



Commentary

To accompany Banas et al., Time for a Paradigm Shift

Atul K. Sharma^{a,*}, Tom D. Blydt-Hansen^b^a Department of Pediatrics and Child Health, University of Manitoba, Canada^b Department of Pediatrics, University of British Columbia, Canada

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The first successful human kidney transplant was performed in 1954, between identical twins without immunosuppression. By the early 1960s, non-identical donors saw reports of acute inflammatory allograft rejection, treatment with corticosteroids, and percutaneous allograft biopsies [1,2]. Despite steady advances in surgical technique, tissue matching, and anti-rejection therapies, the fundamental paradigm did not change much in the subsequent 60 years. Even today, acute rejection (AR) episodes and smoldering subclinical rejection remain the principle determinants of long-term graft survival [3,4], and renal biopsy—despite well-documented risks and costs—remains the diagnostic gold-standard [5] in the face of typically non-specific clinical findings e.g. declining renal function, which may only appear late in the course.

As the most accessible fluid associated with the kidney, urine is an obvious source for biomarkers related to alloimmunity, including proteins, peptides, and mRNAs [6]. In contrast, urinary metabolomics focuses on small molecules that reflect tissue metabolism, which has practical advantages in being economical, quantitative, and readily-available. Despite early promise [7,8], it has nevertheless been slow to move from bench to bedside. For this reason, we welcome the report by Banas et al. [9], which promises a practical approach to risk assessment in renal allograft recipients with and without clinical signs of AR.

In an earlier publication, these authors applied NMR spectroscopy to 1883 urines from a training cohort of 180 renal transplant recipients with and without AR in the UMBRELLA study [10]. Through a combination of statistical and biological criteria, they proposed and tested a multivariable logistic regression model to assign rejection risk based on a panel of urinary metabolites: alanine, citrate, lactate, urea and creatinine (for normalization). When applied to a test cohort of 589 urine specimens from 178 pa-

tients, discrimination performance was moderate, with a receiver-operator area under the curve (AUC) of 0.72–0.74. While tempting to dismiss such findings as non-diagnostic, the authors have now validated their assay as a decision-making aid for distinguishing patients needing additional histopathologic evaluation from those who can be managed expectantly.

The current report, published in *EBioMedicine*, is a prospective, observational study in an independent validation cohort consisting of 986 urines from 109 consecutively enrolled renal transplant recipients. In addition to validating their existing scoring system (AUC = 0.75, 95% CI 0.68–0.83), the investigators combined it with the estimated glomerular filtration rate (eGFR, ml/min/1.73 m²) to show superior performance for the resulting ratio compared to either alone (AUC = 0.84, 95% CI 0.76–0.91, based on 42 cases and 468 controls). These estimates may be biased by the exclusion of $N=89$ samples with other biopsy findings, which would not normally be known at the time of assessment. In terms of performance, useful discrimination was limited to patients ≥ 15 days post-operatively, where investigators were able to confirm two thresholds from their previous study: Cut-off values of 3.0 and 13.0 were associated, respectively, with 91% sensitivity (low risk) and 89% specificity (high risk). Nevertheless, false positives and negatives remain. At the lower threshold, specificity was 34%, and sensitivity at the upper threshold was 48%, creating a sizeable “intermediate” zone where the test was uninformative. But rather than a single cut-off, the combination of differing high/low thresholds and conventional clinical indicators (eGFR) represents a novel approach to more graded risk assessment.

In total, there were 85 samples with biopsies, same-day urines and concurrent eGFRs to be evaluated. A substantial proportion ($N=25/85$ or 29.4%) were assigned intermediate risk with a metabolite score < 13 and eGFR < 30 or metabolite score ≥ 13 and eGFR ≥ 30 , and 12/25 (48%) were found to have AR. Of those considered high-risk, 8/13 (61.5%) had biopsy-confirmed AR versus 8/47 at low-risk (17%). Particularly intriguing were the results

* Corresponding author.

E-mail addresses: atul.sharma@umanitoba.ca (A.K. Sharma), tom.blydthansen@cw.bc.ca (T.D. Blydt-Hansen).

from urine samples in the 6–10 days prior to biopsy: with AR, average pre-biopsy metabolite scores were ≥ 13 ; without rejection, pre-biopsy scores were consistently < 13 , offering the tantalizing prospect that sequential testing may improve decision-making regarding risk and need for biopsy, particularly for those at intermediate risk. Although non-invasive serial monitoring allows for closer surveillance than protocol biopsies, the optimal approach in terms of thresholds and timing will clearly be an important avenue for ongoing application research.

Despite decades of progress, long-term renal allograft survival is compromised by acute episodes of inflammatory rejection and more indolent processes believed to be harbingers of chronic injury. Not without risks, renal biopsy remains the criterion-standard for diagnosis, and clinicians have waited patiently for better, non-invasive diagnostic tools. As an instrument for assigning patients to high- or low-risk categories for AR, the work of Banas et al. offers a real possibility that our patience is finally being rewarded. Their studies have been careful and methodical— in the technical details of their assay, their use of independent training and validation cohorts, and statistical methodology. Further work is needed: appropriate management of the intermediate risk category is far from clear. The impact of potential confounders —e.g. borderline rejection, interstitial fibrosis, urine infection, or BK nephropathy— needs to be determined, as does the feasibility of refining their model using additional clinical predictors, such as anti-rejection prophylaxis, tissue mismatches, or evidence of antibody-mediated rejection. Rather than discouraging real-world applications, it's time to recognise that many of these issues will only be resolved in real-time in the clinic. Given the urgency, we believe that it's time to begin carefully implementing and evaluating new bedside applications even as we look forward to further improvements in an out-dated diagnostic model.

CRedit authorship contribution statement

Drs. Sharma and Blydt-Hansen contributed equally to drafting, reviewing, and editing this commentary.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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