# Toward Assessment of Tubular Function in Kidney Disease

*Sebastian B. Beckmann*[1], *Madonna Salib*[1], *Robert A. Fenton*[2], *and Ewout J. Hoorn*[1]

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The current assessment of kidney health relies on eGFR and albuminuria and has served the nephrology community well. However, there is a pressing need to improve this assessment to better identify early kidney injury and determine optimal treatments for specific patients. On critical evaluation of current kidney health assessment, three limitations stand out: (*1*) the lack of an index of tubular function, (*2*) reliance on a single urinary biomarker, and (*3*) assessment based solely on static testing. In this Perspective, we propose that kidney health assessment could be enhanced by integrating urinary biomarkers that reflect tubular function and using dynamic testing methods. Tubular injury is increasingly recognized as a driving force for kidney disease progression, and most kidney-protective drugs target the kidney tubule.[1] Beyond CKD progression, tubular biomarkers may also play a role in interstitial nephritis, post-transplant monitoring, drug dosing, and kidney diseases with limited therapeutic options, such as AKI and AKI-CKD transition. Thus, there is a strong pathophysiologic and clinical rationale to include tubular function in kidney health assessment and develop a severity grading system for tubular dysfunction. Urinary biomarkers can effectively capture changes in tubular function or the secretion profile of tubular epithelial cells. Although tubular biomarkers for CKD have not yet achieved widespread clinical use, those that have been identified correlate with clinically significant outcomes irrespective of eGFR and albuminuria.[2]

## Sources of Urinary Tubular Biomarkers

There are five routes through which biomarkers of kidney health can appear in urine: (*1*) glomerular filtration, (*2*) impaired proximal tubule endocytosis, (*3*) proximal tubular secretion of protein-bound substances, (*4*) secretion by tubular epithelial cells, and (*5*) as cargo within extracellular vesicles (Figure 1). The classic example of a urinary biomarker for kidney injury is albumin, which appears in urine after damage to the glomerular filtration barrier. Smaller proteins are filtered and then endocytosed by the proximal tubule—impairment of this process results in tubular proteinuria. Notably, albumin also undergoes filtration and endocytosis in healthy kidney, suggesting that albuminuria is also a marker of proximal tubular injury.[3] The metabolism of filtered proteins along the proximal tubule reveals a remarkable spatiotemporal organization with a multistep degradation process from the S1 to S3 segment.[3] Defects in endocytosis trigger compensatory protein uptake downstream, which implies that tubular proteinuria may underestimate the actual degree of proximal tubular injury. The proximal tubule is also responsible for active secretion of protein-bound solutes from the peritubular capillaries. Reduced proximal tubular secretory clearance has been linked to more severe interstitial fibrosis, tubular atrophy, CKD progression, and mortality.[2] Stressed proximal tubular epithelial cells release proteins that can also serve as kidney disease biomarkers such as kidney injury molecule 1. Other biomarkers representing fibrotic and inflammatory responses can be secreted by all tubular epithelial cells but can also originate from the interstitium. Two biomarkers unique to the distal tubule, uromodulin and EGF, are released into urine as they undergo proteolytic cleavage. Their urinary excretion *decreases* as kidney function declines, possibly due to nephron loss.[4] However, sodium-glucose cotransporter 2 inhibitors can increase urine EGF, suggesting its potential use in assessing treatment response.[4] In addition, low urine EGF is associated with incident CKD in the general population, especially in individuals without hypertension, helping to identify at-risk individuals who might otherwise go undetected.[4] Similarly, the urinary excretion of the lipid biomarker PGE2 was recently found to be an early marker for the risk of CKD and cardiovascular mortality in the general population.[5] The effectiveness of any specific tubular biomarker can vary based on the clinical context, leading to an interest in evaluating biomarkers as a collective tubular panel.[2] Initiatives like the Kidney Precision Medicine Project could enhance our understanding of the origins and functional significance of urine biomarkers.

## Extracellular Vesicles

An exciting development in biomarker discovery comes from examining urinary extracellular vesicles.[6] The main

[1]Division of Nephrology and Transplantation, Department of Internal Medicine, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands
[2]Department of Biomedicine, Aarhus University, Aarhus, Denmark

**Correspondence:** Prof. Ewout J. Hoorn, email: e.j.hoorn@erasmusmc.nl

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S.B.B. and M.S. contributed equally to this work.



