Expanding the Toolbox
Novel Urinary Biomarkers for Kidney Allograft Monitoring

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The landscape of kidney transplantation is undergoing a quiet but potentially transformative evolution. As long-term outcomes of kidney allografts remain suboptimal and invasive biopsy continues to serve as the clinical gold standard for diagnosing graft dysfunction, research has redoubled efforts to identify noninvasive, accurate biomarkers to guide diagnosis, prognosis, and therapeutic decision making. Within the context of kidney transplantation, two manuscripts published in this current issue of *Kidney360*[1,2] bring fresh perspectives to the field of urinary biomarkers, addressing the dual needs of diagnostic differentiation and pathophysiologic insight in transplant nephrology.

Differentiating the Causes of Graft Dysfunction

In their study, Wu *et al.*[1] address a central clinical problem: the inability to noninvasively differentiate between the various causes of kidney transplant dysfunction, most notably acute tubular necrosis (ATN), acute rejection, and interstitial fibrosis and tubular atrophy (IFTA). Although each of these entities shares overlapping clinical and laboratory features, their treatment and prognostic implications diverge significantly. The current reliance on kidney biopsy is both invasive and resource-intensive, with risks of bleeding, sampling error, and delayed diagnosis. The authors propose an innovative, noninvasive diagnostic modality: multispectral autofluorescence analysis of exfoliated proximal tubular cells in urine. The idea is elegant in its simplicity—the metabolic and structural states of these shed cells carry disease-specific fingerprints that can be captured and analyzed using autofluorescence microscopy and machine learning. Using a relatively small but clinically well-phenotyped cohort (ten patients per group), the investigators used both random forest and AutoGluon machine learning approaches to classify autofluorescence-derived features. The performance metrics are impressive, especially for AutoGluon, achieving an area under the curve of 0.95 (ATN versus rejection), 0.92 (ATN versus IFTA), and 0.91 (rejection versus IFTA), respectively. These results point toward the feasibility of a noninvasive, cell-based, image-informed platform that could augment—and potentially, in certain contexts, replace—the need for biopsy.

The greatest strength of this study lies in its proof-of-concept innovation. It leverages autofluorescence, a largely underexploited intrinsic property of cells, and bypasses the need for extrinsic labeling or costly multiplexed panels. In addition, it builds on a biologically grounded hypothesis—that cell autofluorescence correlates with metabolic states and injury patterns—and applies it to a clinically urgent diagnostic problem. That said, several caveats are warranted. First, the sample size is limited, and while rigorous cross-validation was used, the generalizability of these findings awaits external validation in larger, multicentric cohorts. The immunomagnetic cell isolation process (using alanine aminopeptidase and sodium-glucose cotransporter-2 antibodies) may also be difficult to standardize across centers, potentially limiting scalability. Furthermore, although autofluorescence metrics are powerful, they are not yet widely understood by clinicians, potentially creating barriers to clinical adoption unless incorporated into a user-friendly diagnostic workflow. In addition, the study is diagnostic in nature: neither does it provide prognostic data nor does it correlate autofluorescence patterns with clinical outcomes such as graft survival, recurrence, or response to therapy. Most importantly, however, the study fails to demonstrate independence of autofluorescence

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See related articles, "Noninvasive Assessment of Urinary Exfoliated Proximal Tubule Cell Multispectral Autofluorescence May Differentiate between Causes of Kidney Transplant Dysfunction," and "Higher Urinary Iron Levels are Associated with Kidney Dysfunction, Tubular Damage, and Increased Mortality in Kidney Transplant Recipients," on pages 1853–1862 and 1970–1980, respectively.

from time post-transplant, which is expected to be significantly shorter in ATN compared with IFTA. Ruling out such bias and correlating autofluorescence with clinical outcomes will be important next steps for future studies.

