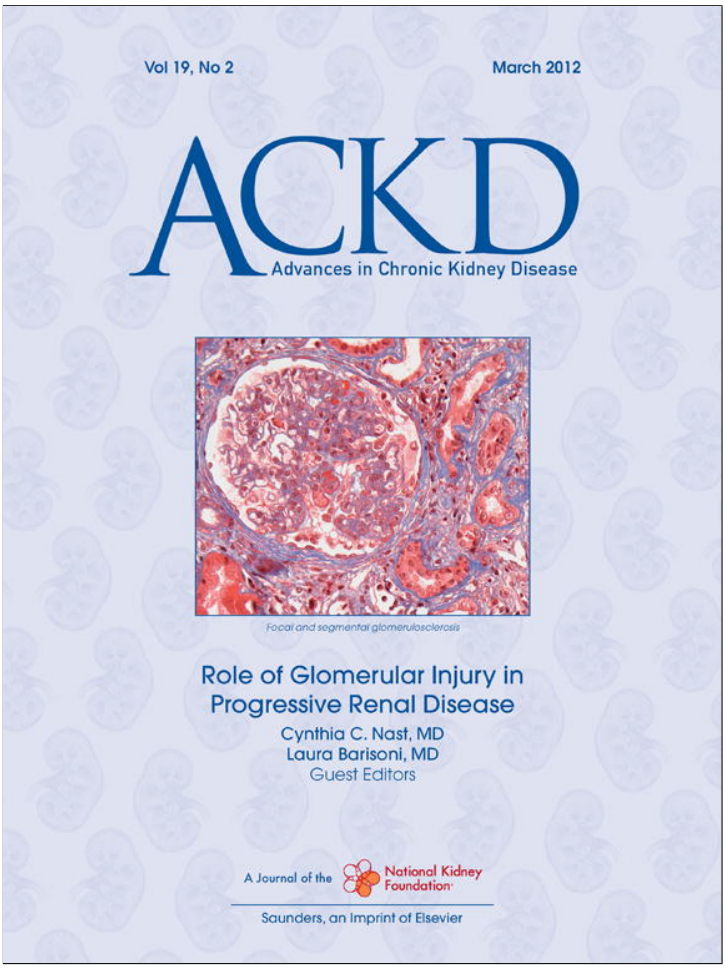

Novel Insights into the Relationship Between Glomerular Pathology and Progressive Kidney Disease

Bancha Satirapoj, Cynthia C. Nast, and Sharon G. Adler

Both glomerular and tubulointerstitial damage are important factors in the pathophysiology and progression of nephropathy. Glomerular injury is associated with tubulointerstitial inflammation, and many studies show that tubulointerstitial changes correlate well with progressive renal functional decline. Strong evidence supports the concept that once established, proteinuric glomerular injury can cause tubular injury. This review briefly summarizes the pathophysiological consequences of glomerular damage that are responsible for tubulointerstitial injury. It further focuses on tubule-derived renal injury biomarkers that may be used to monitor the progression of kidney disease. This monitoring is predicted to become increasingly useful as novel therapeutic interventions preventing progressive renal damage are introduced. In particular, biomarkers of kidney dysfunction, such as urinary podocytes, kidney injury molecule-1, neutrophil gelatinase–associated lipocalin, hematopoietic growth factor–inducible neurokinin 1, or periostin, might be useful in the diagnosis or detection of early nephropathy and risk assessment of kidney disease. However, these biomarkers require further study before they are used in routine screening or in guiding patient therapy.

Key Words: Chronic glomerular disease, Glomerulosclerosis, Tubulointerstitial fibrosis, Chronic kidney disease, Biomarker

Progressive CKD is characterized by compromise of the glomerular, tubular, vascular, and/or interstitial renal compartments. Although disease may start in glomeruli, tubules, or interstitium, or even in the renal vessels, progressive structural injury evolves into patterns with the shared histopathologic characteristics of glomerulosclerosis, tubulointerstitial inflammation, and tubulointerstitial fibrosis (TIF) (Fig 1). Significant progress has been made in understanding the pathophysiological links between glomerular injury and downstream tubulointerstitial inflammation, fibrosis, atrophy, and progressive CKD. The pathogeneses of glomerular diseases are complex and multifactorial, and recently have been reviewed[1]; an in-depth discussion is beyond the scope of this chapter. However, humoral, cellular, and innate immunity,[2] as well as hemodynamic alterations and other nonimmune pathways of glomerular injury, may participate in the induction of damaging downstream tubulointerstitial events, both by altering the glomerular ultrafiltration barrier and by directly contributing proinflammatory peptides into the glomerular ultrafiltrate. In animal models, regression of glomerular disease is readily accomplished by numerous pharmacological interventions.[3-5] Indeed, treatment is expected to induce regression for minimal-change nephropathy and lupus nephritis, whereas a significant number of treated patients with membranous nephropathy, focal and segmental glomerulosclerosis, small-vessel vasculitis, Goodpasture's disease, and type 1 diabetic nephropathy after pancreas transplantation experience regression or cure of the glomerulopathy. Additionally, spontaneous remission frequently is observed in membranous nephropathy, Henoch–Schonlein purpura, and poststreptococcal glomerulonephritis. However, in patients who do not experience a complete or partial remission, renal functional loss occurs in parallel with histological derangement.[6] Glomerular extracellular matrix (ECM) expansion, including collagen IV and VI, laminin, and fibronectin, accumulates in the mesangium and occludes capillary lumens. Glomerular cell apoptosis occurs in parallel with sclerosis, and ECM progressively fills the spaces left by dead and detached cells. Basement membranes are denuded of endothelial cells, and damaged podocytes contribute to the loss of glomerular permselectivity. Glomerulus-derived cytokines, chemokines, and growth factors promote interstitial inflammation and fibrosis.[7] Thus in most CKDs, the loss of selectivity of the glomerular filtration barrier, compounded with apoptotic glomerular cell death, inflammatory activation of the remaining glomerular cells, and infiltration by leukocytes, leads to sclerosis and reduced glomerular flow and filtration. Strong evidence supports the concept that once established, proteinuric glomerular injury also causes downstream tubular injury.

