ISHLT JOURNAL WATCH
October 2025 | Pathology

Maja Nackenhorst, MD
Medical University of Vienna, Vienna, Austria

Alexander N. Wein, MD, PhD
ISSUE EDITOR

ISHLT.ORG

Correlation between donor-derived cell-free DNA and tissue gene expression in heart transplant patients undergoing for-cause endomyocardial biopsies

Valledor AF, Moeller CM, Oren D, Baranowska J, et al. *JHLT* 2025 Sep;44(9):1384-1393. | DOI: 10.1016/j.healun.2025.04.019

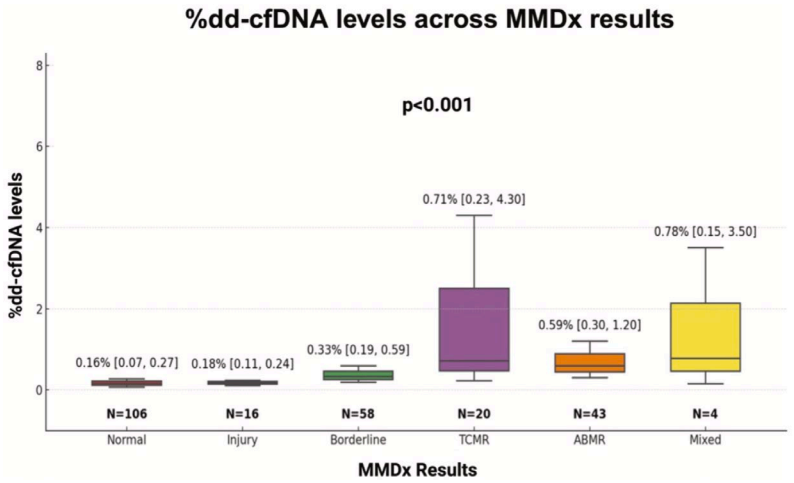
Study Highlights

Objective: This study evaluates the accuracy of donor-derived cell-free DNA (dd-cfDNA) in detecting heart transplant (HT) rejection, compared to Molecular Microscope Diagnostic System (MMDx) and histology, aiming to identify an optimal dd-cfDNA threshold.

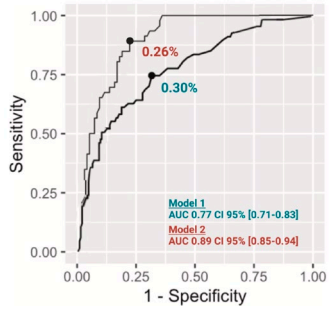
Methods: This single-center prospective study of heart transplant recipients used paired MMDx results and dd-cfDNA levels from for-cause biopsies. It evaluates dd-cfDNA's ability to detect rejection via ROC analysis and examines its correlation with MMDx rejection scores as well as histology, using a mixed-effects model for repeated samples.

Results: Among 247 for-cause biopsies (median 21 months post-HT, 11 days from dd-cfDNA), 56.7% had dd-cfDNA ≥0.20%. MMDx detected rejection in 27.1%, versus 7.7% by histology. Elevated dd-cfDNA was linked to a 4-fold increase in rejection, mainly a 5-fold rise in antibody-mediated rejection. dd-cfDNA predicted rejection better by MMDx (AUC 0.77; cutoff 0.30%), improving to 0.89 with a mixed-effects model (cutoff 0.26 %), while histology-based prediction had a lower AUC of 0.64.

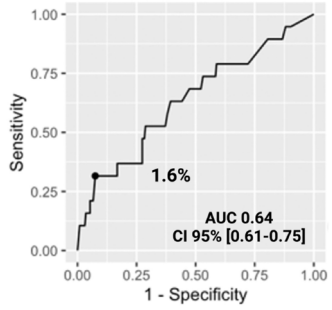
Conclusions: In a for-cause biopsy population, elevated dd-cfDNA levels were more predictive of rejection on MMDx than histology. A dd-cfDNA cutoff of 0.26% provided the highest predictive accuracy for rejection by MMDx when applied within a mixed-effects model for repeated measures.



dd-cfDNA levels stratified by molecular microscope results.



