NIH Public Access
Author Manuscript
Adv Chronic Kidney Dis. Author manuscript; available in PMC 2011 November 1.

Published in final edited form as:
Adv Chronic Kidney Dis. 2010 November ; 17(6): 469–479. doi:10.1053/j.ackd.2010.09.002.

The Use of Targeted Biomarkers for Chronic Kidney Disease

Prasad Devarajan, M.D.
Nephrology and Hypertension, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, MLC 7022, 3333 Burnet Avenue, Cincinnati, OH 45229-3039, Ph: 513-636-4531; FAX: 513-636-7407

Prasad Devarajan: prasad.devarajan@cchmc.org

Abstract

There is a paucity of sensitive and specific biomarkers for the early prediction of chronic kidney disease (CKD) progression. The recent application of innovative technologies such as functional genomics, proteomics, and biofluid profiling has uncovered a number of new candidates that are emerging as predictive biomarkers of CKD. The most promising among these include urinary proteins such as neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), and liver-type fatty acid binding protein (L-FABP). In addition, an improved understanding of the complex pathophysiologic processes underlying CKD progression has also provided discriminatory biomarkers of CKD progression that are being actively evaluated. Candidates included in this category are plasma proteins such as asymmetric dimethylarginine (ADMA), adiponectin, apolipoprotein A-IV (apoA-IV), fibroblast growth factor 23 (FGF23), NGAL, and the natriuretic peptides, as well as urinary *N*-acetyl-β-D-glucosaminidase (NAG). This review represents a critical appraisal of the current status of these emerging CKD biomarkers. At the present time, none are ready for routine clinical use. Additional large, multicenter prospective studies are needed to validate the biomarkers, identify thresholds and cut-offs for prediction of CKD progression and adverse events, assess the effects of confounding variables, and establish the ideal assays.

Keywords

neutrophil gelatinase-associated lipocalin; kidney injury molecule-1; asymmetric dimethylarginine; adiponectin; fibroblast growth factor 23

The Urgent Need For Targeted Chronic Kidney Disease Biomarkers

Chronic kidney disease (CKD) is a global epidemic with an increasing prevalence worldwide (1). The prevalence of CKD in the US adult population was reported in 2003 to be 11% (2), but is clearly increasing at an alarming rate (3). Recent refinements to the kidney function estimating equations (4,5) as well as the widespread adoption of a five-stage classification system (6) have increased awareness, standardized staging, and facilitated management of CKD. In addition, effective strategies are now available to potentially slow the progression of CKD and reduce the risk of cardiovascular events (1). However, while

Financial disclosure: Dr. Devarajan is a co-inventor of patents on the use of NGAL as a biomarker of acute kidney injury and chronic kidney disease, and is a consultant to Abbott Diagnostics and Biosite, Inc.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

several clinical risk factors for the progression of CKD have been identified (7), there remain at least two issues that have impeded progress. First, there is a lack of a consensus definition for CKD progression. Several definitions have been used in published studies, depending on the clinical setting and the duration of the study (Table 1). This lack of a uniform definition adds difficulties in interpreting the literature, and will likely continue to be a limitation for future observational and interventional studies.

Second, there is a paucity of sensitive and specific biomarkers for the early prediction of CKD progression. In current clinical practice, the most commonly employed markers of CKD progression are formulas to estimate glomerular filtration rate (eGFR) and measurement of proteinuria, both easily available but flawed. Estimations of GFR (4,5) reflect late functional changes, and not early structural alterations in the kidney that would identify subtle damage. Functional changes are inherently delayed due to the well known concept of “renal reserve” – irrespective of the underlying disease, with progressive destruction of nephrons, the kidney has an innate ability to maintain GFR by hyperfiltration and compensatory hypertrophy of the remaining healthy nephrons (8). This nephron adaptability allows for continued clearance of plasma solutes, so that the plasma markers used in calculating eGFR (creatinine and urea) show significant increases only after about 50% of the GFR has been lost. In addition, these plasma markers are confounded by a large number of variables, including age, gender, race, muscle mass, muscle metabolism, hydration status, and medications (9,10). Furthermore, the enhanced tubular secretion of creatinine that is characteristically encountered at lower rates of GFR results in an overestimation of renal function.

Proteinuria (or more specifically, albuminuria and microalbuminuria) has also been used as a marker of CKD progression. Recent reports have supported the use of proteinuria for prediction of adverse outcomes in CKD (11,12) and as a surrogate outcome to facilitate clinical trials (13). However, a large number of glomerular, tubular, and interstitial pathophysiologic mechanisms can lead to proteinuria, and significant structural damage has typically already occurred before proteinuria is measureable (14). Progressive renal function decline has usually already commenced at the onset of microalbuminuria (15,16). The predictive value of microalbuminuria has recently been questioned, since a large proportion of diabetic patients with microalbuminuria revert to normoalbuminuria, and only a minority progress to overt proteinuria (17).

Thus, improved biomarkers are clearly needed to stratify subjects who are at greatest risk for CKD progression, who might maximally benefit from increased surveillance, early prevention, and specific interventions. Such biomarkers may also serve as important surrogate end points in clinical trials. In addition, they may provide an improved understanding of the underlying pathogenesis, and identify novel therapeutic targets. Desirable properties of clinically applicable CKD biomarkers are outlined in Table 2.

The Search For Novel CKD Biomarkers

The quest for early markers of CKD and its progression is an area of intense contemporary research. The recent application of innovative technologies such as functional genomics, proteomics, and biofluid profiling has uncovered a number of new candidates that are emerging as predictive biomarkers of both acute kidney injury (AKI) and CKD (9,10,18-21). The most promising among these include urinary proteins such as neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), and liver-type fatty acid binding protein (L-FABP), as shown in Table 3. In addition, an improved understanding of the complex pathophysiologic processes underlying CKD progression has also provided discriminatory biomarkers of CKD progression that remain under active evaluation (7,14).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Candidates included in this category are plasma proteins such as asymmetric dimethylarginine (ADMA), adiponectin, apolipoprotein A-IV (apoA-IV), fibroblast growth factor 23 (FGF23), NGAL, and the natriuretic peptides, as well as urinary *N*-acetyl-β-D-glucosaminidase (NAG). In this review, the current status of these emerging CKD biomarkers will be critically appraised. For each candidate, an attempt will be made to outline the discovery, biologic plausibility, clinical status, and limitations as a CKD biomarker.

Neutrophil Gelatinase-associated Lipocalin (NGAL)

Preclinical cDNA microarray studies in animal models of AKI identified *Ngal* (also known as lipocalin 2 or *lcn2*) to be one of the earliest and most upregulated genes in the kidney (18,22). Downstream proteomic analyses also revealed NGAL to be one of the most highly induced proteins in the kidney after ischemic or nephrotoxic AKI in animal models. (23-25). The serendipitous finding that NGAL protein was easily detected in the urine soon after AKI in animal studies (23-25) has inspired a number of translational human studies, and NGAL has emerged as an excellent biomarker in the urine and plasma for early diagnosis, therapeutic monitoring, and prediction of prognosis in common clinical AKI scenarios (26-32). The deployment of standardized clinical platforms for the rapid and accurate measurement of NGAL in urine (33) and plasma (34) has further facilitated the widespread use and validation of NGAL as a biomarker.