Chronic Tubulointerstitial Disease in Glomerular Injury

The concept of hemodynamically mediated glomerular injury was introduced by Brenner as the primary pathogenetic process driving progressive glomerular sclerosis. This theory provided the rationale for the highly successful angiotensin inhibitor treatment strategies for diabetic nephropathy and other proteinuric renal diseases that are currently in use as therapeutic cornerstones.[8] However,

From the Division of Nephrology, Phramongkutklao Hospital and College of Medicine, Bangkok, Thailand; Cedars-Sinai Medical Center, Los Angeles, CA; and Harbor-UCLA, Los Angeles Biomedical Research Institute, Torrance, CA.

Address correspondence to Bancha Satirapoj, Division of Nephrology, Phramongkutklao Hospital and College of Medicine, 315 Rajavithee Road, Payathai, Bangkok 10400, Thailand. E-mail: satirapoj@yahoo.com

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close analysis shows that functional impairment of the kidney is better correlated with the degree of tubulointerstitial injury than with glomerular injury.[9-11] This finding has led to the broad recognition that the final common pathway of kidney failure operates principally in the tubulointerstitium. Characteristic features of tubulointerstitial damage in chronic glomerulonephritis include tubular atrophy, obliteration of peritubular capillaries, accumulation of ECM causing interstitial fibrosis, loss of tubular epithelial cell differentiation markers, de novo expression of mesenchymal markers on tubular epithelial cells, and the appearance of a tubulointerstitial inflammatory cell infiltrate, the latter resulting in a significant increment in interstitial volume.

Tubular atrophy and damage increase fluid delivery to the macula densa, triggering a decrease in glomerular filtration rate (GFR) via tubuloglomerular feedback. TIF is associated with the development of atubular glomeruli and impairment of renal blood flow with consequent ischemic nephron injury. Similar findings are observed in the setting of protein overload nephropathy, in which megalin/cubilin-mediated endocytosis of increased quantities of filtered proteins by proximal tubular cells appears to evoke cell stress responses, resulting in increased inflammatory cytokines, which then lead to tubulointerstitial inflammation and fibrosis.[12] An increased rate of apoptosis also has been observed in the proximal tubules of protein-overload proteinuric animals. The changes in this model are similar to those observed during heavy proteinuria from glomerular disease, suggesting that tubular protein overload, independent of cause, can induce tubular atrophy and progressive kidney failure.[13] Indeed, albumin peptides derived from glomerular ultrafiltration and processed by renal dendritic cells behave as an autoantigen in experimental models of CKD,[14,15] inducing tubulointerstitial lymphocytes to undergo replication and secrete interferon.

Peritubular capillaries are downstream of the glomerular efferent arterioles. It has been suggested that capillary loss in glomerular disease is the result of nitric oxide synthesis inhibition, as hydrolysis of the endogenous nitric oxide synthase inhibitor reduces the extent of capillary loss and renal damage. In a model of accelerated glomerulosclerosis induced by repeated injections of anti-Thy1 antibody in uninephrectomized animals, stagnation of blood flow in peritubular capillaries induced chronic hypoxia at an early stage followed by progressive TIF and a loss of peritubular capillaries.[16] Another study demonstrated that elevated efferent arteriolar resistance and decreased peritubular capillary flow were associated with reversible renal functional impairment and tubular dysfunction in patients with severe glomerulonephritis and normoalbuminuric diabetic kidney disease.[17] These data support the concept that peritubular capillary obliteration may induce tubulointerstitial injury in patients with a variety of kidney diseases.

Ultimately, TIF results from dysregulation of the normal balance between ECM synthesis and turnover that is required for maintenance of tissue structure. Matrix accumulates as a consequence of both increased production and decreased turnover.[18] Matrix turnover is regulated primarily by the family of matrix metalloproteinases, their endogenous inhibitors (the tissue inhibitors of metalloproteinase [TIMPs]), and by the plasmin plasminogen activator inhibitor cascade. TIF is characterized by increased levels of TIMPs and plasminogen activator inhibitor I acting to suppress ECM turnover and promote ECM accumulation. Matrix metalloproteinases–independent effects of TIMPs on cell proliferation, survival, and differentiation also may be relevant.