ROC curves for dd-cfDNA in predicting rejection based on MMDx.



ROC curve for dd-cfDNA in predicting rejection on histology.

Reviewer's Comments

- Interesting analysis that attempts to shed more light into the accuracy of dd-cfDNA
- The in-depth analysis would have benefited from the inclusion of DSA titers. They are listed in the tables only and have interesting implications
- The median time between dd-cfDNA assessment and biopsy was 11 days, which could at least in part account for the discrepancies in the analysis
- There is no pathologist listed in the author list, which is surprising for a paper so heavily involving and commenting on histology

Limitations

- Single-center study
- In some cases, only one biopsy specimen or MMDx analysis instead of two
- Median time between the dd-cfDNA assessment and biopsy was 11 days
- Training and validation cohorts not separate

ISHLT JOURNAL WATCH
October 2025 | Pathology

Margaret L. Axelrod, MD, PhD
Washington University in St. Louis, MO USA

Alexander N. Wein, MD, PhD
ISSUE EDITOR

ISHLT.ORG

Suppression of cardiac allograft vasculopathy by a macrophage efferocytosis receptor

Shah S, Ananthakrishnan A, DeBerge M, Glinton K, et al. *Am J Transplant* 2025 Aug 26:S1600-6135(25)02940-5. | DOI: 10.1016/j.ajt.2025.08.026

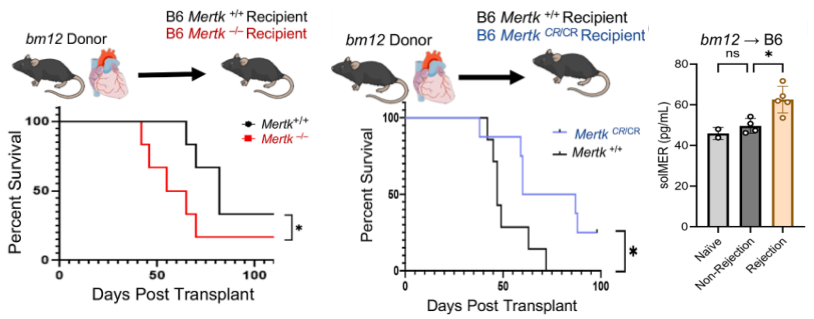
Study Highlights

Objective: This study aims to assess the role of MerTK, a receptor tyrosine kinase involved in macrophage efferocytosis, in the pathogenesis of cardiac allograft vasculopathy (CAV).

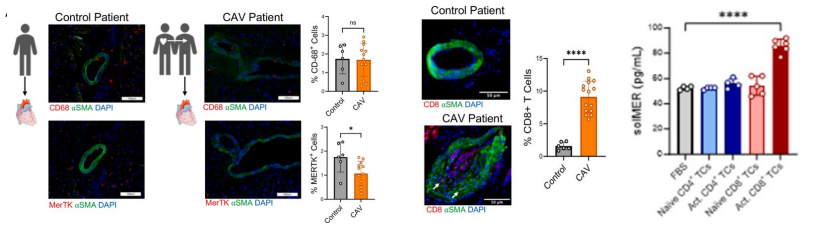
Methods: The authors used a mouse model of CAV (*bm12)* in heterotopic transplant and *Mertk*-altered mice, including mice with a cleavage-resistant MerTK (*Mertk*$^{CR/CR}$). The authors also obtained human samples from explants with CAV and control non-transplanted donor hearts for comparison of MerTK expression. Co-culture experiments evaluated the effect of immune cell subsets on bone marrow derived macrophages (BMDMs) activation and metabolism.

Results: In *bm12* heterotopic transplants, *Mertk*$^{-/-}$ recipients had decreased survival and increased CAV, while *Mertk*$^{CR/CR}$ recipients had increased survival and decreased CAV. There was increased plasma soluble MerTK (solMER) in rejection compared to naïve or non-rejection recipients. In human CAV, MerTK+ cells were decreased, while CD8+ cells were increased, compared to donor hearts. Co-culture experiments showed that activated CD8+ T cells, but not other immune cell types, induced activation of MerTK in BMDMs through secretion of solMER and decreased efferocytosis. Activated CD8+ conditioned media also induced glycolytic capacity and IL-1β production in BMDMs, which was abrogated in *Mertk*$^{CR/CR}$.