From Marker to Mechanism: The Role of Urinary Iron

In contrast to this diagnostic focus, Kremer *et al.*[2] explore the pathophysiologic and prognostic relevance of urinary iron excretion in stable kidney transplant recipients (KTRs). Drawing on data from nearly 700 KTRs in the TransplantLines cohort, this study shows that elevated urinary iron correlates with worse kidney function, greater tubular injury (as reflected by urinary liver-type fatty acid binding protein and vascular endothelial growth factor/creatinine ratio), and significantly higher mortality risk. The authors find that urinary iron does not correlate with systemic iron status but does increase with oral iron supplementation. This raises the provocative possibility that iron, while essential for systemic function, might act as a tubulotoxic agent when present in excess in the renal tubular lumen, potentially driving oxidative stress, epithelial damage, and long-term nephron loss.

This study benefits from a large, well-characterized cohort, robust multivariate modeling, and longitudinal follow-up over more than 5 years. The association of urinary iron with both hard outcomes (mortality, graft failure) and intermediate tubular injury markers lends strong biologic plausibility to the observed findings. Importantly, the results raise therapeutic and management implications. If confirmed, clinicians might need to re-evaluate iron supplementation practices in KTRs, especially in the absence of absolute systemic deficiency. Moreover, urinary iron could potentially serve as a biomarker for early tubular stress, guiding closer monitoring or modification of nephrotoxic exposures. However, the study is observational, and causality cannot be inferred. It remains unclear whether urinary iron is simply a marker of preexisting injury or a mediator of progressive damage. Although oral iron use was adjusted for, the study could not fully disentangle the role of supplementation in elevating urinary iron versus intrinsic tubular dysfunction. Further mechanistic and interventional studies—*e.g.*, trials evaluating the effect of iron chelation or changes in supplementation practices—will be required to clarify this point. In addition, the mechanisms by which iron enters the urine in KTRs are not fully dissected here. Increased glomerular permeability, defective tubular reuptake, or altered local iron handling may all play a role, and these processes likely differ across patient subgroups. Finally, the cohort's homogeneity (predominantly Dutch recipients) and its single-center design may limit generalizability to diverse populations.

Toward an Integrated Biomarker Paradigm

Recent advances in urinary biomarker research for kidney transplantation reflect a rapidly evolving landscape that extends beyond traditional chemokines and mRNA signatures. As highlighted in recent reviews, the integration of multiomic approaches—including transcriptomics, proteomics, and metabolomics—has enabled the identification of novel urinary biomarkers with potential for early detection of both subclinical and overt allograft injury, as well as for prognostication and mechanistic insight into chronic dysfunction.[3,4] Notably, urinary chemokines such as chemokine (C-X-C motif) ligand 9 and chemokine (C-X-C motif) ligand 10 have demonstrated robust performance in large clinical trials for diagnosing acute and subclinical rejection, while urinary cell mRNA panels continue to show promise for noninvasive immune monitoring and prediction of graft outcomes.[4–6] Another study found that combining urinary T cells with either tubular epithelial cells or podocalyxin-positive cells enabled noninvasive detection of post-transplant graft rejection with high diagnostic accuracy.[7] In addition, the clinical implementation of donor-derived cellfree DNA has further expanded the biomarker paradigm, with donor-derived cellfree DNA now recognized as a sensitive marker of severe allograft injury, regardless of etiology, and as a predictor of adverse graft outcomes when persistently elevated.[4,8] However, it needs to be emphasized that these biomarkers require rigorous multicenter validation, standardization of assays, and integration with clinical and histopathologic data to maximize their clinical utility.[9]

A particularly innovative direction is the application of single-cell RNA sequencing to urine-derived kidney cells, demonstrating that urinary single-cell transcriptomics can capture the full spectrum of kidney and immune cell types, providing a noninvasive window into intrarenal cellular dynamics and potentially enabling real-time, high-resolution monitoring of allograft health.[10] This approach may facilitate the discovery of cell-type–specific injury signatures and inform precision immunosuppression strategies. Urinary biomarkers have several inherent advantages: They are noninvasive, can be obtained serially, and may reflect real-time intrarenal processes. However, as these studies illustrate, moving from concept to clinic will require careful validation, standardization, and contextualization within broader clinical workflows. Ultimately, the future of urine-derived biomarkers lies in multimodal integration—combining molecular, cellular, and clinical data to enable individualized, minimally invasive monitoring and intervention, with the goal of improving long-term graft survival and patient outcomes.

