risk of relapse. Active urinary sediment and increasing ANCA titer are traditional biomarkers of relapse, but these can be unreliable. Recently, a proteome analysis of urine from patients with ANCA disease and matched controls found that a panel of 47 biomarkers could reliably distinguish ANCA disease from other causes of kidney dysfunction and also correlated with disease activity and response to therapy. Most of these biomarkers were related to breakdown products of hemoglobin or albumin.

The diagnosis and treatment of membranous nephropathy may be aided in the future by the discovery that ~80% of patients with primary membranous nephropathy have IgG4 antibodies to the M-type phospholipase A2 receptor in their serum that is not present in healthy controls. Levels of this antibody correlate with disease activity and response to treatment. Interestingly, the presence of these antibodies in patients prior to transplantation does not necessarily predict recurrence, suggesting that there is an interplay of risk factors that predisposes to this disease. Other autoantibodies, including antibodies to bovine serum albumin and superoxide dismutase, are being evaluated for their roles in the pathogenesis of idiopathic membranous nephropathy. Other biomarkers that may have a role in understanding the pathogenesis, clinical identification, and management of patients with nephrotic syndrome include circulating soluble urokinase receptor in focal segmental glomerulosclerosis and podocyte-secreted angiopoietin-like-4 in minimal change disease.

Additional Readings

» Avihingsanon Y, Phumesin P, Benjachat T, et al. Measurement of urinary chemokine and growth factor messenger RNAs: a noninvasive monitoring in lupus nephritis. *Kidney Int*. 2006;69(4):747-753.

» Bazzi C, Rizza V, Raimondi S, Casellato D, Napodano P, D'Amico G. In crescentic IgA nephropathy, fractional excretion of IgG in combination with nephron loss is the best predictor of progression and responsiveness to immunosuppression. *Clin J Am Soc Nephrol.* 2009;4(5):929-935.

» Brunner HI, Bennett MR, Mina R, et al. Non-invasive renal protein biomarkers are associated with histological features of lupus nephritis. *Arthritis Rheum.* 2012;64(8):2687-2697.

» Haubitz M, Good DM, Woywodt A, et al. Identification and validation of urinary biomarkers for differential diagnosis and evaluation of therapeutic intervention in anti-neutrophil cytoplasmic antibody-associated vasculitis. *Mol Cell Proteomics.* 2009;8(10):2296-2307.

» Herrmann SM, Sethi S, Fervenza FC. Membranous nephropathy: the start of a paradigm shift. *Curr Opin Nephrol Hypertens*. 2012;21(2):203-210.

» Pitashny M, Schwartz N, Qing X, et al. Urinary lipocalin-2 is associated with renal disease activity in human lupus nephritis. *Arthritis Rheum.* 2007;56(6):1894-1903.

» Romick-Rosendale LE, Brunner HI, Bennett MR, et al. Identification of urinary metabolites that distinguish membranous lupus nephritis from proliferative lupus nephritis and focal segmental glomerulosclerosis. *Arthritis Res Ther*. 2011; 13(6):R199.

» Rubinstein T, Pitashny M, Levine B, et al. Urinary neutrophil gelatinase-associated lipocalin as a novel biomarker for disease activity in lupus nephritis. *Rheumatology (Oxford)*. 2010;49(5):960-971.

» Stangou M, Alexopoulos E, Papagianni A, et al. Urinary levels of epidermal growth factor, interleukin-6 and monocyte chemoattractant protein-1 may act as predictor markers of renal function outcome in immunoglobulin A nephropathy. *Nephrology (Carlton)*. 2009;4(6):613-620.

» Wei C, El Hindi S, Li J, et al. Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. *Nat Med*. 2011;17(8):952-960.

» Zhang X, Nagaraja HN, Nadasdy T, et al. A composite urine biomarker reflects interstitial inflammation in lupus nephritis biopsies. *Kidney Int.* 2012;81(4):401-406.

FUTURE CHALLENGES

The convergence of basic science investigations and modern clinical epidemiologic techniques has ushered in an era of discovery and early validation of a number of novel biomarkers of kidney disease. A number of challenges face clinicians and scientists before the widespread adoption of novel biomarkers of kidney disease in the clinic. These include the need for larger studies with clinically important end points, comparisons of conventional versus novel biomarkers for their added clinical utility, correlation of severity of disease with biomarker levels and duration of elevation, clarification of the treatment implications from measurement of novel biomarkers, and development of reliable assays and point-of-care testing if appropriate.

ACKNOWLEDGEMENTS

Support: Dr Waikar is supported by grants DK093574, DK085660, and DK075941 from the National Institute of Diabetes and Digestive Kidney Diseases (NIDDK).

Financial Disclosure: Dr Waikar has served as a consultant to CVS Caremark, BioTrends Research Group, and Takeda; provided expert testimony for GE Heathcare, Northstar Rx, and Salix; and received grants from the NIDDK, Otsuka, Merck, Genzyme, and Satellite Healthcare. Dr McMahon declares that he has no relevant financial interests.