ISHLT October 2024 | Pathology
JOURNAL WATCH

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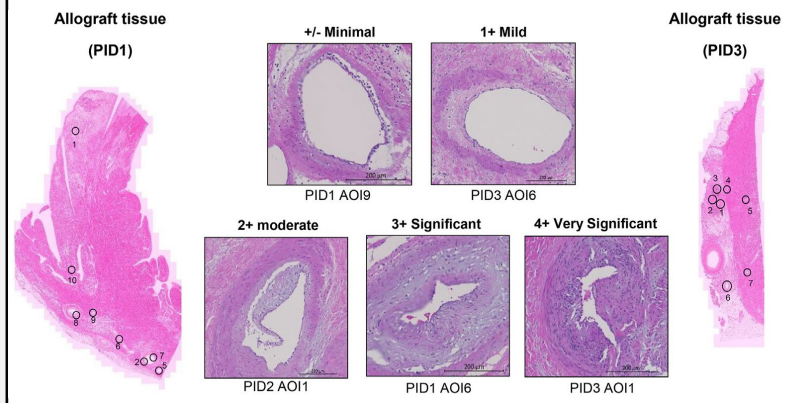
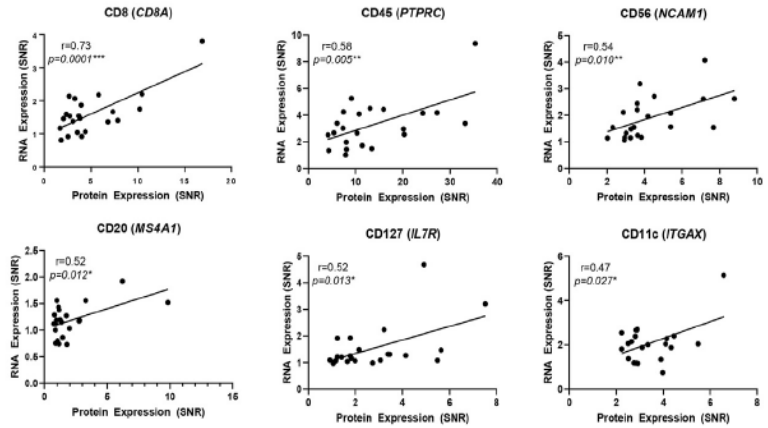
Spatial multiomics of arterial regions from cardiac allograft vasculopathy (CAV) rejected grafts reveal novel insights into the pathogenesis of chronic antibody-mediated rejection

Navarez-Mejia J, Pickering H, Sosa R, Valenzuela NM, et al. *American Journal of Transplantation* 2024 Jul;24(7):1146-1160 | DOI: 10.1016/j.ajt.2024.01.004

Study Highlights

- **Aim:** to employ GeoMx digital spatial profiling to conduct a patho-molecular and spatial analysis of CAV and to analyze arterial areas of interests (AOIs) from CAV+DSA+ rejected cardiac allografts
- **Methods:** a total of 22 geometric AOIs were selected across 3 cardiac allografts explants from CAV+DSA+ patients. Multiplex Digital Spatial Profilig of protein and RNA in fixed tissues was performed by nanostring techonology.
- **Results:** arterial AOIs from CAV+DSA+ patients were spatially profiled using 73 protein-panel. Among the 22 arterial regions examined, the authors identified similarly expressed protein markers relating to innate and adaptive immune cells, cell activation, and cell death. AOIs with low neointima exhibit higher inflammatory and cell death profiles, while AOIs with high neointima exhibit lesions undergoing proliferation, migration.
- **Conclusions:** Our findings further accentuate on the degree of vessel heterogeneity and inflammatory profiles not usually identified by pathology





Reviewer's Comments

- This is the first study that investigated the molecular aspects of CAV empolying GeoMx digital spatial profilig.
- low neointima mainly exhibit features of burnt-out vasculitis and endothelilitis
- high neointima mainly demonstrated features of ongoing neointima expansion as seen by an increase in transcripts involved in myofibroblast differentiation and remodeling.

Limitations

- This study did not distiguish the three different layers of the vessel
- The low number of the cases is in contrast with the great variability of the CAV in term of timing and clinical charactestics of the patients.
- Future additional studies including atherosclerosis comparison would complete CAV pathways signaling specificity