Conclusions: These data suggest activated CD8+ T cells induce MerTK cleavage on macrophages, which leads to a proinflammatory state that is associated with CAV and worse outcomes in mouse models.



Mertk$^{-/-}$ recipients have decreased survival in a heterotopic heart transplant model of CAV (left), however *Mertk*$^{CR/CR}$ recipients have increased survival in the same model (center). There is increased plasma solMER in rejection compared to naïve or non-rejection recipients (right).



While macrophage (CD68+) abundance is similar, hearts from CAV patients have fewer MerTK+ cells than unused donor control hearts (left). There are also increased CD8+ T cells in human CAV (center). Co-culture experiments show that activated CD8+ T cells, but not other immune cells induce solMER by BMDMs (right).

Reviewer's Comments

- CAV is a major barrier to long-term cardiac allograft function and is incompletely understood.
- This study proposes a model in which activated CD8+ T cells induce cleavage of MerTK on macrophages, which results in a proinflammatory state that correlates with worsened CAV.
- These data suggest that blocking MerTK cleavage may be a therapeutic approach in CAV.
- The authors also propose that plasma solMER may be a biomarker of rejection, although more study is needed in humans.

Limitations

- The use of mice with whole body *MerTK* alterations raises the possibility of *MerTK* expression by other cells types (in addition to macrophages) playing a key role in CAV outcomes.
- More robust study is needed, particularly in human patients with appropriate controls, to explore solMER as a potential biomarker.
- The significance of decreased MerTK+ cells in human CAV vs. donor hearts is unclear. Overall, there is limited human data to support the interesting mechanistic work in animal models.

ISHLT JOURNAL WATCH
October 2025 | Pathology

Jose Coelho Lima, MD, PhD
The Newcastle Hospitals NHS Foundation Trust, Newcastle Upon Tyne, UK

Alexander N. Wein, MD, PhD
ISSUE EDITOR

ISHLT.ORG

Persistent and progressive acute lung allograft dysfunction is linked to cell compositional and transcriptional changes in small airways

Brunet-Ratnasingham E, Yellamilli S, Guo R, Mohanty RP, et al. *JHLT* 2025 Sep;44(9):1482-1492. | DOI: 10.1016/j.healun.2025.03.010

Study Highlights

Objective: Acute lung allograft dysfunction (ALAD) is a poorly-understood clinical syndrome defined by >10% reduction in baseline FEV1, which may precede chronic allograft dysfunction (CLAD) development. This study aimed to investigate whether cellular compositional and cell-specific gene expression changes in small airways were associated with ALAD progression outcomes.

Methods: Prospective study including lung transplant recipients at least one year post-transplant with clinical suspicion of ALAD and controls with stable allograft function. Participants underwent bronchoscopy with small airway brushing for cellular composition and single-cell RNA sequencing analysis. Patients were followed-up for 12 months and retrospectively stratified as "recovered", "persisted", and "progressed" for ALAD outcome based on FEV1.

Results: A total of 12 ALAD and 8 control cases were included (**Fig. 1A**). In 4 patients FEV1 recovered to baseline, in 5 FEV1 decline persisted, and in 3 FEV1 decline progressed. Cell composition changed according to ALAD outcome groups. Non-recovering ALAD was associated with decreased basal and club cell populations and alveolar macrophages but increased proportion of ciliated epithelial cells and cytotoxic CD8 T lymphocytes (**Fig. 1B**). Worsening ALAD outcome correlated with increased AI2 score (a 32-gene subset associated with CLAD), especially in epithelial cells. Pathway enrichment analysis showed increased interferon/TCR signaling and apoptosis regulatory programs in epithelial cells from patients with worsening ALAD (**Fig. 1C**).