The tubulointerstitial injury of glomerular disease also is characterized by hypercellularity. The increase in interstitial cell number is due to increased proliferation and decreased apoptosis of resident interstitial cells, as well as migration of hematopoietic-derived inflammatory cells into the tubulointerstitium. Interstitial myofibroblasts increase in number by differentiation of interstitial fibroblasts and possibly also by the transendothelial migration of fibrocytes.[19] Although some have suggested that transdifferentiation of tubular epithelial to mesenchymal cells also plays a role,[20] others have provided evidence that interstitial fibroblasts are derived from pericytes and not from transdifferentiated tubular epithelium.[21] In CKD, the tubulointerstitium is persistently inflamed, either focally or more diffusely, and to varying degrees. The inflammatory infiltrate persists, escaping normal host defense and restorative mechanisms. This leukocytic infiltrate results from both activation of resident and recruitment of circulating inflammatory cells and consists of lymphocytes, macrophages, dendritic or antigen-presenting cells, and,

CLINICAL SUMMARY

- Glomerular injury induces downstream injury responses and counter-regulatory adaptations leading to tubulointerstitial scarring and inflammation.
- Tubular protein overload, independent of cause, can induce tubular atrophy and progressive kidney failure.
- There is a need for simple, noninvasive, sensitive and specific biomarkers to identify and follow chronic kidney injury.
- Glomerular and tubular markers such as urinary podocytes, kidney injury molecule-1, neutrophil gelatinase–associated lipocalin, or hematopoietic growth factor–inducible neurokinin 1 may represent a "kidney injury panel" of the future.
- Candidate biomarkers are still being validated, and are not yet ready for routine use in screening and guiding therapy in patients with CKD.

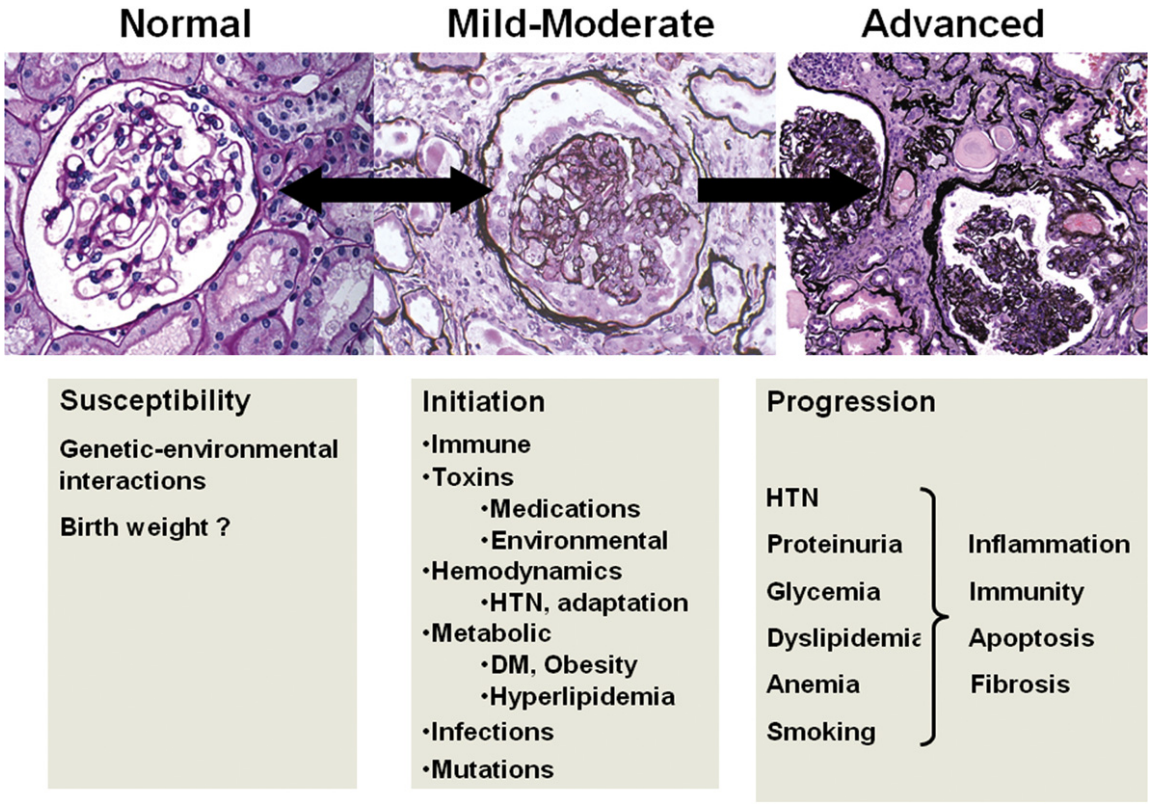

Figure 1. Variable factors and steps of progression in chronic glomerular disease.

occasionally, plasma cells.[22,23] Lymphocytes actively participate in the progression of experimental CKD.[24,25] Indeed, the renal interstitium in CKD expresses features of lymphoid neogenesis, a characteristic of tissue injury observed whenever inflammatory and immune responses are unable to eradicate antigen, as would be anticipated when the antigen is endogenous.[26] The data from the studies by Macconi and colleagues[14] and Heymann and colleagues[27] for albumin, and our own data for oxidized low-density lipoprotein,[23] support the hypothesis that the tubulointerstitial inflammatory infiltrate accompanying CKD reflects, at least in part, the development of autoimmunity to novel antigens generated during renal injury.[28] Moreover, a number of experimental studies have shown that anti-inflammatory agents can limit interstitial injury and preserve kidney function.[3-5]

