



Editorial

Graft biopsy reimaged: Integrating morphology and molecular maps

Commentary on [<https://doi.org/10.1016/j.ajt.2025.04.025>]Umberto Maggiore¹ , Paolo Cravedi^{2,*} ¹ UO Nefrologia, Dipartimento di Medicina e Chirurgia, Università di Parma, Parma, Italy² Translational Transplant Research Center, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Gene expression profiling assays for core biopsies were developed to enhance the diagnostic accuracy of Banff histological assessments, which are limited by interobserver variability and low resolution in grading acute and chronic lesions.¹ Pioneered by Halloran's group, this approach led to the creation of the Molecular Microscope Diagnostic System (MMDx), which identifies rejection phenotypes and provides continuous probabilistic scores for parenchymal injury and rejection.¹ MMDx has been used to complement histology, calibrate biomarkers, and serve as an endpoint in antibody-mediated rejection (AMR) clinical trials.¹ However, broader adoption has been limited by the need for fresh-frozen tissue, centralized processing, and high cost. Conclusive evidence that adoption of MMDx improves graft survival also remains lacking.

In 2019, the Banff Molecular Pathology Working Group introduced the Banff Human Organ Transplant (B-HOT) panel, a curated gene set designed to capture key molecular features of allograft injury and rejection.² Of the more than 20,000 genes in the human genome, the B-HOT panel includes 770 genes selected through expert consensus for their strong association with clinically relevant phenotypes in allograft biopsies.² These genes represent core biological pathways involved in tissue injury, such as inflammation and viral infection, as well as alloimmune responses and rejection.² The B-HOT panel offers a focused molecular framework to enhance graft pathology interpretation beyond conventional histology. Compared to MMDx, it

offers compatibility with formalin-fixed paraffin-embedded samples, potential for local implementation, and significantly lower cost.²

In this issue, Zielinski et al.³ present a molecular framework for assessing AMR and T cell-mediated rejection (TCMR) using selected genes from the B-HOT panel. The study analyzed 950 kidney allograft biopsies from 10 transplant centers across Europe and North America. The development cohort included 664 biopsies, split into a training set ($n = 537$) and a test set ($n = 127$), with 2 independent external validation cohorts ($n = 286$).³ The models demonstrated strong discriminatory power in both test and validation sets; however, using a 50% probability cutoff, discrepancies between molecular predictions and histologic diagnoses were observed in 30% of cases.³

The authors also developed an automated diagnostic pipeline (HistoMx) that generates standardized reports, including sample quality metrics and molecular scores for AMR and TCMR, reported on a continuous scale.³ The uncertainty of these estimates can be assessed via confidence intervals and the interquartile range of each score. This system operates solely on raw gene expression count data, enhancing reproducibility, interpretability, and accessibility across decentralized sites. As such, this automated approach holds considerable promise for improving the consistency and clinical utility of rejection diagnosis, both in routine clinical practice and in multicenter trials.

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However, like MMDx, the B-HOT panel does not fully capture the multifaceted nature of allograft injury, which often involves combinations of intrinsic, drug-induced, alloimmune, and non-alloimmune parenchymal abnormalities. Alternative diagnoses, such as disease recurrence and BK polyomavirus-associated nephropathy, further complicate interpretation. Histologic assessment still requires specialized expertise to capture the full heterogeneity and dynamic range of injury and rejection. Moreover, B-HOT intersample variability across different labs remains untested, and further studies are needed to demonstrate clinical utility and support routine implementation.

There is a common perception that molecular analyses of graft biopsies offer greater sensitivity and reproducibility than conventional histology. However, transcriptomic and morphological assessments provide complementary—not fully overlapping—information. Although convergence between molecular and histologic findings is expected, in some instances, they may reflect different stages (eg, probable AMR) or dimensions of the same process (eg, microvascular inflammation, donor-specific antibody [DSA]-negative or C4d-negative rejection), making discrepancies highly informative.

In the study by Zielinski et al,³ mismatches between histology-based diagnoses and gene expression-based predictions were attributed to factors such as subthreshold histologic abnormalities, limited specificity of certain 2019 and 2022 Banff lesions, and the interpretive challenges posed by advanced fibrosis. Rather than reflecting limitations of either modality, these divergences highlight their complementary strengths and the value of integrating both approaches for a more comprehensive understanding of graft pathology.

Of note, neither histological nor molecular analyses per se can directly inform about the underlying causality of pathophysiological processes in the allograft.^{4,5} Thanks to its compatibility with formalin-fixed paraffin-embedded samples, the B-HOT platform facilitates large retrospective studies, enabling the generation of multimodal datasets. These can incorporate B-HOT profiles with clinical variables, donor characteristics (eg, eplet mismatches, natural killer cell complex repertoire), noninvasive biomarkers (eg, donor-derived cell-free DNA), posttransplant DSA (including low mean fluorescence intensity DSA), and prognostic tools such as iBox.

Pending further mechanistic studies, artificial intelligence (AI)-driven analyses of these datasets could uncover new prognostic patterns, support novel classification frameworks beyond traditional rejection categories, and better define poorly characterized conditions such as mixed rejection, microvascular inflammation, DSA-negative rejection, C4d-negative

rejection, and minimal TCMR or AMR. AI may also help delineate distinct clinical endpoints for trials targeting DSA-positive vs DSA-negative late rejection. To fully harness this potential, not only should the B-HOT gene list and HistoMX application be open source, but ideally, the algorithms used to calculate rejection probabilities should also be openly available, both for B-HOT and other validated transcriptomic platforms.

In summary, B-HOT offers a potentially reproducible and cost-effective tool to enhance biopsy interpretation. Its integration into large-scale, multimodal datasets could accelerate discovery through AI-driven approaches and ultimately refine surrogate endpoints in transplant clinical trials.

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Declaration of competing interest

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