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JOURNAL WATCH

Darshan H. Brahmbhatt, MB BChir MD(Res)
University of Toronto, Toronto, Canada

Forum Kamdar, MD, PhD
EDITOR
ISHLT.ORG

Validation of the clinical utility of microRNA as noninvasive biomarkers of cardiac allograft rejection: A prospective longitudinal multicenter study

G Coutance, et al. *JHLT* November 2023 | https://doi.org/10.1016/j.healun.2023.07.010

Study Highlights

Objective: To validate the association between 3 previously identified circulating microRNAs (miRs 10a, 92a, 155) and histopathological rejection.

Methods: A prospective observational study aimed at validating circulating miR signature of cardiac rejection (defined as acute cellular rejection ≥2R and/or antibody-mediated rejection ≥pAMR1). Patients <10 years post-isolated heart transplant undergoing endomyocardial biopsy (EMB) were included from 11 centers across France between August 2016-March 2018. Sera samples were collected just before the EMB (i) for all for-cause biopsies; (ii) at 1-, 3-, 6-, and 12- months post-transplant for de-novo transplant recipients; and (iii) during annual visits after 1 year. The miRs were measured in triplicate.

Results: 461 patients were included representing 831 EMB. 79 rejection episodes were diagnosed (25 ACR & 56 AMR). An interim analysis based on 258 EMB from 204 patients, including 49 rejection episodes, no association between the relative expression of any circulating miR and rejection was found (Figure 1)

Conclusions: There was no clinical utility of circulating miR 10a, 92a, and 155 monitoring in heart transplant rejection.

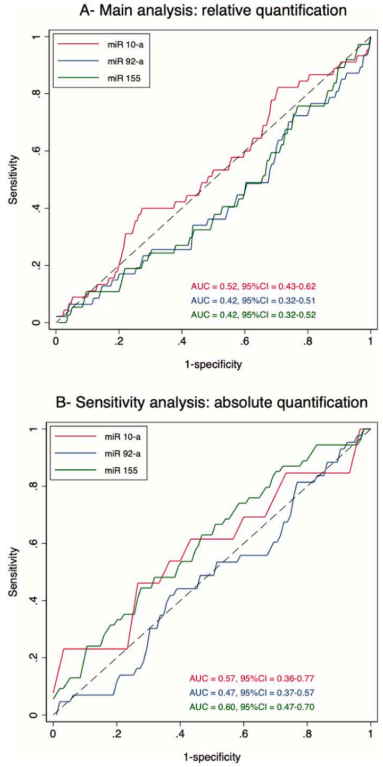

Figure 1: A) Relative quantification of miRsB) Absolute quantification of miRs

Reviewer's Comments

- Prospective, longitudinal, multi-center study using ISHLT definitions of rejection and previously identified miRs associated with rejection
- Large sample size and interim analysis was enriched for episodes of rejection, with a 4:1 ratio of controls for every rejection case.
- When main analysis of relative quantification of miR was negative a further sensitivity analysis of absolute quantification of miR corroborated the negative results.

Limitations

- Study was halted for futility based on interim analysis alone, though this is convincingly negative.
- Previous studies have identified differential tissue expression of miRs, alongside changes in serum concentration, this study did not examine tissue levels of miRs: absent changes in tissue miRs could explain why serum levels were unchanged.
- It may be that miRs expression changes longitudinally within an individual when they undergo rejection (DOI: 10.1002/ehf2.13238), therefore, changes in serum miR before and after rejection within the same individual may demonstrate efficacy in predicting rejection.

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Darshan H. Brahmbhatt, MB BChir MD(Res)
University of Toronto, Toronto, Canada

Forum Kamdar, MD, PhD
EDITOR
ISHLT.ORG

Metabolomic profiling during ex situ normothermic perfusion before heart transplantation defines patterns of substrate utilization and correlates with markers of allograft injury

L Truby, et al. *JHLT* December 2023 | https://doi.org/10.1016/j.healun.2023.12.002

Study Highlights

Objective: Metabolomic profiling during ex-situ normothermic perfusion before heart transplantation (HT) would provide insight into myocardial substrate utilization and primary graft dysfunction (PGD).