Additional novel pathogenetic mechanisms are becoming increasingly appreciated as contributors to progressive renal injury consequent to glomerular damage. In conditions of heavy proteinuria, the increased tubular reabsorbtion of proteins activates the production of cytokines by tubular cells, which, in turn, promotes the infiltration of immune cells and the activation of an immunoinflammatory response.[29,30] Several cytokines, chemokines, growth factors, and complement proteins, through the activation of nuclear factor κB–related pathways, initiate the release of chemotactic factors. Expressed at the site of kidney injury, chemokines are involved in the recruitment of specific leukocyte subsets to the injured kidney.[31,32] Moreover, "danger" recognition is facilitated by various innate immune receptor families, including the Toll-like receptors, which detect danger signals in extracellular and intracellular compartments. Renal injury-triggered inflammation induces the expression of danger-associated molecules that act as immunostimulatory agonists for Toll-like receptors.[33] These ligand–receptor interactions further induce cytokine and chemokine secretion, leukocyte recruitment, and tissue remodeling.[34] An additional novel concept in autoimmune kidney injury involves the role of autophagy in tubulointerstitial injury and repair. Autophagy is a cellular response to injury in which repair is implemented by the removal of injurious proteins, cell organelles, or even bacteria or viral material, through entrapment of materials in a cytoplasmic vacuole followed by lysosomal fusion for removal. It has been proposed that autophagy-related processing of self-proteins provides a source of immunostimulatory molecules and autoantigens.[34] Although triggered as a repair process, there is potential for extreme injury to overwhelm repair and promote renal immunopathology and progressive kidney disease. Thus, in glomerular disease, upstream injury induces a complex combination of downstream responses and counter-regulatory adaptations, which, if they fail to

achieve repair, ultimately contribute to peritubular vascular obstruction, accumulation of ECM, tubular atrophy, and/or an inflammatory cell infiltrate through immune recognition of molecules and danger-associated molecules, eventually leading to nephron loss.

Markers of Progression in Chronic Glomerular Disease

Blood urea nitrogen, serum creatinine, cystatin C, formulas to eGFR, proteinuria, and albuminuria are measures currently used to assess the presence and progress of CKD. However, these measures are imprecise, are not direct measures of renal tissue injury, and are relatively insensitive to small changes in renal function. Thus, availability of novel biomarkers that are sensitive, specific, and precise, as well as detect kidney injury, predict clinically significant outcomes, and are useful broadly in CKD, would represent an advance in clinical management of patients and for clinical trials in nephrology. Early detection of CKD could translate into more favorable outcomes, as renoprotective treatments might be implemented in a more timely manner. Thus, there is a need for simple, noninvasive, sensitive and specific biomarkers that could monitor and report on the pathophysiological processes occurring within the kidney.

Many of the discovered biomarkers for CKD were identified by transcriptomic and proteomic analyses of renal tissues after injury. As such, there tends to be a bias toward the identification of markers from the renal tubulointerstitium, reflecting its greater mass compared with the vascular and glomerular compartments. Recently, certain biomarkers, which were initially identified in acute kidney injury (AKI), also have been reported to confer value in the evaluation of patients with CKD. Biomarkers such as kidney injury molecule-1 (KIM-1), neutrophil gelatinase–associated lipocalin (NGAL), hematopoietic growth factor–inducible neurokinin 1 (HGFIN, also known as glycoprotein nonmetastatic melanoma protein b [gpnmb] and osteoactivin), and periostin reflect tubular injury. In this article, we focus on the potential applications of these biomarkers in chronic glomerular disease (Table 1).

Markers of Glomerular Damage

Proteinuria and Albuminuria

Urinary albumin is filtered through the glomerulus and later reabsorbed by tubular cells through the megalin–cubulin pathway. Albuminuria is a well-established marker of glomerular damage, and more severe and persistent proteinuria correlates with more rapid progression of disease in proteinuric nephropathies, with the obvious exception of minimal change disease.[30,35] Thus, proteinuria has been used as a surrogate marker for chronic glomerular disease progression, and albuminuria, specifically, has been used as a marker of glomerular injury. Albuminuria in glomerular disease has traditionally been thought to result from structural injury resulting in basement membrane pores of large size and relatively low size selectivity; however, partial loss of charge selectivity also may contribute.[29] Proteinuria reduction is strongly associated with a slower decline in progression of CKD, and treatments that fail to reduce proteinuria are associated with a faster decline.[36] More specifically, albuminuria has been recognized as the "gold standard" marker of impaired glomerular permselectivity, and a predictor of both microvascular and macrovascular disease in diabetes[37] and even in otherwise healthy subjects. However, the negative predictive value of microalbuminuria for the early detection of diabetic nephropathy among type 2 diabetic patients was 77%, whereas the positive predictive value was only 43%, demonstrating limitations of the precision of this marker.[38] Furthermore, in some diseases, including hypertensive nephrosclerosis, tubulointerstitial injury progresses to end-stage kidney failure in the absence of severe albuminuria. Therefore, several authors have suggested the need for more sensitive and specific markers for renal injury than urinary albumin, particularly markers that are measureable in the urine before, or even without, the onset of microalbuminuria.

Table 1. Various Biomarkers in CKD

Biomarkers	Urine/Serum	Source Cell	AKI/CKD	Description
Podocytes (nephrin, podocin, synaptopodin)	Urine	Podocytes	CKD	Monitoring podocyte loss, increased in response to proteinuric kidney diseases
Cystatin C	Urine/serum	Nucleated cells	AKI/CKD	Early marker of impaired glomerular filtration
KIM-1	Urine	Proximal tubule	AKI > CKD	Increased in response to ischemic-reperfusion injury, increased in proteinuric CKD
NGAL	Urine/serum	Renal tubule/ neutrophil	AKI/CKD	Increased in response to ischemic-reperfusion injury, increased in advanced CKD
HGFIN	Urine	Distal tubule	CKD	Increased in response to progressive kidney injury, increased in proteinuric and nonproteinuric CKD

Abbreviations: AKI, acute kidney injury; KIM-1, kidney injury molecule-1; HGFIN, hematopoietic growth factor–inducible neurokinin 1; NGAL, neutrophil gelatinase–associated lipocalin.