Conclusions: Non-resolving ALAD was associated with bronchiolar cell compositional and transcriptional program changes

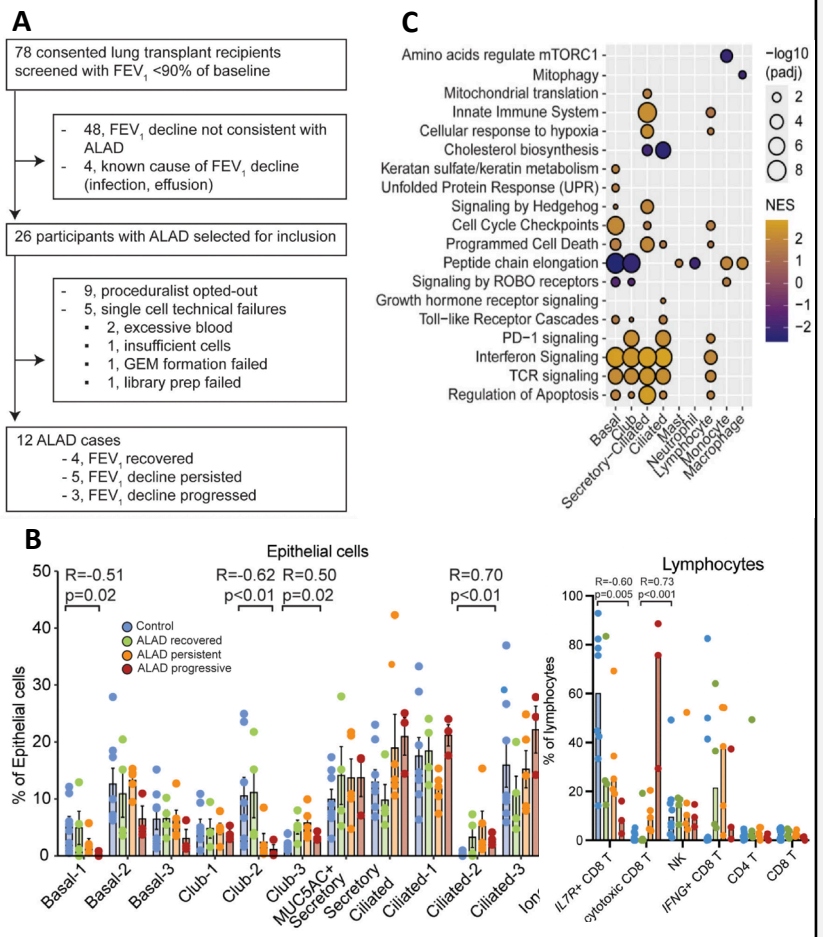


Figure 1. Main study results. A. Study design and cohort. **B.** Proportion of epithelial cells and lymphocyte subsets across ALAD outcome groups. **C.** Gene enrichment pathway analysis reveals a shift in transcriptomic profiles in epithelial cells and lymphocytes with worsening ALAD outcomes. NES, normalized enrichment score per unit increase in ALAD outcome severity group.

Reviewer's Comments

- This well-conducted, exploratory study unveils important biological features of non-resolving ALAD based on single-cell RNA sequencing of material obtained from small airways, a key compartment in CLAD pathogenesis.
- The results provide evidence for the potential use of cell subtyping and gene expression profiling from small airway brushing material as minimally-invasive biomarkers to risk-stratify patients for ALAD outcome and CLAD development, which warrants further validation.
- The observed cellular and transcriptional changes suggest that interventions aimed at interferon-dependent antigen presentation, macrophage/T cell dysregulation, and basal/club cell dedifferentiation could be further investigated as potential therapeutic avenues in ALAD.

Limitations

- Small sample size (n=20 patients), out of which only 8 had non-resolving ALAD (although large by single-cell RNA sequencing standards with 68,140 cells sequenced).
- The findings lack validation in external, larger cohorts for biomarker application purposes.
- Lack of histologic validation (e.g. in concurrent transbronchial biopsies) for confirmation of transcriptional changes at protein expression level.
- Limited number of immune cells within the analyzed cellular milieu, which could have impaired robustness to identify molecular changes across immune cell subtypes.