The biologic plausibility of NGAL as a biomarker of kidney disease is now reasonably well established. Human NGAL protein was originally isolated from secondary granules of human neutrophils, and subsequently shown to be a 25-kDa protein covalently bound to neutrophil gelatinase. NGAL mRNA is normally expressed in a variety of adult human tissues, including prostate, salivary gland, stomach, colon, trachea, lung, liver, and kidney (32). Several of these tissues are prone to exposure to microorganisms, and constitutively express NGAL protein at low levels. The major ligands for NGAL are siderophores, small iron-binding molecules. Siderophores are synthesized by bacteria to scavenge iron from the surroundings, and use specific transporters to recover the siderophore:iron complex, ensuring their iron supply. The siderophore-chelating property of NGAL therefore renders it as a bacteriostatic agent. On the other hand, siderophores produced by eukaryotes participate in NGAL-mediated iron shuttling that is critical to various cellular responses such as proliferation and differentiation (35). This property provides a potential molecular mechanism for the documented role of NGAL in enhancing the epithelial phenotype (36). In the context of kidney injury, the biological role of early and rapid NGAL induction is one of marked preservation of function, attenuation of apoptosis, and an enhanced proliferative response (25,32). Not surprisingly, gene expression studies in AKI have demonstrated a rapid and massive upregulation of NGAL mRNA in the distal nephron segments – specifically in the thick ascending limb of Henle's loop and the collecting ducts (35). The resultant synthesis of NGAL protein in the distal nephron and secretion into the urine comprises the major fraction of urinary NGAL. Although plasma NGAL is freely filtered by the glomerulus, it is largely reabsorbed in the proximal tubules. Thus, any urinary excretion of NGAL is likely only when a kidney disease precludes proximal tubular NGAL reabsorption, and/or induces distal tubular *de novo* NGAL synthesis. With respect to plasma NGAL, the kidney itself does not appear to be a major source. NGAL protein released into the circulation from distant organs such as the liver and lung constitute a distinct systemic pool. Additional contributions to the systemic pool may derive from activated neutrophils, macrophages, and other immune cells. Furthermore, any decrease in GFR would decrease the renal clearance of NGAL, with subsequent accumulation in the systemic circulation in patients with CKD.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Interestingly, animal models of AKI-to-CKD transition have identified NGAL and KIM-1 as two of the most upregulated genes and proteins in the kidney, revealing a possible role for these proteins as biomarkers of the chronically injured kidney (37). NGAL protein expression was first noted to be markedly increased in kidney biopsy samples from humans with a variety of chronic glomerular and tubular diseases (38,39). Cross-sectional studies revealed that CKD is also associated with increased concentrations of NGAL in both urine and plasma when compared to normal individuals, although not to the same extent as in AKI (38). Plasma and urine NGAL in CKD were shown to correlate well with measured (ioversol clearance) or estimated GFR (40,41). In a study of subjects with CKD due to autosomal dominant polycystic kidney disease, both urine and plasma NGAL were found to be elevated and inversely correlated with GFR, and a sub-set of patients with higher cystic growth displayed the highest concentrations of urine and plasma NGAL, indicating a correlation with disease severity and progression (42). A recent report has shown increased urinary NGAL expression in patients with biopsy-proven HIV-associated nephropathy (HIVAN), and abundant NGAL mRNA expression in dilated microcystic tubules in a transgenic mouse model of HIVAN, suggesting the utility of urine NGAL for the early diagnosis of CKD due to HIVAN (43). Elevated levels of NGAL, as well as an inverse correlation with GFR, have now been documented in a number of publications examining patients with CKD due to membranous nephropathy (43-45), primary focal segmental glomerulosclerosis (43), type-2 diabetic nephropathy (46,47), and in a mixed population with CKD stages 2-4 (48,49). Urinary NGAL levels also highly correlated with the degree of proteinuria in patients with CKD due to IgA nephropathy (39), membranous nephropathy (43-45), type-2 diabetic nephropathy (46,47), and early type-1 diabetic nephropathy (50,51), further indicating a relationship between biomarker concentration and disease severity. Urinary NGAL has also been correlated with the degree of histologic damage in IgA nephropathy (39). Both serum and urinary NGAL levels are significantly elevated in type-1 diabetes mellitus despite normal urinary albumin excretion, suggesting NGAL as a more sensitive and predictive biomarker than microalbuminuria in this population (51). Both serum and urine NGAL concentrations increased in subjects chronically treated with cyclosporine, with a positive correlation between biomarker levels and serum cyclosporine levels despite absence of changes in eGFR, suggesting the use of NGAL for the early detection of kidney damage in chronic nephrotoxicity (52). Finally, a number of studies have documented NGAL as a useful measure of kidney disease activity in chronic lupus nephritis, and as an early predictive marker for relapses (53-57).

However, only a few studies have examined NGAL as a discriminatory marker of CKD progression. In a small study of 23 patients with CKD from membranous nephropathy who were followed up for 12 months (45), those with the highest baseline levels of urine NGAL displayed the greatest reduction in kidney function, with a 3.36 risk ratio of developing a ≥50% decrease in eGFR. In another study of 78 patients with CKD of various etiologies who were followed up over a mean time of 200 days (58), urine NGAL levels at baseline significantly correlated with future changes in serum creatinine ($r = 0.77$; $p = 0.0002$) and eGFR ($r = -0.40$; $p = 0.02$). Similarly, in a study of 74 individuals with type-2 diabetic nephropathy followed for 1 year (47), urine NGAL concentrations at baseline correlated with follow up levels of eGFR ($r = -0.57$, $p<0.001$), serum creatinine ($r = 0.57$, $p<0.001$), and cystatin C ($r = 0.56$, $p<0.001$). In the largest study reported to date (49), 96 subjects with CKD stages 2-4 due to a variety of causes were followed for a median of 18.5 months, during which 32% reached the composite end-point for CKD progression (doubling of baseline serum creatinine and/or onset of end-stage renal disease). Both urine and plasma NGAL at baseline predicted CKD progression, independent of other factors such as eGFR and age. High baseline values for both serum and urine NGAL were associated with a significantly faster evolution to the composite end-point. The area under the receiver

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

operating characteristic curve (AUC) for predicting CKD progression was 0.78 for baseline urine NGAL and 0.70 for baseline serum NGAL, both indicating good diagnostic accuracy.

Recent preliminary studies have also suggested a role for NGAL as an efficacy biomarker during treatments for CKD. A decrease in urinary NGAL levels have been shown to herald the response to therapies in patients with nephrotic syndrome (59), membranous nephropathy treated with intravenous immunoglobulin (60), and hypertensive subjects treated with angiotensin receptor blockers (61). In CKD subjects treated with chronic hemodialysis, plasma NGAL concentrations were found to correlate well with measures of dialysis adequacy, suggesting a role for this biomarker in guiding the management of dialysis prescriptions (62,63).

Collectively, the studies reported herein indicate that NGAL is emerging as a promising biomarker for the early detection and staging of CKD, for predicting progression, and for monitoring the response to interventions. However, there are several limitations that should be acknowledged. First, they represent single center studies with relatively small numbers of cases, and the results will need to be confirmed in larger multi-center trials. Second, the majority of studies report only statistical associations, but do not provide sensitivity, specificity, and AUCs for the diagnosis of CKD progression, which are essential to determine the accuracy of the biomarker. Third, the majority of publications have reported on results obtained using research-based ELISA-type assays, which are impractical from the clinical utility standpoint. Fourth, the definitions of CKD and its progression in the published studies varied widely, but were based largely on changes in creatinine-based eGFR measurements, which raise the challenge of using a flawed outcome variable to analyze the performance of a novel assay. Fifth, increases in NGAL levels are not specific to CKD. It is well known that NGAL is an early responder to AKI, and clinical situations where AKI is superimposed on CKD are common. However, the differentiation between AKI and CKD using NGAL levels has been easy in the clinical setting, given the much more robust increase in biomarker levels that are characteristic of AKI (31). Indeed, urine NGAL levels increase by about 20-fold when subjects with CKD develop AKI (64). Plasma NGAL measurements may be influenced by a number of coexisting variables such as chronic hypertension, systemic infections, inflammatory conditions, anemia, hypoxia, and malignancies (65-67). In addition, NGAL has been showed to be expressed in human atherosclerotic plaques (68), which may also influence plasma NGAL measurements. Urine NGAL levels may be increased in urinary tract infections (69). Future studies should be aimed at addressing all of these limitations, taking into account these potential confounding variables. For now, the routine measurements of NGAL in patients with CKD cannot be recommended.