Urinary Podocytes

The podocyte slit diaphragm plays an important role in the regulation of glomerular permeability. The concept that podocyte loss is a major culprit underlying development and progression of glomerular diseases is well established.[39] Podocyte loss also will trigger tubulointerstitial damage by misdirected filtration into the periglomerular interstitium, resulting in the replacement of damaged nephrons by fibrosis, which may itself contribute to progressive renal injury.[39] Detection of podocytes is common in the urinary sediment of patients with glomerular diseases, including micro- and macroalbuminuric diabetes, IgA nephropathy, Henoch–Schonlein purpura, lupus nephritis, and focal and segmental glomerulosclerosis.[40-43] Urinary mRNA expression of podocyte constituents, such as nephrin and podocin, is are detected in the sediment of patients with proteinuric disease. Furthermore, urine nephrin and podocin mRNA levels correlate with the rate of decline in renal function.[44] In an animal model of podocyte injury, persistently increased urinary podocin:aquaporin 2 and nephrin:aquaporin 2 molar ratios were determinants of the progression of renal disease and correlated with global podocyte depletion and interstitial scarring.[45] Parallel human studies showed that biopsy-proven lupus nephritis was associated with increased urinary podocin:aquaporin 2 and nephrin:aquaporin 2 molar ratios.[45] In another animal model, podocyturia was limited to phases of active glomerular injury, suggesting that it may be a more sensitive means of assessing the activity of glomerular damage than proteinuria.[46] One recent study demonstrated that intrarenal mRNA expression levels of nephrin, podocin, and synaptopodin were lower, and urinary mRNA expression levels were higher, in patients with hypertensive nephropathy than in healthy subjects.[47] Moreover, podocyte density and intrarenal gene expression of podocyte-specific molecules significantly correlated with GFR and inversely correlated with blood pressure and the degree of renal fibrosis.[47] Taken together, the data suggest that urinary podocyte gene expression may be a useful noninvasive tool for measuring continuing glomerular injury and specifically monitoring podocyte loss, thereby providing additional clinically important information for the management of proteinuric diseases.

Markers of Tubular Damage

KIM-1

Tubulointerstitial damage plays an important role in the progression of chronic glomerular disease. KIM-1 is a type I transmembrane glycoprotein, which was initially found to be markedly upregulated in proximal tubules in response to ischemic or toxic tubular injury in animal models.[48] It plays a role in the removal of apoptotic cells. Urinary KIM-1 results from ectodomain shedding from injured tubules and is considered to be a sensitive biomarker for early tubular damage. However, it is becoming increasingly apparent that KIM-1 identifies more than AKI. In an experimental animal model of tubulointerstitial damage from overload proteinuria, tubular KIM-1 expression was limited to areas with inflammation, fibrosis, and tubular damage.[49] A recently published clinical study confirmed that KIM-1 is associated with tubulointerstitial inflammation and is overexpressed in the tubules of patients with various chronic proteinuric kidney diseases, such as focal and segmental glomerulosclerosis, membranoproliferative glomerulonephritis, IgA nephropathy, and diabetic nephropathy.[50] In an additional study involving 34 patients with CKD, urinary KIM-1 levels were significantly higher in patients with nondiabetic proteinuric kidney disease. In this study, urine KIM-1 levels were significantly reduced by antiproteinuric regimens including losartan alone or in combination with hydrochlorothiazide. The reduction in urinary KIM-1 paralleled the decline in proteinuria, but it did not correlate with blood pressure.[51] The Food and Drug Administration and the European Medicines Evaluation Agency approved KIM-1 as 1 of 7 biomarkers (KIM-1, albumin, total protein, β2-microglobulin, cystatin C, clusterin, and trefoil factor-3) whose results they would take into consideration when evaluating for kidney injury in preclinical data and, in a limited manner, in some phase 1 studies in the context of new drug applications. Urinary KIM-1 excretion could become a noninvasive biomarker of both acute and chronic tubulointerstitial damage and, when expressed chronically in the context of glomerular disease, may be a biological marker of a tubular pathophysiological process that contributes to disease progression.

NGAL

The novel inflammatory marker NGAL is a protein of the lipocalin family. NGAL is a critical component of innate immunity and is normally expressed at very low levels in several human tissues including uterus, prostate, salivary gland, lung, colon, and kidney as well as by neutrophils. In tubular epithelium, it initially was reported expressed in proximal tubular cells, but it now appears that it is predominantly, if not exclusively, expressed in distal tubular cells. NGAL is highly expressed in human kidney cortical tubules and in the blood and urine after nephrotoxic and ischemic injury. Similar to KIM-1, NGAL was initially identified as a biomarker for AKI,[52] and it too appears to have renal applications beyond AKI. In patients with CKD and in nondiabetic kidney transplant patients, overexpression of NGAL occurs in renal tubular cells, compared with expression in non-CKD subjects.[53] NGAL expression rises gradually,

reaching the highest value in a late stage of CKD. In a recently reported study, NGAL levels closely reflected the GFR decline in CKD, and both urinary and serum NGAL levels predicted CKD progression independently of other potential confounders, including eGFR and age.[54] It also was postulated that in the setting of CKD, the rise in NGAL levels occurred independently of the decreased GFR, and was a reflection of tubular injury and inflammation, in contrast to increments in serum creatinine levels, which largely reflect GFR loss.[55]