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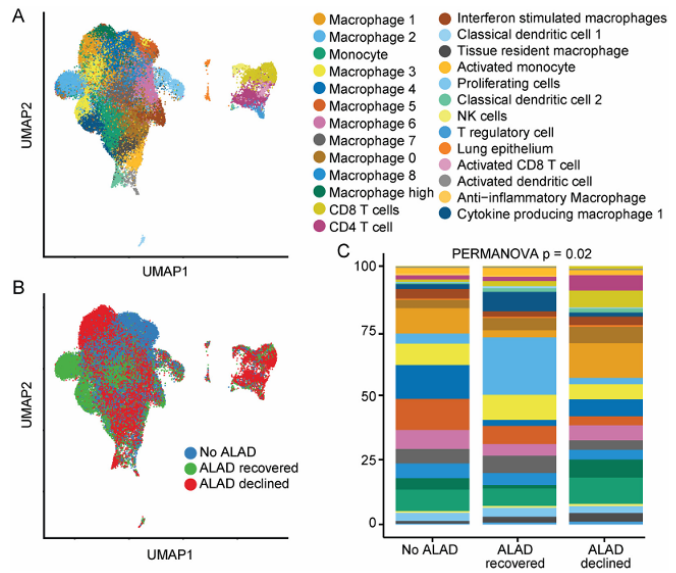
Macrophage and CD8 T Cell Discordance are Associated with Acute Lung Allograft Dysfunction Progression

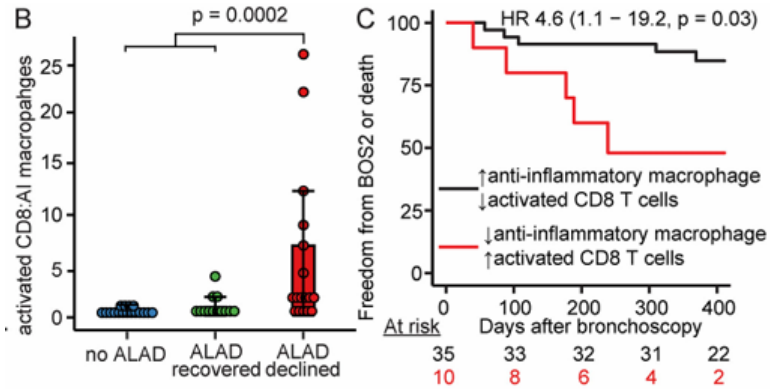
Calabrese DR, Ekstrand CA, Yellamilli S, Singer JP, et al. *JHLT* 2024 Jul;43(7):1074-1086 | DOI: 10.1016/j.healun.2024.02.007

Study Highlights

- **Objective:** To identify immune cell subsets in bronchoalveolar lavage (BAL) fluid associated with progression from acute lung allograft dysfunction (ALAD) to chronic lung allograft dysfunction (CLAD).
- **Methods:** BAL samples from 45 lung transplant recipients were collected, including 17 with declining ALAD, 13 with recovering ALAD, and 15 stable controls. Single-cell RNA sequencing was performed to analyze immune cell populations. Differences between ALAD groups were assessed using machine learning and survival analysis, with key findings validated in external datasets.
- **Results:** A distinct CD8 T cell cluster and an anti-inflammatory macrophage cluster were associated with ALAD progression. Recipients with discordant levels of high CD8 T cells and low anti-inflammatory macrophages had a 5-fold increased risk of severe graft dysfunction or death. Communication between these cell types was linked through HLA, fibronectin, and galectin pathways. Key findings were validated in two external cohorts.



Cell Populations in Bronchoalveolar Lavage (BAL)



Discordance in Macrophages and CD8 T Cells Predict Graft Failure

Reviewer's Comments

This study combines advanced sequencing techniques and detailed immune profiling, providing valuable insights with broad implications for clinical practice. Key points include:

- **Novel use of single-cell RNA sequencing** to identify immune cell populations linked to ALAD progression.
- **Large-scale cell analysis** with over 51,000 cells sequenced, providing detailed immune profiling.
- **Identification of key immune cell discordance** (CD8 T cells and anti-inflammatory macrophages) as predictors of graft dysfunction.
- **Validated in two external cohorts**, enhancing the robustness and generalizability of the findings.
- **Potential therapeutic targets** identified through cell-cell communication analysis, opening avenues for future interventions.

Limitations

The study's generalizability is limited by a small sample size and single-center design, with additional constraints posed by the macrophage dominance in BAL samples, a focus on short-term outcomes that may not reflect long-term patterns, and the exclusion of infection and rejection cases, which could overlook critical interactions.

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Surveillance with dual noninvasive testing for acute cellular rejection after heart transplantation: Outcomes from the Surveillance HeartCare Outcomes Registry

Khush K, Hall S, Kao A, Raval N, et al. *JHLT* 2024 Sep;43(9):1409-1421 | DOI: 10.1016/j.healun.2024.05.003

Study Highlights

- **Objective:** To evaluate the utility of combined noninvasive molecular testing for acute cellular rejection (ACR) surveillance in heart transplant patients

- **Methods**: Prospective observational registry of heart transplant recipients managed with noninvasive molecular testing in 52 centres in the USA enrolled from Dec/2018 to Nov/2021. Gene expression profile (GEP; AlloMap, CareDx) and donor-derived cell free DNA (dd-cfDNA; AlloSure, CareDx) were quantified in peripheral blood. Patients were categorized as: dual positive; GEP +/dd-cfDNA -; GEP -/dd-cfDNA +; or dual negative. The incidence of ACR in endomyocardial biopsy (EMB) for each group was assessed. Positive likelihood ratios (LR+) and follow-up EMB rates following molecular test results were analysed