Kidney Injury Molecule 1 (KIM-1)

Preclinical subtractive hybridization screens identified kidney injury molecule 1 (*Kim-1*) as a gene that is markedly up-regulated in ischemic rat kidneys (70). Downstream proteomic studies have also shown KIM-1 to be one of the most highly induced proteins in the kidney after AKI in animal models. KIM-1 is a transmembrane protein that is not expressed in normal kidney but is specifically upregulated in dedifferentiated proximal tubule cells after ischemic or nephrotoxic AKI. It has been identified as a phosphatidylserine receptor that transforms epithelial cells into phagocytes by recognizing cell surface-specific epitopes expressed by apoptotic tubular epithelia (71). A proteolytically processed extracellular domain of KIM-1 is detectable in the urine soon after AKI (72). KIM-1 represents a promising biomarker for the early diagnosis of AKI and its clinical outcomes (73-75). The recent availability of a rapid urine dipstick test for KIM-1 will facilitate its further evaluation in preclinical and clinical studies (76).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Preliminary studies have reported on the potential utility of KIM-1 as a CKD biomarker. In a kidney biopsy study of 74 patients with CKD from various etiologies, KIM-1 was primarily expressed at the luminal side of dedifferentiated proximal tubules, in areas with fibrosis and inflammation (77). KIM-1 staining in the kidney correlated positively with morphological damage and negatively with renal function. Urinary KIM-1 levels measured in a subset of these patients were also negatively correlated with eGFR ($r = -0.37$; $p = 0.016$). In a prospective study of 145 kidney transplant patients followed for an average of 4 years, elevated urinary KIM-1 levels were associated with a 5.1-fold increased risk of graft loss (78). Prediction of graft loss by KIM-1 was independent of donor age, creatinine clearance, and proteinuria. In a retrospective study of non-diabetic proteinuric subjects, anti-proteinuric therapies reduced the urinary excretion of KIM-1, suggesting its use as an efficacy marker (79). Finally, KIM-1 has proven to be an excellent marker of nephrotoxicity in preclinical studies (80), but published human data are lacking to date.

Many of the limitations mentioned above for NGAL as a CKD biomarker also pertain to KIM-1, and additional large long-term studies are required to confirm the utility of KIM-1 in the CKD setting. A combination of biomarkers such as NGAL and KIM-1 may provide complementary information, with NGAL reflecting acute inflammatory events in real time and KIM-1 indicating the more chronic fibrotic changes.

Liver-type Fatty Acid Binding Protein (L-FABP)

L-FABP is a protein expressed in the proximal tubule of the kidney. Increased expression and urinary excretion have been described in animal models as well as humans with AKI (81,82). Although its precise function is unknown, L-FABP in the kidney has been postulated to represent an endogenous antioxidant capable of suppressing tubulointerstitial damage (83). Its urinary excretion is also increased in the setting of CKD. A clinical trial in 120 patients with nondiabetic CKD evaluated urinary L-FABP levels as a marker of CKD and its progression (84). Urine L-FABP levels correlated with urine protein and serum creatinine levels. Notably, L-FABP levels were significantly higher in the group of patients with mild CKD who progressed to more severe disease ($p = 0.05$). Neither serum creatinine nor urine protein differed between those same groups. In another small study of patients with type-2 diabetes, urinary L-FABP levels were associated with degree of proteinuria (85). In a large health screening study, urinary excretion of L-FABP was found to be increased in subjects with hypertension and diabetes mellitus, even in the absence of overt kidney damage (86). Additional longitudinal studies are needed to demonstrate L-FABP's ability to predict CKD and its progression. Standardized clinical platforms for the measurement of urinary L-FABP are not currently available.

Asymmetric dimethylarginine (ADMA)

ADMA is a naturally occurring amino acid and the most potent endogenous inhibitor of nitric oxide synthase (NOS). Circulating ADMA is generated from the hydrolysis of methylated proteins containing ADMA residues (87). It is largely metabolized by the enzyme dimethylarginine dimethylaminohydrolase, which is co-localized along with NOS in glomerular endothelial and tubular epithelial cells. Even mild kidney dysfunction can interfere with ADMA metabolism, leading to increased circulating levels. By competing with arginine for binding sites on NOS, the high ADMA levels result in decreased local nitric oxide (NO) production, with resultant progressive kidney damage as has been demonstrated in animal models (88). Therefore, ADMA represents both a biomarker as well as a potential target for therapy in CKD (89).

Accumulation of circulating ADMA and resultant inhibition of NOS in humans was first demonstrated in patients on hemodialysis (90). ADMA levels were also shown to be

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

increased early in the course of CKD due to IgA nephropathy and autosomal dominant polycystic kidney disease, preceding a reduction in renal function when measured by inulin clearance (91). A number of prospective studies have now demonstrated the utility of ADMA as a marker of CKD progression. The Mild to Moderate Kidney Disease Study (92) recruited 227 patients with nondiabetic CKD (stage 1-3) to demonstrate a strong correlation between plasma ADMA and GFR measured by iothalamate clearance ($r = -0.591$, $p<0.01$). During follow up for an average of 7 years, those who progressed (defined as a doubling of serum creatinine or renal replacement therapy) displayed significantly higher baseline ADMA levels. Each 0.1 μmol/l increase in plasma ADMA concentration was associated with a 47% increase in the probability for CKD progression, after correction for baseline serum creatinine levels. In another cohort of 131 subjects with CKD stages 2-5 (93), plasma ADMA concentrations were moderately correlated with GFR estimated by MDRD formula ($r = -0.22$, $p = 0.01$). During a mean follow up of 27 months, ADMA levels predicted progression to ESRD or death (21% increased risk per 0.1 μmol/l increase in plasma ADMA concentration). In a large prospective study of 397 type-1 diabetic patients with overt nephropathy who were followed for a median of 11.3 years (94), ADMA levels above the median were associated with an increased rate of major cardiovascular events (adjusted hazard ratio 2.05, $p = 0.002$), decline in measured GFR (increased by 1.2 ml/min/1.73m^2 per year, $p = 0.004$), and development of end-stage kidney disease (adjusted hazard ratio 1.85, $p = 0.055$). In another prospective study of 225 subjects with type 2 diabetes followed for 5.2 years (95), ADMA levels above the median predicted progression of nephropathy (defined as advancing albuminuria) with an adjusted hazard ratio of 2.72 ($p = 0.012$). Finally, in a prospective randomized trial, 111 obese CKD patients received a low-protein diet supplemented with either keto-amino acids or placebo for 36 months (96). Those receiving keto-amino acids demonstrated decreases of ADMA, visceral body fat, proteinuria, glycated hemoglobin, LDL-cholesterol and pentosidine, and experienced a significantly lower decrease in eGFR.

Taken together, these studies indicate that plasma ADMA is a promising biomarker for the early detection and staging of CKD, for predicting progression, and perhaps for monitoring the response to interventions. However, there are limitations to this biomarker. Specificity for CKD is a major issue, since increased plasma concentrations of ADMA are encountered in a variety of clinical settings characterized by endothelial dysfunction, including hypertension, hypercholesterolemia, diabetes mellitus, and congestive heart failure (95). Also lacking to date are human data indicating that lowering plasma ADMA levels is clinically beneficial (87). Finally, measurement of ADMA still requires research-based assays such as high-performance liquid chromatography (87), mass spectrometry (92), or ELISA (93). Hence, it is not yet time to recommend measuring ADMA in patients with CKD on a routine basis (87).