HGFIN (Also Known as Gpnmb and Osteoactivin)

HGFIN is a type 1 transmembrane glycoprotein that is expressed in numerous cells, including osteoclasts, macrophages, and dendritic cells.[56] It is involved in the regulation of autophagy, and is a negative regulator of inflammation. Uremic macrophages exhibit increased HGFIN gene and protein expression, heightened expression of proinflammatory cytokines, and suppressed expression of anti-inflammatory cytokines.[57] Lymphocytes from mice with a stop codon in the gpnmb (HGFIN) gene respond to endotoxin challenge with higher rates of interferon elaboration, consistent with an immunomodulating function for gpnmb.[58] We identified HGFIN by microarray and real-time polymerase chain reaction as a kidney injury biomarker in cortical tissue of rats with 5/6 nephrectomy.[59] In urine, HGFIN increased over time after 5/6 nephrectomy, demonstrating potential as both an AKI and a CKD renal injury biomarker. Increased renal cortical tissue HGFIN mRNA also was observed in streptozotocin-induced diabetic rats, and in patients with micro- and macroalbuminuric diabetic nephropathy. Urine HGFIN distinguished patients with proteinuric and nonproteinuric renal disease from healthy controls, with a sensitivity of 96.2% and a specificity of 80%, comparing favorably with urine NGAL. HGFIN was expressed de novo in distal nephron cortical tubules after kidney injury and colocalized with markers of lysosomal trafficking and autophagy in injured tubules. A protective role for gpnmb/HGFIN in the autophagic renal repair of tubules after injury has been described.[60] Thus, a unique distinguishing feature of urine HGFIN is that it may reflect injury in distal nephron tubular cells that are utilizing autophagy as a repair mechanism for survival. Our initial data suggest that HGFIN is a novel biomarker of renal injury in experimental models of renal disease and in CKD.[59]

Conclusion

The health and survival of the glomerular and tubular compartments of the kidney are tightly interconnected. Tubulointerstitial diseases ultimately induce glomerulosclerosis, and glomerular diseases eventually cause tubulointerstitial damage. Irrespective of the cause, CKD progresses via pathogenetic processes that engender variable amounts of tubulointerstitial inflammation, tubular cell dropout, and fibrosis. Importantly, the risk of kidney disease progression closely correlates with the extent of TIF, regardless of etiology. Tubulointerstitial damage in chronic glomerular disease is characterized by an increase in tubular atrophy, accumulation of ECM, obliteration of the peritubular capillaries, the emergence of interstitial and tubular cells expressing a mesenchymal phenotype, and the influx of an inflammatory cell infiltrate. Novel biomarkers of the processes that induce these tubulointerstitial changes may ultimately prove to be better predictors of disease progression and long-term prognosis than our current markers, which predominantly focus on glomerular filtration and permselectivity. There are several early sensitive markers of kidney injury that we briefly highlighted, and which are windows to biological processes that contribute to progression in CKD. Renoprotection afforded by current antiproteinuric strategies, such as inhibition of the rennin–angiotensin–aldosterone system, indirectly confers tubulointerstitial protection in glomerular disease. The unique contributions of the novel tubule injury biomarkers may be in highlighting injury pathways to directly target tubule survival as a primary strategy for renoprotection in glomerular disease. Glomerular and tubular markers, such as urinary podocytes, KIM-1, NGAL, or HGFIN, separately and/or together may represent a "kidney injury panel" of the future. For now, these candidate biomarkers are still being validated, and there is much work yet to be done to explore their role before their use in routine screening and guiding therapy in patients with CKD. The National Institutes of Health has designated this area as one of high priority, and an ongoing CKD Biomarker Research Group will undoubtedly spearhead additional work in this area.

References

1. Couser WG. Pathogenesis of glomerulonephritis: from chickens to humans. *Nephron Physiol.* 2009;112:7-23.
2. Anders HJ, Banas B, Schlondorff D. Signaling danger: toll-like receptors and their potential roles in kidney disease. *J Am Soc Nephrol.* 2004;15:854-867.
3. Romero F, Rodriguez-Iturbe B, Parra G, et al. Mycophenolate mofetil prevents the progressive renal failure induced by 5/6 renal ablation in rats. *Kidney Int.* 1999;55:945-955.
4. Utimura R, Fujihara CK, Mattar AL, et al. Mycophenolate mofetil prevents the development of glomerular injury in experimental diabetes. *Kidney Int.* 2003;63:209-216.
5. Huugen D, Tervaert JW, Heeringa P. TNF-alpha bioactivity-inhibiting therapy in ANCA-associated vasculitis: clinical and experimental considerations. *Clin J Am Soc Nephrol.* 2006;1:1100-1107.
6. Kang SW, Natarajan R, Shahed A, et al. Role of 12-lipoxygenase in the stimulation of p38 mitogen-activated protein kinase and collagen alpha5(IV) in experimental diabetic nephropathy and in glucose-stimulated podocytes. *J Am Soc Nephrol.* 2003;14:3178-3187.
7. Eardley KS, Zehnder D, Quinkler M, et al. The relationship between albuminuria, MCP-1/CCL2, and interstitial macrophages in chronic kidney disease. *Kidney Int.* 2006;69:1189-1197.