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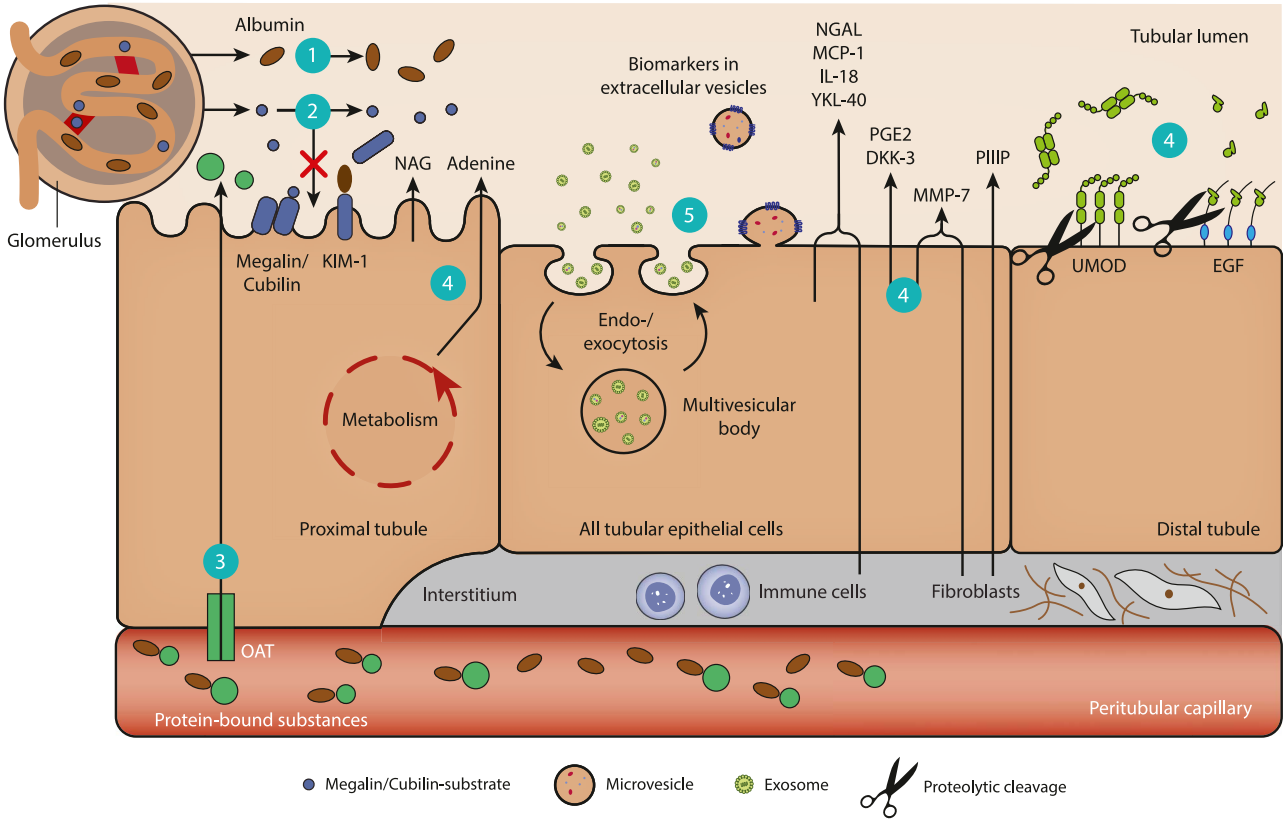



**Figure 1. Overview of five urinary excretion routes of biomarkers for kidney disease.** First, glomerular injury causes filtration of proteins such as albumin. Second, impaired endocytosis in the proximal tubule causes tubular proteinuria. Examples of tubular proteinuria include the urinary excretion of low–molecular weight proteins such as $\alpha$1- and $\beta$2-microglobulin and retinol-binding protein. Most of these proteins are substrates for megalin and cubilin. Because albumin is also filtered and endocytosed in healthy kidney, albuminuria reflects glomerular and proximal tubular injury. Albumin is endocytosed by megalin/cubilin and by KIM-1 when it is bound to fatty acids. Third, kidney disease can impair the proximal tubule secretion of blood-derived protein-bound substances, which are normally transported through OATs. Fourth, tubular epithelial cells can increase the secretion of biomarkers on stress or injury. This includes biomarkers specific to the proximal tubule (KIM-1, NAG, and adenine), distal tubule (UMOD and EGF), and those that can be secreted by all tubular epithelial cells (DKK-3 and PGE2). Furthermore, both tubular epithelial cells and infiltrating immune cells can secrete inflammatory biomarkers, including MCP-1, IL-18, and chitinase-3-like protein-1 (also called chitinase three-like protein 1). Fibrosis biomarkers MMP-7 and PIIIP are (also) derived from the interstitium. Fifth route consists of the secretion of extracellular vesicles. Extracellular vesicles include both exosomes and microvesicles, which are derived from an endocytosis–exocytosis process involving multivesicular bodies and plasma membrane budding, respectively. Extracellular vesicles contain proteins, RNA, microRNA, DNA, lipids, and metabolites that can be harnessed as kidney biomarkers. DKK-3, Dickkopf-3; EV, extracellular vesicle; KIM-1, kidney injury molecule 1; MCP-1, monocyte chemoattractant protein-1; MMP-7, matrix metalloproteinase 7; NAG, *N*-acetyl-$\beta$-glucosaminidase; OAT, organic anion transporter; PIIIP, procollagen III amino-terminal propeptide; UMOD, uromodulin.