Adiponectin

Adiponectin is a 30-kDa protein secreted exclusively from adipose tissue, but its serum concentration is inversely associated with adiposity. It improves insulin sensitivity and decreases the adverse effects of inflammatory mediators in vascular cells (97). The association between adiponectin levels and kidney disease is complex. There is a direct correlation between adiponectin levels and macroalbuminuria, but an inverse correlation between adiponectin and normoalbuminuria in type 2 diabetes (98). Several studies have demonstrated elevated levels of plasma adiponectin in patients with CKD. The proposed mechanisms include a reduction in clearance (since adiponectin levels normalize rapidly after kidney transplantation in patients with ESRD), a state of adiponectin resistance, and a counter-regulatory response to the complex metabolic derangements in CKD (7).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Adiponectin has also been proposed as a biomarker for CKD progression. In a study of 177 patients with CKD who were prospectively followed for 7 years, those who reached a progression endpoint defined as doubling of serum creatinine or dialysis requirement displayed higher baseline adiponectin concentrations (99). Interestingly, this effect was gender-specific; high adiponectin levels were an independent predictor of CKD progression in men but not in women. As part of a large study of 438 subjects with nephropathy due to type 1 diabetes followed for an average of 8 years (100), 198 has serum adiponectin levels measured. Within this sub-group, those with progression of CKD (defined as ESRD, n=40) showed significantly higher plasma adiponectin levels at baseline. The unadjusted hazard ratio for ESRD in patients with adiponectin concentrations above the median was 2.72 ($p = 0.010$). Similarly, in a sub-group of 296 patients with type 1 diabetic nephropathy and macroalbuminuria (101), adiponectin levels were significantly elevated among those who progressed to ESRD (n=83). The regression analysis revealed a hazard ratio for progression of 1.022 ($p = 0.011$). However, adiponectin levels were not predictive of progression to ESRD in patients with normoalbuminia or microalbuminuria, which limits the utility of this marker in milder cases of CKD.

There are other limitations to this biomarker. Adiponectin levels are lowered in subjects with obesity, type II diabetes and coronary artery disease, increased with ACE inhibitor therapy, and influenced by genetic variations in the adiponectin gene (100). Finally, the immunofluorometric assay for adiponectin remains a specialized research-based measurement. Therefore, the routine measurement of adiponectin in patients with CKD cannot yet be recommended.

Apolipoprotein A-IV (apoA-IV)

Human apoA-IV is a 46 kDa glycoprotein synthesized in intestinal enterocytes during fat absorption and incorporated onto the surface of chylomicrons (102-104). While originally thought to be involved in fat absorption, recent evidence suggests an important role for apoA-IV in the reverse cholesterol transport pathway, which removes cholesterol from peripheral cells and directs it to the liver for metabolism. ApoA-IV thus has anti-atherogenic properties, and low levels are found in patients with coronary artery disease.

A number of studies have demonstrated elevated apoA-IV levels in ESRD as well as the early stages of CKD (102,103), likely related either to decreased clearance and/or decreased renal catabolism. In terms of CKD progression, one study of 177 patients with mild-to-moderate CKD followed prospectively for 7 years (104) showed increased baseline apoA-IV levels in those who progressed (doubling of serum creatinine or renal replacement therapy, n=65). ApoA-IV predicted progression by a 11 ml/min decrease in GFR for an increment of 1 mg/dl, with an AUC of 0.792 ($p<0.001$) and a hazard ratio of 1.062 ($p=0.006$). However, the corresponding AUC for baseline measured GFR was superior (AUC=0.842).

Some confounding variables do exist. Serum apoA-IV levels are directly correlated with serum albumin concentrations, and inversely correlated with degree of proteinuria in nephrotic subjects (103), likely related to increased renal losses. The effects of genotype polymorphisms have not been fully explored. Additional studies of apoA-IV in CKD are warranted.

Fibroblast growth factor 23 (FGF23)

FGF23 is a protein primarily secreted by osteocytes, and has recently occupied center stage in the bone-kidney axis, the regulation of calcium-phosphate metabolism, and implications for CKD outcomes (105,106). FGF23 induces phosphaturia by decreasing phosphate reabsorption in the proximal tubule via downregulation of luminal sodium-phosphate co-

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

transporters; it reduces circulating levels of calcitriol by inhibiting 1-α hydroxylase and stimulating 24-hydroxylase in the kidney; and it inhibits secretion of parathyroid hormone. While several studies have established the link between hyperphosphatemia and adverse clinical outcomes in patients with CKD, serum phosphorus itself is an inadequate biomarker. FGF23 has therefore been proposed and examined as a more sensitive biomarker of phosphorus metabolism. Of particular interest is the potential use of FGF23 to guide phosphorus-related therapies in CKD. Sensitive ELISA-based assays for both intact FGF23 and the C-terminal fragment are now available, but a wide distribution between these assay results have been reported, and data demonstrating their relative utilities are currently lacking.

Circulating FGF23 levels increase progressively as GFR declines, beginning in early stages of CKD (107,108). It remains unclear whether this is a cause or a consequence of disturbed mineral metabolism in CKD. Proposed mechanisms include decreased clearance, a compensatory increase in response to hyperphosphatemia, or a response to vitamin D therapy. In a prospective study of 177 patients with mild-to-moderate nondiabetic CKD followed for a median of 53 months (109), increased FGF23 levels were associated with increased risk for CKD progression (defined as a doubling of serum creatinine or dialysis requirement). Baseline FGF23 levels independently predicted progression of CKD after adjustment for age, gender, GFR, proteinuria, and serum levels of calcium, phosphate, and parathyroid hormone. However, the AUCs for prediction of CKD prognosis for FGF23 (0.81 for C-terminal FGF23 and 0.72 for intact FGF23) were somewhat inferior to that of measured GFR (AUC=0.84). In a recent study of 21 subjects with CKD stages 3 to 4, therapy with the phosphate binder sevelemer for 6 weeks resulted in a significant reduction of circulating FGF23 levels (110).

While promising, additional studies are clearly needed before FGF23 can be integrated into routine CKD practice. Potential confounding effects of age, body mass index, assay type, steroid therapy, acute kidney injury, and dietary intake of phosphorus and Vitamin D also need to be resolved (111).

Natriuretic Peptides

Natriuretic peptides such as adrenomedullin (ADM), A-type natriuretic peptide (ANP), B-type natriuretic peptide (BNP), and its precursor N-terminal pro-brain natriuretic peptide (NT-proBNP) all play critical roles in the cardio-renal axis by causing natriuresis, diuresis, and vasodilation (112). All have been shown to predict cardiac dysfunction, and recent evidence for their role in CKD has emerged (113,114). Plasma concentrations of natriuretic hormones are increased in CKD and correlate with the estimated GFR. Postulated mechanisms include diminished renal clearance as well as a compensatory homeostatic response of the heart to impaired renal function.

In terms of CKD progression, these peptides have been examined in a prospective study of 177 patients with mild-to-moderate nondiabetic CKD followed for a median of 53 months for CKD progression (defined as a doubling of serum creatinine or dialysis requirement). BNP and NT-proBNP at baseline were significantly elevated among the progressors (113). Each increment of 1 SD in BNP and NT-proBNP increased the risk of CKD progression by hazard ratios of 1.38 ($p = 0.009$) and 2.28 ($p<0.001$) respectively. The AUC for CKD progression was 0.758 for NT-proBNP but only 0.603 for BNP. After adjustment for known risk factors of CKD progression, only NT-proBNP remained a significant independent predictor. Although NT-proBNP is not the biologically active peptide, the reason for its better performance is likely related to the longer half life of NT-proBNP compared to BNP

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

(120 vs 22 min). Additionally, the use of BNP may be limited by the assay, since about 20% of patients with CKD displayed BNP concentrations below the threshold for detection (113).

In the same cohort of patients, ANP and ADM were analyzed using novel sandwich immunoassays covering the mid-regional epitopes of the stable prohormones (MRproANP and MR-proADM). Increased plasma concentrations at baseline were documented for both at baseline in progressors (114). The AUCs for the prediction of CKD progression were similar for GFR (0.838), MR-proANP (0.810), and MRproADM (0.876). Increased plasma concentrations of both peptides were each strongly predictive of the progression of CKD after adjustments for age, gender, GFR, proteinuria and amino-terminal pro-B-type natriuretic peptide. Each increment of 1 SD in ADM and ANP more than doubled the risk of CKD progression.