8. Brenner BM, Meyer TW, Hostetter TH. Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. *N Engl J Med.* 1982;307:652-659.
9. Nangaku M. Mechanisms of tubulointerstitial injury in the kidney: final common pathways to end-stage renal failure. *Intern Med.* 2004;43:9-17.
10. Bohle A, Mackensen-Haen S, von Gise H. Significance of tubulointerstitial changes in the renal cortex for the excretory function and concentration ability of the kidney: a morphometric contribution. *Am J Nephrol.* 1987;7:421-433.
11. Gilbert RE, Cooper ME. The tubulointerstitium in progressive diabetic kidney disease: more than an aftermath of glomerular injury? *Kidney Int.* 1999;56:1627-1637.
12. Nakhoul N, Batuman V. Role of proximal tubules in the pathogenesis of kidney disease. *Contrib Nephrol.* 2011;169:37-50.
13. Thomas ME, Brunskill NJ, Harris KP, et al. Proteinuria induces tubular cell turnover: a potential mechanism for tubular atrophy. *Kidney Int.* 1999;55:890-898.
14. Macconi D, Chiabrando C, Schiarea S, et al. Proteasomal processing of albumin by renal dendritic cells generates antigenic peptides. *J Am Soc Nephrol.* 2009;20:123-130.
15. Le Goff B, Soltner E, Charrier C, et al. A combination of methotrexate and zoledronic acid prevents bone erosions and systemic bone mass loss in collagen induced arthritis. *Arthritis Res Ther.* 2009;11:R185.
16. Matsumoto M, Tanaka T, Yamamoto T, et al. Hypoperfusion of peritubular capillaries induces chronic hypoxia before progression of tubulointerstitial injury in a progressive model of rat glomerulonephritis. *J Am Soc Nephrol.* 2004;15:1574-1581.
17. Futrakul N, Vongthavarawat V, Sirisalipotch S, et al. Tubular dysfunction and hemodynamic alteration in normoalbuminuric type 2 diabetes. *Clin Hemorheol Microcirc.* 2005;32:59-65.
18. Eddy AA. Progression in chronic kidney disease. *Adv Chronic Kidney Dis.* 2005;12:353-365.
19. Sakai N, Wada T, Yokoyama H, et al. Secondary lymphoid tissue chemokine (SLC/CCL21)/CCR7 signaling regulates fibrocytes in renal fibrosis. *Proc Natl Acad Sci USA.* 2006;103:14098-14103.
20. Liu Y. Epithelial to mesenchymal transition in renal fibrogenesis: pathologic significance, molecular mechanism, and therapeutic intervention. *J Am Soc Nephrol.* 2004;15:1-12.
21. Humphreys BD, Lin SL, Kobayashi A, et al. Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. *Am J Pathol.* 2010;176:85-97.
22. Truong LD, Farhood A, Tasby J, Gillum D. Experimental chronic renal ischemia: morphologic and immunologic studies. *Kidney Int.* 1992;41:1676-1689.
23. Satirapoj B, Wang Y, Chamberlin M, et al. Overexpression of oxidized low-density lipoprotein (Ox-LDL) in the remnant kidney after 5/6 nephrectomy (5/6Nx) and antigen transport to renal lymph nodes (RLN). *J Am Soc Nephrol.* 2009;20:134A.
24. Kramer S, Binder E, Loof T, et al. The lymphocyte migration inhibitor FTY720 attenuates experimental hypertensive nephropathy. *Am J Physiol Renal Physiol.* 2009;297:F218-F227.
25. D'Amico G. Influence of clinical and histological features on actuarial renal survival in adult patients with idiopathic IgA nephropathy, membranous nephropathy, and membranoproliferative glomerulonephritis: survey of the recent literature. *Am J Kidney Dis.* 1992;20:315-323.
26. Drayton DL, Liao S, Mounzer RH, Ruddle NH. Lymphoid organ development: from ontogeny to neogenesis. *Nat Immunol.* 2006;7:344-353.
27. Heymann F, Meyer-Schwesinger C, Hamilton-Williams EE, et al. Kidney dendritic cell activation is required for progression of renal disease in a mouse model of glomerular injury. *J Clin Invest.* 2009;119:1286-1297.
28. Zoja C, Abbate M, Remuzzi G. Progression of chronic kidney disease: insights from animal models. *Curr Opin Nephrol Hypertens.* 2006;15:250-257.
29. Kanwar YS, Liu ZZ, Kashihara N, Wallner EI. Current status of the structural and functional basis of glomerular filtration and proteinuria. *Semin Nephrol.* 1991;11:390-413.
30. Ruggenenti P, Perna A, Mosconi L, Pisoni R, Remuzzi G. Urinary protein excretion rate is the best independent predictor of ESRF in non-diabetic proteinuric chronic nephropathies. "Gruppo Italiano di Studi Epidemiologici in Nefrologia" (GISEN). *Kidney Int.* 1998;53:1209-1216.
31. Segerer S, Schlondorff D. Role of chemokines for the localization of leukocyte subsets in the kidney. *Semin Nephrol.* 2007; 27:260-274.
32. Anders HJ, Ninichuk V, Schlondorff D. Progression of kidney disease: blocking leukocyte recruitment with chemokine receptor CCR1 antagonists. *Kidney Int.* 2006;69:29-32.
33. Lech M, Garlanda C, Mantovani A, et al. Different roles of TiR8/Sigirr on toll-like receptor signaling in intrarenal antigen-presenting cells and tubular epithelial cells. *Kidney Int.* 2007;72:182-192.
34. Anders HJ, Schlondorff DO. Innate immune receptors and autophagy: implications for autoimmune kidney injury. *Kidney Int.* 2010;78:29-37.
35. Thomas GN, Lin JW, Lam WW, et al. Albuminuria is a marker of increasing intracranial and extracranial vascular involvement in type 2 diabetic Chinese patients. *Diabetologia.* 2004; 47:1528-1534.
36. Wright JT Jr, Bakris G, Greene T, et al. Effect of blood pressure lowering and antihypertensive drug class on progression of hypertensive kidney disease: results from the AASK trial. *JAMA.* 2002;288:2421-2431.
37. Parving HH, Chaturvedi N, Viberti G, Mogensen CE. Does microalbuminuria predict diabetic nephropathy? *Diabetes Care.* 2002;25:406-407.
38. Tabaei BP, Al-Kassab AS, Ilag LL, Zawacki CM, Herman WH. Does microalbuminuria predict diabetic nephropathy? *Diabetes Care.* 2001;24:1560-1566.
39. Kriz W, Gretz N, Lemley KV. Progression of glomerular diseases: is the podocyte the culprit? *Kidney Int.* 1998;54:687-697.
40. Vogelmann SU, Nelson WJ, Myers BD, Lemley KV. Urinary excretion of viable podocytes in health and renal disease. *Am J Physiol Renal Physiol.* 2003;285:F40-F48.
41. Hara M, Yanagihara T, Kihara I. Cumulative excretion of urinary podocytes reflects disease progression in IgA nephropathy and Schonlein-Henoch purpura nephritis. *Clin J Am Soc Nephrol.* 2007;2:231-238.
42. Hara M, Yanagihara T, Itoh M, Matsuno M, Kihara I. Immunohistochemical and urinary markers of podocyte injury. *Pediatr Nephrol.* 1998;12:43-48.
43. Nakamura T, Ushiyama C, Suzuki S, et al. Urinary excretion of podocytes in patients with diabetic nephropathy. *Nephrol Dial Transplant.* 2000;15:1379-1383.
44. Szeto CC, Lai KB, Chow KM, et al. Messenger RNA expression of glomerular podocyte markers in the urinary sediment of acquired proteinuric diseases. *Clin Chim Acta.* 2005;361:182-190.
45. Sato Y, Wharram BL, Lee SK, et al. Urine podocyte mRNAs mark progression of renal disease. *J Am Soc Nephrol.* 2009; 20:1041-1052.
46. Yu D, Petermann A, Kunter U, et al. Urinary podocyte loss is a more specific marker of ongoing glomerular damage than proteinuria. *J Am Soc Nephrol.* 2005;16:1733-1741.
47. Wang G, Lai FM, Kwan BC, et al. Podocyte loss in human hypertensive nephrosclerosis. *Am J Hypertens.* 2009;22:300-306.
48. Ichimura T, Bonventre JV, 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;273:4135-4142.

