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EDITORIAL

# Molecular diagnostics for lung transplant rejection: An information pipeline or a pipe dream?

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Long-term survival after lung transplantation remains limited by the development of chronic lung allograft dysfunction (CLAD), which affects approximately 50% of lung recipients by 5 years post-transplant.[1] Given the lack of a proven therapy once CLAD is diagnosed, risk factor mitigation is a key preventive strategy. The principal risk factor for CLAD is A-grade acute cellular rejection (ACR). A-grade ACR is diagnosed by transbronchial biopsy (TBB), exhibiting perivascular mononuclear cell infiltrates that extend into the interstitium with higher grade rejection.[2] Although definitive data are lacking, treatment of A-grade ACR with augmented immunosuppression is considered to be a key strategy for reducing the risk of CLAD. However, inappropriate treatment increases the risk of opportunistic infections and malignancy, which makes an accurate diagnosis essential. Although considered the current "gold standard," the utility of TBB to diagnose A-grade ACR is limited by frequent sub-optimal or inadequate sampling, as well as poor interobserver agreement.[3–5] New tools are needed to facilitate a reliable diagnosis of A-grade ACR and thus impact the clinician's ability to prevent CLAD.

In this issue of the Journal, Halloran et al[6] report their study of microarray assessment of single TBB pieces for the diagnosis of lung allograft rejection. The authors used a discovery-based approach and unsupervised analysis to determine whether gene expression relates to histologic A-grade ACR (also referred to T-cell–mediated rejection [TCMR] in the study), as well as antibody-mediated rejection (ABMR) and donor-specific antibody (DSA) status. Four extreme phenotypes, or archetypes, were distinguished based on the expression of a pre-specified list of rejection-associated transcripts (RATs). RATs were established in renal transplant and validated in heart transplant biopsies.[7,8] The 4 archetypes identified were A1 (normal), A2 (TCMR), A3 (ABMR-like), and A4 (injury), and each biopsy was assigned 4 corresponding scores, reflecting the weighted similarity to each archetype. High scores for the TCMR archetype were strongly associated with histopathologic A-grade ACR. There was no association of the ABMR-like archetype, or any other archetype, with a clinical diagnosis of ABMR or DSA positivity. The authors argued this lack of relationship between molecular scores and ABMR may be due to the low frequency of ABMR diagnoses in the cohort. In addition, the lack of association may be a reflection of the diagnostic uncertainty of ABMR in lung transplantation. However, the strong association of TCMR molecular scores with histopathologic A-grade ACR suggests promise as a novel tool for ACR diagnosis and deserves additional reflection.

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There are drawbacks in their study that should be considered in interpreting the results. First, an important rationale for the research is the relatively high rate of TBBs that were ungradeable for A-grade ACR (15% in the study). However, with the 1 TBB piece used for the molecular diagnostic test, the authors had to exclude 37% of samples from the analysis due to low surfactant expression, indicative of the low amounts of alveolated tissue. Inclusion of additional TBB pieces should solve this issue, but with some incremental risk due to the added biopsies. Safety was not addressed by the authors, but most centers perform 6 to 10 biopsies for

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histopathologic grading. Thus, replacement of the current standard with a molecular diagnostic test that relies on fewer biopsies should be at least safety-neutral, if not safer, than the current standard.

An additional drawback of the study is that the primary analysis relied on a list of RATs that were previously identified in kidney transplants.[8] Subsequently, the authors demonstrated RATs to be shared with heart transplantation.[7] However, the study does not prove that RATs are applicable to lung transplantation. There are reasons to believe that lung allograft rejection may include unique molecular pathways: ACR is more common in lung transplantation despite the use of more intense immunosuppression, and pre-clinical rodent models indicate rejection of lungs is more rapid,[9] can occur in the absence of secondary lymphoid organs,[10] and is not dependent on $CD4^+$ T cells,[11] in contrast to other solid-organ transplants. Nevertheless, the strong relationship between TCMR scores and A-grade ACR in spite of these limitations is exciting. It is possible the relationship would be further strengthened by the development of lung-allograft–specific RATs, but this will require a much larger number of biopsies with A-grade ACR. Eventually, a larger sample size should also allow for analyses of other important outcomes, including B-grade rejection, infections, and CLAD.

Given the problems with TBB histopathology for ACR diagnosis, the findings by Halloran and colleagues suggest that molecular diagnostic testing of TBB tissue will eventually be the better diagnostic test once the "kinks" are worked out. In addition, molecular testing will result in a better understanding of lung allograft pathobiology. However, it is not clear how TBB molecular diagnostics will stack up against other novel diagnostics also being studied. For example, cryobiopsy provides more tissue and better preservation of architecture than TBB. Several small studies in lung transplantation have suggested that cryobiopsy improves the diagnostic yield of ACR compared with TBB.[12–14] However, cryobiopsy would likely incur added risk for the patient. Several small studies have also tested molecular diagnostics in bronchoalveolar lavage (BAL) fluid for ACR diagnosis and suggested gene expression in the BAL cell pellet is informative about ACR.[15,16] BAL is easier to perform, has less risk, and provides sampling from a larger area of lung than TBB or cryobiopsy. Furthermore, the ultimate goal of a minimally invasive diagnostic test for ACR may also be achievable. Several groups are investigating cell-free DNA in peripheral blood as a diagnostic test for lung allograft ACR.[17,18]

Undoubtedly, the standard of care for the diagnosis of lung allograft ACR is overdue for an overhaul. Halloran et al demonstrated that molecular diagnostics are feasible and provide a wealth of information beyond what we can abstract with standard histopathology, yet they have only scratched the surface of what is possible. Through continued work in this area, a reliable diagnosis of ACR is achievable, which will enhance our ability to prevent CLAD and improve outcomes among patients with lung transplantation.

## Disclosure statement

The authors have no conflicts of interest to disclose.

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