Additional studies examining the natriuretic peptides are required, especially in the diabetic CKD patients. The coexistence of congestive heart failure will present a major confounding variable for the use of these peptides in CKD progression.

N-acetyl-β-D-glucosaminidase (NAG)

NAG is a lysosomal enzyme that is constitutively expressed by the proximal tubule, and a well-studied urinary marker of established proximal tubule cell injury (9). In a recent nested case-control study of type 1 diabetic subjects who participated in the Diabetes Control and Complications Trial (115), baseline levels of urinary NAG independently predicted the development of macroalbuminuria (adjusted odds ratio 2.26, $p<0.001$), as well as microalbuminuria (adjusted odds ratio 1.86, $p<0.001$) during the follow up period of 9 years. The lack of data about the use of ACE inhibitors in this study limits its generalizability to the diabetic population as a whole, and NAG has not been studied in other CKD populations. In addition, several studies have demonstrated a direct correlation between NAG excretion and hyperglycemia; this confounding variable may further limit the utility of NAG as a biomarker in diabetic subjects.

Summary

The tools of modern science continue to unveil promising novel biomarkers that are induced in the kidney, and reflect the severity and progression of CKD when measured non-invasively in urine. These include NGAL, KIM-1 and L-FABP. In addition, a number of constitutively expressed circulating proteins accumulate in CKD, and are being tested as biomarkers of CKD progression. Examples include ADMA, adiponectin, apoA-IV, FGF23, NGAL, and natriuretic peptides. The current status of these analytes as CKD biomarkers has been explored in this review. At the present time, none are ready for prime time. Additional large, multicenter prospective studies are needed to validate the biomarkers, identify thresholds and cut-offs for prediction of CKD progression and adverse events, assess the effects of confounding variables, and establish the ideal assays. Additional studies are also required to determine whether the biomarkers continue to predict CKD progression longitudinally, in addition to merely the baseline levels that appear to correlate with progression. It is likely that a panel of CKD biomarkers will provide more information than any one biomarker alone. Based on pathophysiologic considerations, it is also likely that CKD biomarkers may need to be context-specific. For example, distinct panels may emerge for prediction of CKD due to diabetes, hypertension, and primary glomerulonephritides. Discrete CKD biomarker panels may also emerge that reflect the major underlying pathologic feature such as tubulointerstitial fibrosis and inflammation. Finally, ongoing discoveries using techniques such as proteomics, peptidomics, urinary transcriptomics, and

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

microRNA analysis are continuing to reveal novel biomarkers and therapeutic targets that could dramatically change the outcome for patients with CKD in the near future.

Acknowledgments

Studies cited in this review that were performed by the author's laboratory were supported by grants from the NIH (R01 DK53289, RO1 DK069749 and R21 DK070163).

References

1. James MT, Hemmelgarn BR, Tonelli M. Early recognition and prevention of chronic kidney disease. Lancet 2010;375:1296–309. [PubMed: 20382326]
2. Coresh J, Astor BC, Greene T, Eknoyan G, Levey AS. Prevalence of chronic kidney disease and decreased kidney function in the adult US population: Third National Health and Nutrition Examination Survey. Am J Kidney Dis 2003;41:1–12. [PubMed: 12500213]
3. Coresh J, Selvin E, Stevens LA, et al. Prevalence of chronic kidney disease in the United States. JAMA 2007;298(17):2038–47. [PubMed: 17986697]
4. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150(9):604–12. [PubMed: 19414839]
5. Levey AS, Coresh J, Greene T, et al. Expressing the Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values. Clin Chem 2007;53(4):766–72. [PubMed: 17332152]
6. Levey AS, Eckardt KU, Tsukamoto Y, et al. Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney Int 2005;67:2089–100. [PubMed: 15882252]
7. Kronenberg F. Emerging risk factors and markers of chronic kidney disease progression. Nat Rev Nephrol 2009;5:677–89. [PubMed: 19935815]
8. Woods LL. Intrarenal mechanisms of renal reserve. Semin Nephrol 1995;15:386–95. [PubMed: 8525140]
9. Parikh CR, Lu JC, Coca SG, Devarajan P. Tubular proteinuria in acute kidney injury: a critical evaluation of current status and future promise. Ann Clin Biochem. 2010 May 28; Epub ahead of print.
10. Devarajan P. Proteomics for biomarker discovery in acute kidney injury. Semin Nephrol 2007;27(6):637–51. [PubMed: 18061846]
11. Hemmelgarn BR, Manns BJ, Lloyd A, et al. Relation between kidney function, proteinuria, and adverse outcomes. JAMA 2010;303(5):423–9. [PubMed: 20124537]
12. Hallan SI, Ritz E, Lydersen S, Romundstad S, Kvenild K, Orth SR. Combining GFR and albuminuria to classify CKD improves prediction of ESRD. J Am Soc Nephrol 2009;20:1069–77. [PubMed: 19357254]
13. Levey AS, Cattran D, Friedman A, et al. Proteinuria as a surrogate outcome in CKD: report of a scientific workshop sponsored by the National Kidney Foundation and the US Food and Drug Administration. Am J Kidney Dis 2009;54:205–226. [PubMed: 19577347]
14. Chaudhary K, Phadke G, Nistala R, Weidmeyer CE, McFarlane SI, Whaley-Connell A. The emerging role of biomarkers in diabetic and hypertensive chronic kidney disease. Curr Diab Rep 2010;10:37–42. [PubMed: 20425065]
15. Merchant ML, Perkins BA, Boratyn GM, et al. Urinary peptidome may predict renal function decline in type 1 diabetes and microalbuminuria. J Am Soc Nephrol 2009;20:2065–74. [PubMed: 19643930]
16. Perkins BA, Ficociello LH, Ostrander BE, et al. Microalbuminuria and the risk for early progressive renal function decline in type 1 diabetes. J Am Soc Nephrol 2007;18:1353–61. [PubMed: 17329575]
17. Perkins BA, Ficociello LH, Silva KH, Finkelstein DM, Warram JH, Krolewski AS. Regression of microalbuminuria in type 1 diabetes. N Engl J Med 2003;348:2285–93. [PubMed: 12788992]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