49. van Timmeren MM, Bakker SJ, Vaidya VS, et al. Tubular kidney injury molecule-1 in protein-overload nephropathy. *Am J Physiol Renal Physiol.* 2006;291:F456-F464.
50. van Timmeren MM, van den Heuvel MC, Bailly V, et al. Tubular kidney injury molecule-1 (KIM-1) in human renal disease. *J Pathol.* 2007;212:209-217.
51. 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;53:16-25.
52. 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;115:610-621.
53. Malyszko J, Malyszko JS, Bachorzewska-Gajewska H, et al. Neutrophil gelatinase-associated lipocalin is a new and sensitive marker of kidney function in chronic kidney disease patients and renal allograft recipients. *Transplant Proc.* 2009;41:158-161.
54. 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;4:337-344.
55. Mori K, Nakao K. Neutrophil gelatinase-associated lipocalin as the real-time indicator of active kidney damage. *Kidney Int.* 2007; 71:967-970.
56. Safadi FF, Xu J, Smock SL, et al. Cloning and characterization of osteoactivin, a novel cDNA expressed in osteoblasts. *J Cell Biochem.* 2001;84:12-26.
57. Pahl MV, Vaziri ND, Yuan J, Adler SG. Upregulation of monocyte/macrophage HGFIN (Gpnmb/Osteoactivin) expression in end-stage renal disease. *Clin J Am Soc Nephrol.* 2010;5:56-61.
58. Ripoll VM, Irvine KM, Ravasi T, Sweet MJ, Hume DA. Gpnmb is induced in macrophages by IFN-gamma and lipopolysaccharide and acts as a feedback regulator of proinflammatory responses. *J Immunol.* 2007;178:6557-6566.
59. Patel-Chamberlin M, Wang Y, Satirapoj B, et al. Hematopoietic growth factor inducible neurokinin-1 (Gpnmb/Osteoactivin) is a biomarker of progressive renal injury across species. *Kidney Int.* 2011;79:1138-1148.
60. Li B, Castano AP, Hudson TE, et al. The melanoma-associated transmembrane glycoprotein Gpnmb controls trafficking of cellular debris for degradation and is essential for tissue repair. *FASEB J.* 2010;24:4767-4781.