source of urinary extracellular vesicles is tubular epithelial cells; they encompass cargo from the cytosol and the plasma membrane, including proteins, RNA, microRNA, lipids, and metabolites. Why cells secrete extracellular vesicles remains a rather teleological question, but their role in cell–cell communication, particularly through microRNA delivery, is increasingly recognized.[6] Extracellular vesicles are also linked to the progression of kidney disease, making them attractive both for biomarker discovery and as therapeutic targets. Mass spectrometry advancements allow for the analysis of thousands of proteins within urinary extracellular vesicles isolated from only a few milliliters of urine. We conducted proteomic analysis of urinary extracellular vesicles to identify novel biomarkers for polycystic kidney disease progression.[7] Matrix metalloproteinase 7 (MMP-7) was identified in urinary extracellular vesicles as a biomarker for rapid disease progression, but not in whole urine. An unbiased kidney tissue proteomics study in patients with diabetic kidney disease also linked MMP-7 to fibrosis and found that blood MMP-7 correlates with eGFR in the general population.[8] This illustrates the potential of unbiased approaches to identify novel tubular biomarkers for high-throughput clinical application. Urinary extracellular vesicle biomarkers also have the potential to predict both short-term and long-term outcomes after kidney transplantation, including rejection.[6] Several technical barriers that limited the application of urinary extracellular vesicles have largely been resolved, including approaches to characterize, quantify, and normalize urinary extracellular vesicles.[9] The next step to move urinary extracellular vesicles forward is the ability to investigate

urinary extracellular vesicle cargo without the need to isolate urinary extracellular vesicles. Once biomarker candidates have been identified in urinary extracellular vesicles, a targeted mass spectrometry approach on whole urine may enable this isolation-free, high-throughput detection.

## Tubular Stress Test for CKD

The kidney is a complex organ, yet nephrology relies on static tests for assessing kidney function, which often use dynamic tests. Kidney functional reserve is a dynamic measure of kidney function that has been linked to CKD prognosis and progression.[10] Recently, the idea of expanding kidney functional reserve to include tubular function has been proposed, with stress tests for proximal tubular secretion, urinary concentration, and acid-base handling, all of which have been linked to CKD progression.[10] These tests include administration of compounds such as creatinine or diuretics to assess proximal tubular secretion, water deprivation or desmopressin for identifying concentrating defects, and bicarbonate or an acid load to test acid-base retention. Tubular stress tests may also provide information on therapeutic benefit (*e.g.*, alkali therapy). Diuretic stress testing may be an effective approach to evaluate both proximal and distal tubular function, as diuretics are secreted through the proximal tubule and affect sodium reabsorption in the distal tubule. In patients with CKD, diuretic clearance reduces as eGFR declines, while diuretic sensitivity remains intact, indicating that sodium reabsorption increases in more advanced stages of CKD.[11] This also illustrates that CKD can affect the proximal and distal tubule differently, which may have implications for prognosis and treatment.

## Moving Forward

Assessing tubular function can provide valuable insights not only into kidney disease but also into cardiovascular and cognitive health. The clinical application of tubular biomarkers requires them to demonstrate minimal biologic and analytical variability, fill a diagnostic or prognostic gap, or guide treatment, all while remaining cost-effective. Integrating tubular biomarkers into clinical trials would be a logical next step to expedite their implementation. In addition, if tubular stress tests outperform static ones, the added workload would be justified. The ultimate paradigm shift would be to expand the current CKD classification (C, G, A) by adding a fourth character, T, for tubular function.

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### Author Contributions

**Conceptualization:** Ewout J. Hoorn.
**Supervision:** Ewout J. Hoorn, Madonna Salib.
**Visualization:** Sebastian Beckmann.
**Writing – original draft:** Sebastian Beckmann, Ewout J. Hoorn, Madonna Salib.
**Writing – review & editing:** Sebastian Beckmann, Robert Fenton, Ewout J. Hoorn, Madonna Salib.

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