18. Supavekin S, Zhang W, Kucherlapati R, Kaskel FJ, Moore LC, Devarajan P. Differential gene expression following early renal ischemia/reperfusion. Kidney Int 2003;63:1714–24. [PubMed: 12675847]
19. Devarajan P. Update on mechanisms of ischemic acute kidney injury. J Am Soc Nephrol 2006;17:1503–20. [PubMed: 16707563]
20. Devarajan P. Emerging biomarkers of acute kidney injury. Contrib Nephrol 2007;156:203–12. [PubMed: 17464129]
21. Nguyen MT, Devarajan P. Biomarkers for the early detection of acute kidney injury. Pediatr Nephrol 2008;23:2151–7. [PubMed: 17394022]
22. Devarajan P, Mishra J, Supavekin S, Patterson LT, Potter SS. Gene expression in early ischemia renal injury: clues towards pathogenesis, biomarker discovery, and novel therapeutics. Mol Genet Metab 2003;80:365–376. [PubMed: 14654349]
23. Mishra J, Mori K, Ma Q, et al. Neutrophil gelatinase-associated lipocalin: a novel early urinary biomarker for cisplatin nephrotoxicity. Am J Nephrol 2004;24(3):307–15. [PubMed: 15148457]
24. 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):2534–43. [PubMed: 14514731]
25. Mishra J, Mori K, Ma Q, et al. Amelioration of ischemic acute renal injury by neutrophil gelatinase-associated lipocalin. J Am Soc Nephrol 2004;15(12):3073–82. [PubMed: 15579510]
26. Haase M, Bellomo R, Devarajan P, et al. 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):1012–24. [PubMed: 19850388]
27. Mishra J, Dent C, Tarabishi R, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet 2005;365(9466):1231–8. [PubMed: 15811456]
28. Hirsch R, Dent C, Pfriem H, et al. NGAL is an early predictive biomarker of contrast-induced nephropathy in children. Pediatr Nephrol 2007;22(12):2089–95. [PubMed: 17874137]
29. Mishra J, Ma Q, Kelly C, et al. Kidney NGAL is a novel early marker of acute injury following transplantation. Pediatr Nephrol 2006;21(6):856–63. [PubMed: 16528543]
30. Wheeler DS, Devarajan P, Ma Q, et al. Serum neutrophil gelatinase-associated lipocalin (NGAL) as a marker of acute kidney injury in critically ill children with septic shock. Crit Care Med 2008;36(4):1297–303. [PubMed: 18379258]
31. Nickolas TL, O'Rourke MJ, Yang J, et al. Sensitivity and specificity of a single emergency department measurement of urinary neutrophil gelatinase-associated lipocalin for diagnosing acute kidney injury. Ann Intern Med 2008;148(11):810–9. [PubMed: 18519927]
32. Devarajan P. Neutrophil gelatinase-associated lipocalin: a promising biomarker for human acute kidney injury. Biomarkers Med 2010;4:265–280.
33. Bennett M, Dent CL, Ma Q, et al. Urine NGAL predicts severity of acute kidney injury after cardiac surgery: a prospective study. Clin J Am Soc Nephrol 2008;3(3):665–73. [PubMed: 18337554]
34. Dent CL, Ma Q, Dastrala S, et al. Plasma neutrophil gelatinase-associated lipocalin predicts acute kidney injury, morbidity and mortality after pediatric cardiac surgery: a prospective uncontrolled cohort study. Crit Care 2007;11(6):R127. [PubMed: 18070344]
35. Schmidt-Ott KM, Mori K, Li JY, et al. Dual action of neutrophil gelatinase-associated lipocalin. J Am Soc Nephrol 2007;18:407–13. [PubMed: 17229907]
36. Yang J, Goetz D, Li JY, et al. An iron delivery pathway mediated by a lipocalin. Mol Cell 2002;10:1045–56. [PubMed: 12453413]
37. Ko GJ, Grigoryev DN, Linfert D, et al. Transcriptional analysis of kidneys during repair from AKI reveals possible roles for NGAL and KIM-1 as biomarkers of AKI-to-CKD transition. Am J Physiol Renal Physiol 2010;298(6):F1472–83. [PubMed: 20181666]
38. Mori K, Lee HT, Rapoport D, et al. Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury. J Clin Invest 2005;115:610–21. [PubMed: 15711640]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

39. Ding H, He Y, Li K, et al. Urinary neutrophil gelatinase-associated lipocalin (NGAL) is an early biomarker for renal tubulointerstitial injury in IgA nephropathy. Clin Immunol 2007;123(2):227–34. [PubMed: 17360238]
40. Mitsnefes MM, Kathman TS, Mishra J, et al. Serum neutrophil gelatinase-associated lipocalin as a marker of renal function in children with chronic kidney disease. Pediatr Nephrol 2007;22(1):101–8. [PubMed: 17072653]
41. Nishida M, Kawakatsu H, Okumura Y, Hamaoka K. Serum and urinary NGAL levels in children with chronic renal diseases. Pediatr Int. 2010 Jan 5; Epub ahead of print.
42. Bolignano D, Coppolino G, Campo S, et al. Neutrophil gelatinase-associated lipocalin in patients with autosomal dominant polycystic kidney disease. Am J Nephrol 2007;27(4):373–8. [PubMed: 17570904]
43. Paragas N, Nickolas TL, Wyatt C, et al. Urinary NGAL marks cystic disease in HIV-associated nephropathy. J Am Soc Nephrol 2009;20:1687–92. [PubMed: 19628667]
44. Bolignano D, Coppolino G, Campo S, et al. Urinary neutrophil gelatinase-associated lipocalin (NGAL) is associated with severity of renal disease in proteinuric patients. Nephrol Dial Transplant 2008;23(1):414–6. [PubMed: 17893105]
45. Bolignano D, Coppolino G, Lacquaniti A, Nicocia G, Buemi M. Pathological and prognostic value of urinary neutrophil gelatinase-associated lipocalin in macroproteinuric patients with worsening renal function. Kidney Blood Press Res 2008;31(4):274–9. [PubMed: 18753796]
46. Bolignano D, Lacquaniti A, Coppolino G, et al. Neutrophil gelatinase-associated lipocalin as an early biomarker of nephropathy in diabetic patients. Kidney Blood Press Res 2009;32:91–8. [PubMed: 19321980]
47. Yang YH, He XJ, Chen SR, Wang L, Li EM, Xu LY. Changes of serum and urine neutrophil gelatinase-associated lipocalin in type-2 diabetic patients with nephropathy: one year observational follow-up study. Endocrine 2009;36(1):45–51. [PubMed: 19390997]
48. Malyszko J, Bachorzewska-Gajewska H, Sitniewska E, Malyszko JS, Poniatowski B, Dobrzycki S. Serum neutrophil gelatinase-associated lipocalin as a marker of renal function in non-diabetic patients with stage 2-4 chronic kidney disease. Ren Fail 2008;30:1–4. [PubMed: 18197536]
49. 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;4:337–44. [PubMed: 19176795]
50. Thrailkill KM, Moreau CS, Cockrell GE, et al. Disease and gender-specific dysregulation of NGAL and MMP-9 in type 1 diabetes mellitus. Endocr 2010;37:336–43.
51. Zachwieja J, Soltysiak J, Fichna P, et al. Normal-range albuminuria does not exclude nephropathy in diabetic children. Pediatr Nephrol 2010;25(8):1445–51. [PubMed: 20157738]
52. Wasilewska A, Zoch-Zwierz W, Taranta-Janusz K, Michaluk-Skutnik J. Neutrophil gelatinase-associated lipocalin (NGAL): a new marker of cyclosporine nephrotoxicity? Pediatr Nephrol 2010;25(5):889–97. [PubMed: 20072790]
53. Brunner HI, Mueller M, Rutherford C, et al. Urinary neutrophil gelatinase-associated lipocalin as a biomarker of nephritis in childhood-onset systemic lupus erythematosus. Arthritis Rheum 2006;54(8):2577–84. [PubMed: 16868980]
54. 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):1894–903. [PubMed: 17530720]
55. Suzuki M, Wiers KM, Klein-Gitelman MS, et al. Neutrophil gelatinase-associated lipocalin as a biomarker of disease activity in pediatric lupus nephritis. Pediatr Nephrol 2008;23(3):403–12. [PubMed: 18202859]
56. 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):960–71. [PubMed: 20144927]
57. Hinze CH, Suzuki M, Klein-Gitelman M, et al. Neutrophil gelatinase-associated lipocalin is a predictor of the course of global and renal childhood-onset systemic lupus erythematosus disease activity. Arthritis Rheum 2009;60(9):2772–81. [PubMed: 19714584]
58. Nickolas TL, Barasch J, Devarajan P. Biomarkers in acute and chronic kidney disease. Curr Opin Nephrol Hypertens 2008;17:127–32. [PubMed: 18277143]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