- **Results**: A total of 2,077 subjects [male, n=1,531 (73.7%)] were included. The median (IQR) post-transplant follow-up length was 40 (32–50) months. The incidence of EMB-proven ACR was higher in the molecular test dual-positive group (9.2%) compared to single-positive and dual-negative groups. Dual-positive test results also showed higher specificity [91.7% (95% CI: 91%-92,4%)] and LR+ [3.90, (95% CI: 3.08%-4.96%)] for ACR compared to single-positive results. Dual-positive results prompted a higher rate of follow-up surveillance EMB (35.4% vs. 8.8% in the dual-negative group). However, molecular test monitoring led to reduced need for EMB over time, especially in year 2 post-transplant. At 2 years, overall survival was 94.9% and normal graft function was seen in 97.3% of patients (Figure 1)

- **Conclusions**: Dual molecular testing showed improved performance for ACR monitoring compared to single testing. Dual noninvasive testing was associated with lower EMB rates over time and excellent survival

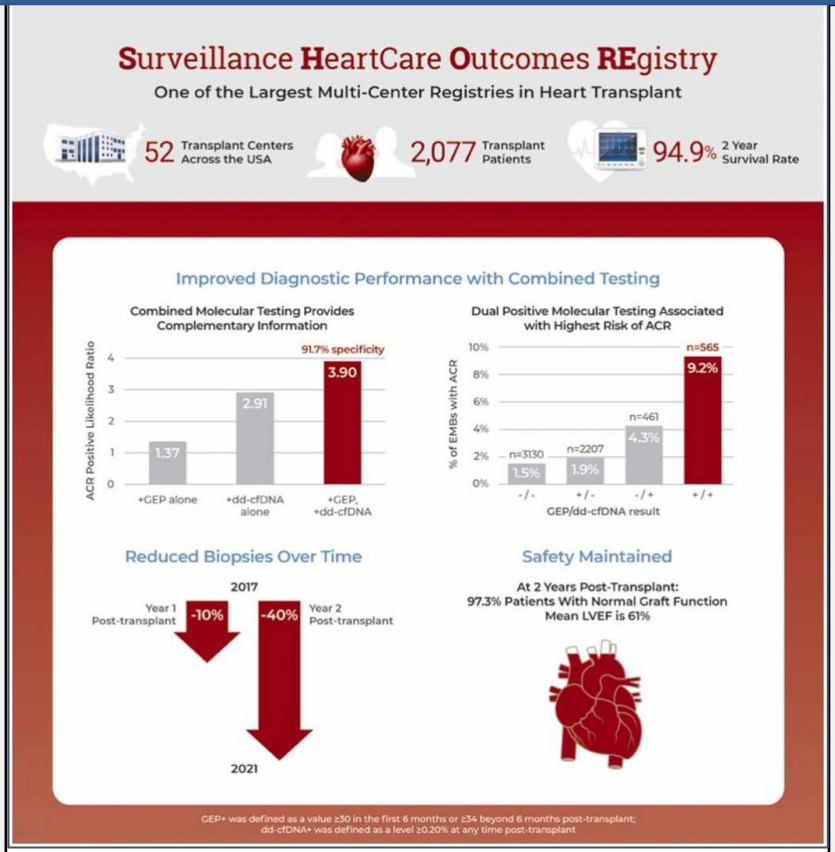

Figure 1. Graphic abstract showing main study findings.
Taken from: Khush K et al. Journal of Heart and Lung Transplantation 2024; 43(9):1409-1421.

Reviewer's Comments

- This is a large, multicentric study of heart transplant recipients showing compelling evidence for the use of dual over single noninvasive molecular testing for ACR surveillance
- It is hypothesized that the synergistic performance of dual noninvasive testing for ACR surveillance derives from GEP and dd-cfDNA assessment of distinct pathologic processes, namely immune cell activation and graft cellular injury, respectively
- This study showed real-world data that illustrates how clinicians have become more familiar with noninvasive ACR monitoring and used molecular test results to inform clinical decisions regarding further surveillance EMB over time
- The excellent survival and graft function outcomes, although not unexpected, show that noninvasive ACR monitoring was safe despite the observed reduction in surveillance EMB
- Noninvasive ACR surveillance may help reducing the risk of side effects and inconvenience associated with EMB for patients whilst refining patient selection for EMB to optimize healthcare resources and expert pathologists' workload

Limitations

- Most episodes of ACR in this cohort occurred within 30 days post-transplant, which could not be assessed by noninvasive tests as these can only be performed from 2 months post-transplant
- EMB and molecular tests associated with antibody-mediated rejection were excluded from the molecular testing performance analysis
- Clinical factors other than molecular results may have influenced clinicians' decisions to perform EMB, as the study did not require molecular tests to be paired with an EMB