Future directions in this space should aim for:

1. Validation across diverse populations and centers, ensuring reproducibility.
2. Multiomic integration with clinical decision tools, combining transcriptomic, proteomic, and metabolomic biomarker data with histopathology, imaging, and traditional laboratory results.
3. Mechanistic insight, especially where biomarkers suggest actionable pathways (*e.g.*, iron-induced tubulotoxicity).
4. Cost-effectiveness and scalability, as ultimately the value of a biomarker lies not just in its area under the curve, but in its ability to change clinical practice.

In conclusion, both studies—while distinct in their approach—move us closer to a future where noninvasive, precision-guided transplant monitoring is not aspirational, but routine. As the transplant community continues to

refine its tools, these contributions serve as important building blocks toward a more nuanced, personalized, and less invasive standard of care.

Disclosures

Disclosure forms, as provided by each author, are available with the online version of the article at http://links.lww.com/KN9/B181.

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References

1. Wu HHL, Lang Y, Handley S, et al. Noninvasive assessment of urinary exfoliated proximal tubule cell multispectral autofluorescence may differentiate between causes of kidney transplant dysfunction. *Kidney360.* 2025;6(11):1853–1862. doi:10.34067/KID.0000000879
2. Kremer D, Rawee P, Knobbe TJ, et al. Higher urinary iron levels are associated with kidney dysfunction, tubular damage, and increased mortality in kidney transplant recipients. *Kidney360.* 2025;6(11):1970–1980. doi:10.34067/KID.0000000878
3. Jin PH, Sarwal RD, Sarwal MM. Urinary biomarkers for kidney allograft injury. *Transplantation.* 2022;106(7):1330–1338. doi:10.1097/tp.0000000000004017
4. Gupta G, Athreya A, Kataria A. Biomarkers in kidney transplantation: a rapidly evolving landscape. *Transplantation.* 2025;109(3):418–427. doi:10.1097/tp.0000000000005122
5. Lubetzky ML, Salinas T, Schwartz JE, Suthanthiran M. Urinary cell mRNA profiles predictive of human kidney allograft status. *Clin J Am Soc Nephrol.* 2021;16(10):1565–1577. doi:10.2215/CJN.14010820
6. Guzzi F, Cirillo L, Buti E, et al. Urinary biomarkers for diagnosis and prediction of acute kidney allograft rejection: a systematic review. *Int J Mol Sci.* 2020;21(18):6889. doi:10.3390/ijms21186889
7. Goerlich N, Brand HA, Langhans V, et al. Kidney transplant monitoring by urinary flow cytometry: biomarker combination of T cells, renal tubular epithelial cells, and podocalyxin-positive cells detects rejection. *Sci Rep.* 2020;10(1):796. doi:10.1038/s41598-020-57524-7
8. Parajuli S, Garg N, Dodin B, et al. Changes in donor-derived cell-free DNA before and after rejection and de novo DSA detection in primary and repeat kidney transplant recipients. *Clin Transplant.* 2024;38(11):e70019. doi:10.1111/ctr.70019
9. Huang E, Mengel M, Clahsen-van Groningen MC, Jackson AM. Diagnostic potential of minimally invasive biomarkers: a biopsy-centered viewpoint from the banff minimally invasive diagnostics working group. *Transplantation.* 2023;107(1):45–52. doi:10.1097/tp.0000000000004339
10. Abedini A, Zhu YO, Chatterjee S, et al.; TRIDENT Study Investigators. Urinary single-cell profiling captures the cellular diversity of the kidney. *J Am Soc Nephrol.* 2021;32(3):614–627. doi:10.1681/ASN.2020050757