59. Kuwabara T, Mori K, Mukoyama M, et al. Urinary neutrophil gelatinase-associated lipocalin levels reflect damage to glomeruli, proximal tubules, and distal nephrons. Kidney Int 2009;75(3):285–94. [PubMed: 19148153]
60. Bolignano D, Coppolino G, Aloisi C, Romeo A, Nicocia G, Buemi M. Effect of a single intravenous immunoglobulin infusion on neutrophil gelatinase-associated lipocalin levels in proteinuric patients with normal renal function. J Investig Med 2008;56(8):997–1003.
61. Kasahara M, Mori K, Satoh N, et al. Reduction in urinary excretion of neutrophil gelatinase-associated lipocalin by angiotensin receptor blockers in hypertensive patients. Nephrol Dial Transplant 2009;24(8):2608–9. [PubMed: 19474274]
62. Malyszko J, Malyszko JS, Koc-Zorawska E, Kozminski P, Mysliwiec M. Neutrophil gelatinase-associated lipocalin in dialyzed patients is related to residual renal function, type of renal replacement therapy and inflammation. Kidney Blood Press Res 2009;32(6):464–9. [PubMed: 20068352]
63. Bolignano D, Coppolino G, Romeo A, Lacquaniti A, Buemi M. Neutrophil gelatinase-associated lipocalin levels in chronic hemodialysis patients. Nephrology (Carlton) 2010;15(1):23–6. [PubMed: 20377767]
64. Sise ME, Barasch J, Devarajan P, Nickolas TL. Elevated neutrophil gelatinase-associated lipocalin can diagnose acute kidney injury in patients with chronic kidney disease. Kidney Int 2009;75:115–6. [PubMed: 19092815]
65. Devarajan P. Neutrophil gelatinase-associated lipocalin: new paths for an old shuttle. Cancer Ther 2007;5(B):463–470. [PubMed: 18449360]
66. Devarajan P. The promise of biomarkers for personalized renal cancer care. Kidney Int 2010;77(9):755–7. [PubMed: 20393489]
67. Malyszko J, Bachorzewska-Gajewska H, Malyszko JS, et al. Serum neutrophil gelatinase-associated lipocalin as a marker of renal function in hypertensive and normotensive patents with coronary artery disease. Nephrology (Carlton) 2008;13:153–6. [PubMed: 18275504]
68. Hemdahl AL, Gabrielsen A, Zhu C, et al. Expression of neutrophil gelatinase-associated lipocalin in atherosclerosis and myocardial infarction. Arterioscler Thromb Vasc Biol 2006;26:136–42. [PubMed: 16254208]
69. Yilmaz A, Sevketoglu E, Gedikbasi A, et al. Early prediction of urinary tract infection with urinary neutrophil gelatinase associated lipocalin. Pediatr Nephrol 2009;24(12):2387–92. [PubMed: 19649660]
70. Ichimura T, Bonventre JC, Bailly V, et al. Kidney Injury Molecule-1 (KIM-1), a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain, is up-regulated in renal cells after injury. J Biol Chem 1998;273:4135–42. [PubMed: 9461608]
71. Ichimura T, Asseldonk EJ, Humphreys BD, Gunaratnam L, Duffield JS, Bonventre JV. Kidney injury molecule-1 is a phosphatidylserine receptor that confers a phagocytic phenotype on epithelial cells. J Clin Invest 2008;118(5):1657–68. [PubMed: 18414680]
72. Vaidya VS, Ramirez V, Ichimura T, et al. Urinary kidney injury molecule-1: a sensitive quantitative biomarker for early detection of kidney tubular injury. Am J Physiol Renal Physiol 2006;290:F517–29. [PubMed: 16174863]
73. Liangos O, Periyanayagam 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):904–12. [PubMed: 17267747]
74. Han WK, Waikar SS, Johnson A, et al. Urinary biomarkers in the early diagnosis of acute kidney injury. Kidney Int 2008;73(7):863–9. [PubMed: 18059454]
75. Liangos O, Tighiouart H, Perianayagam MC, et al. Comparative analysis of urinary biomarkers for early detection of acute kidney injury following cardiopulmonary bypass. Biomarkers 2009;14(6):423–31. [PubMed: 19572801]
76. Vaidya VS, Ford GM, Waikar SS, et al. A rapid urine test for early detection of kidney injury. Kidney Int 2009;76(1):108–14. [PubMed: 19387469]
77. van Timmeren MM, van den Heuvel MC, Bailly V, Bakker SJ, van Goor H, Stegeman CA. Tubular kidney injury molecule-1 (KIM-1) in human renal disease. J Pathol 2007;212(2):209–17. [PubMed: 17471468]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

78. van Timmeren MM, Vaidya VS, van Ree RM, et al. High urinary excretion of kidney injury molecule-1 is an independent predictor of graft loss in renal transplant recipients. Transplantation 2007;84(12):1625–30. [PubMed: 18165774]
79. 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. [PubMed: 18823687]
80. Vaidya VS, Ozer JS, Dieterle F, et al. Kidney injury molecule-1 outperforms traditional biomarkers of kidney injury in preclinical biomarker qualification studies. Nat Biotechnol 2010;28(5):478–85. [PubMed: 20458318]
81. Portilla D, Dent C, Sugaya T, et al. Liver fatty acid-binding protein as a biomarker of acute kidney injury after cardiac surgery. Kidney Int 2008;73(4):465–72. [PubMed: 18094680]
82. Ferguson MA, Vaidya VS, Waikar SS, et al. Urinary liver-type fatty acid-binding protein predicts adverse outcomes in acute kidney injury. Kidney Int 2010;77(8):708–14. [PubMed: 19940842]
83. Kamijo-Ikemori A, Sugaya T, Obama A, et al. Liver-type fatty acid binding protein attenuates renal injury induced by unilateral ureteral obstruction. Am J Pathol 2006;169:1107–17. [PubMed: 17003471]
84. Kamijo A, Sugaya T, Hikawa A, et al. Urinary liver-type fatty acid binding protein as a useful biomarker in chronic kidney disease. Mol Cell Biochem 2006;284(1-2):175–82. [PubMed: 16532260]
85. Nakamura T, Sugaya T, Kawagoe Y, Ueda Y, Osada S, Koide H. Effect of pitavastatin on urinary liver-type fatty acid-binding protein levels in patients with early diabetic nephropathy. Diabetes Care 2005;28(11):2728–32. [PubMed: 16249547]
86. Ishimitsu T, Ohta S, Saito M, et al. Urinary excretion of liver fatty acid-binding protein in health-check participants. Clin Exp Nephrol 2005;9(1):34–9. [PubMed: 15830271]
87. Kielstein JT, Fliser D, Veldink H. Asymmetric dimethylarginine and symmetric dimethylarginine: axis of evil or useful alliance? Semin Dial 2009;22(4):346–50. [PubMed: 19708979]
88. Sharma M, Zhou Z, Miura H, et al. ADMA injures the glomerular filtration barrier: role of nitric oxide and superoxide. Am J Physiol Renal Physiol 2009;296(6):F1386–95. [PubMed: 19297451]
89. Kielstein JT, Zoccali C. Asymmetric dimethylarginine: a novel marker of risk and a potential target for therapy in chronic kidney disease. Curr Opin Nephrol Hypertens 2008;17(6):609–15. [PubMed: 18941355]
90. Vallance P, Leone A, Calver A, Collier J, Moncada S. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. Lancet 1992;339(8793):572–5. [PubMed: 1347093]
91. Kielstein JT, Böger RH, Bode-Böger SM, et al. Marked increase of asymmetric dimethylarginine in patients with incipient primary chronic renal disease. J Am Soc Nephrol 2002;13(1):170–6. [PubMed: 11752034]
92. Fliser D, Kronenberg F, Kielstein JT, et al. Asymmetric dimethylarginine and progression of chronic kidney disease: the mild to moderate kidney disease study. J Am Soc Nephrol 2005;16(8): 2456–61. [PubMed: 15930091]
93. 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):2449–55. [PubMed: 15944335]
94. Lajer M, Tarnow L, Jorsal A, Teerlink T, Parving HH, Rossing P. Plasma concentration of asymmetric dimethylarginine (ADMA) predicts cardiovascular morbidity and mortality in type 1 diabetic patients with diabetic nephropathy. Diabetes Care 2008;31(4):747–52. [PubMed: 18162497]
95. Hanai K, Babazono T, Nyumura I, et al. Asymmetric dimethylarginine is closely associated with the development and progression of nephropathy in patients with type 2 diabetes. Nephrol Dial Transplant 2009;24(6):1884–8. [PubMed: 19131352]
96. Teplan V, Schück O, Racek J, et al. Reduction of plasma asymmetric dimethylarginine in obese patients with chronic kidney disease after three years of a low-protein diet supplemented with keto-amino acids: a randomized controlled trial. Wien Klin Wochenschr 2008;120(15-16):478–85. [PubMed: 18820852]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

97. Ix JH, Sharma K. Mechanisms linking obesity, chronic kidney disease, and fatty liver disease: the roles of fetuin-A, adiponectin, and AMPK. J Am Soc Nephrol 2010;21(3):406–12. [PubMed: 20150538]
98. Looker HC, Krakoff J, Funahashi T, et al. Adiponectin concentrations are influenced by renal function and diabetes duration in Pima Indians with type 2 diabetes. J Clin Endocrinol Metab 2004;89:4010–17. [PubMed: 15292342]
99. Kollerits B, Fliser D, Heid IM, et al. Gender-specific association of adiponectin as a predictor of progression of chronic kidney disease: the Mild to Moderate Kidney Disease Study. Kidney Int 2007;71(12):1279–86. [PubMed: 17457380]
100. Jorsal A, Tarnow L, Frystyk J, et al. Serum adiponectin predicts all-cause mortality and end stage renal disease in patients with type I diabetes and diabetic nephropathy. Kidney Int 2008;74(5): 649–54. [PubMed: 18496510]
101. Saraheimo M, Forsblom C, Thorn L, et al. Serum adiponectin and progression of diabetic nephropathy in patients with type 1 diabetes. Diabetes Care 2008;31(6):1165–9. [PubMed: 18346990]
102. Kronenberg F, Kuen E, Ritz E, et al. Apolipoprotein A-IV serum concentrations are elevated in patients with mild and moderate renal failure. J Am Soc Nephrol 2002;13(2):461–9. [PubMed: 11805176]
103. Lingenhel A, Lhotta K, Neyer U, et al. Role of the kidney in the metabolism of apolipoprotein A-IV: influence of the type of proteinuria. J Lipid Res 2006;47(9):2071–9. [PubMed: 16788210]
104. Boes E, Fliser D, Ritz E, et al. Apolipoprotein A-IV predicts progression of chronic kidney disease: the mild to moderate kidney disease study. J Am Soc Nephrol 2006;17(2):528–36. [PubMed: 16382017]
105. Gutiérrez OM. Fibroblast Growth Factor 23 and Disordered Vitamin D Metabolism in Chronic Kidney Disease: Updating the “Trade-off” Hypothesis. Clin J Am Soc Nephrol. 2010 May 27; Epub ahead of print.
106. Wolf M. Forging Forward with 10 Burning Questions on FGF23 in Kidney Disease. J Am Soc Nephrol. 2010 May 27; Epub ahead of print.
107. Gutiérrez OM, Mannstadt M, Isakova T, et al. Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. N Engl J Med 2008;359(6):584–92. [PubMed: 18687639]
108. Gutiérrez O, Isakova T, Rhee E, et al. Fibroblast growth factor-23 mitigates hyperphosphatemia but accentuates calcitriol deficiency in chronic kidney disease. J Am Soc Nephrol 2005;16(7): 2205–15. [PubMed: 15917335]
109. Fliser D, Kollerits B, Neyer U, et al. Fibroblast growth factor 23 (FGF23) predicts progression of chronic kidney disease: the Mild to Moderate Kidney Disease (MMKD) Study. J Am Soc Nephrol 2007;18(9):2600–8. [PubMed: 17656479]
110. Oliveira RB, Cancela AL, Graciolli FG, et al. Early control of PTH and FGF23 in normophosphatemic CKD patients: a new target in CKD-MBD therapy? Clin J Am Soc Nephrol 2010;5(2):286–91. [PubMed: 19965540]
111. Bacchetta J, Dubourg L, Harambat J, et al. The influence of glomerular filtration rate and age on fibroblast growth factor 23 serum levels in pediatric chronic kidney disease. J Clin Endocrinol Metab 2010;95(4):1741–8. [PubMed: 20157196]
112. Ronco C, Haapio M, House AA, Anavekar N, Bellomo R. Cardiorenal syndrome. J Am Coll Cardiol 2008;52(19):1527–39. [PubMed: 19007588]
113. Spanaus KS, Kronenberg F, Ritz E, et al. B-type natriuretic peptide concentrations predict the progression of nondiabetic chronic kidney disease: the Mild-to-Moderate Kidney Disease Study. Clin Chem 2007;53(7):1264–72. [PubMed: 17478561]
114. Dieplinger B, Mueller T, Kollerits B, et al. Pro-A-type natriuretic peptide and pro-adrenomedullin predict progression of chronic kidney disease: the MMKD Study. Kidney Int 2009;75(4):408–14. [PubMed: 19052536]
115. Kern EF, Erhard P, Sun W, Genuth S, Weiss MF. Early urinary markers of diabetic kidney disease: a nested case-control study from the Diabetes Control and Complications Trial (DCCT). Am J Kidney Dis 2010;55(5):824–34. [PubMed: 20138413]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 1
Commonly used end points as measures of CKD progression

- Doubling of serum creatinine
- Decline in GFR by 50%
- Need for dialysis or kidney transplantation
- Yearly or monthly decline in GFR
- Worsening of proteinuria
- Graft loss

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 2

Desirable Properties of CKD Biomarkers

- Noninvasive – utilize easily accessible samples such as urine or blood
- Easy to perform – utilize standardized clinical laboratory assays
- Biologic plausibility – correlate with pathophysiology, reflect severity of injury
- Sensitive – facilitate early detection, minimize false negatives
- Wide dynamic range – facilitate risk stratification
- Specific – facilitate diagnosis, minimize false positives
- Predictive – predict CKD progression, cardiovascular complications, mortality
- Theranostic – monitor response to interventions

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Table 3
Characteristics of Promising Novel CKD Biomarkers

Biomarker	Sample	Origin	Biological function
NGAL	Urine	Distal tubule, collecting duct	Promotes tubule cell survival and proliferation, limits tubule cell apoptosis
KIM-1	Urine	Proximal tubule	Promotes epithelial regeneration, regulates tubule cell apoptosis
L-FABP	Urine	Proximal tubule	Endogenous antioxidant, suppresses tubulointerstitial damage
NAG	Urine	Proximal tubule	Glycosidase activity in lysosomes of proximal tubule cells
NGAL	Blood	Liver, lung, neutrophils	Acute phase reactant, marker of organ cross-talk following kidney injury
ADMA	Blood	Methylated proteins	Inhibitor of nitric oxide synthase, promotes endothelial damage and sclerosis
Adiponectin	Blood	Adipose tissue	Improves insulin sensitivity, anti-inflammatory, anti-atherogenic
ApoA-IV	Blood	Intestinal enterocytes	Reverse cholesterol transport pathway, anti-atherogenic
FGF23	Blood	Osteocytes, osteoblasts	Phosphaturic, reduction of circulating calcitriol, inhibition of PTH secretion
ANP, BNP	Blood	Stretched heart walls	Natriuresis, diuresis, vasodilation, regulation of cardio-renal axis, anti-fibrotic

Footnote to table 3: NGAL, neutrophil gelatinase-associated lipocalin; KIM-1, kidney injury molecule-1; L-FABP, liver-type fatty acid binding protein; ADMA, asymmetric dimethylarginine; apoA-IV, apolipoprotein A-IV; FGF23, fibroblast growth factor 23; NAG, *N*-acetyl-β-D-glucosaminidase.