Methods: Serial samples of ex-situ normothermic perfusate had biomarkers of myocardial injury (lactate & cardiac troponin I (TnI)) as well as metabolites (including 66 acylcarnitines, amino acids, nonesterified fatty acids, ketones, & 3-hydroxybutyrate). Associations between metabolites, injury biomarkers, and PGD were assessed.

Results: 176 samples from 92 ex-situ perfusion runs were taken from donors. Lactate trends over time differed significantly by recovery strategy, while TnI increased during ex-situ perfusion regardless of DCD vs DBD status **(Figure 1).** Fuel substrates were rapidly depleted during ex-situ perfusion **(Figure 2).** Changes in leucine/isoleucine, arginine, C12:1-OH/C10:1-DC, and C16-OH/C14-DC were associated with increased odds of moderate-severe PGD.

Conclusions: Metabolomic profiling of ex situ normothermic perfusion solution reveals a pattern of fuel substrate utilization that correlates with subclinical and clinical allograft dysfunction.

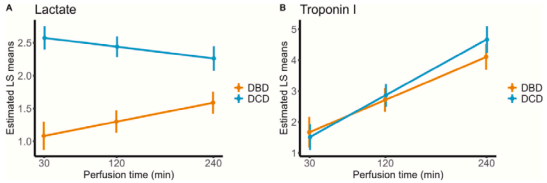

Figure 1: Least squared (LS) mean estimates of (A) lactate and (B) troponin-I at 30, 120, and 240 minutes into perfusion time. DBD, donation after brain death; DCD, donation after circulatory death.

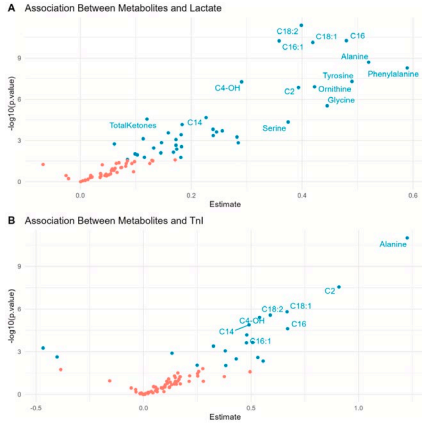

Figure 2: Volcano plot of the association between metabolites and (A) lactate and (B) troponin I (TnI).

Reviewer's Comments

- Pioneering study assessing metabolite levels during ex-situ perfusion of donor hearts.
- Study suggests that during perfusion, the cardiac allograft exhausts the circulating fatty acids and moving away from oxidative phosphorylation leading to a build-up of fatty acid β-oxidation intermediates (increased long-chain acylcarnitines, LCACs). As lactate production is coupled to cardiac metabolism, and correlated with the concentration of several circulating LCACs, it suggests that glucose becomes the primary fuel source, instead of fatty acids and branched-chain amino acids (BCAA)
- This could be a useful foundation to begin manipulation of the perfusate and potentially change the metabolic performance of donor organs to reduce risk of PGD.

Limitations

- Limited donor clinical data were available, in particular, their nutritional status and substrate availability within donor blood may have varied between donors and could not be corrected for.
- In the Organ Care System, the proprietary solution is mixed with donor blood. While this is standardized, exact ratio could vary by donor.

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Kavita Dave, MD, FRCP
Harefield Hospital, UK

Forum Kamdar, MD, PhD
EDITOR
ISHLT.ORG

Airway pepsinogen A4 (PGA4) identifies lung transplant recipients with microaspiration and predicts chronic lung allograft dysfunction (CLAD)

Ramendra, et al. *JHLT* 2024 | https://doi.org/10.1016/j.healun.2024.01.002

Study Highlights

Objective: Microaspiration is associated with increased risk of CLAD. Measuring bile acids is difficult. The utility of airway PGA4 measurement as a biomarker and predictor was evaluated early post-transplant.

Methods: Retrospectively, total pepsin and PGA4 measured in large airway bronchial washings (LABWs) were compared to pre-existing biomarkers, relationship with CLAD assessed, and changes post-reflux surgery reviewed in a subset.

Results: LABW PGA4 is associated with increased frequency of infection, higher levels of LABW conjugated bile acids, and risk of CLAD, though not time to allograft failure. Anti-reflux surgery reduced airway PGA4.

Conclusion: PGA4 is unique to the GI tract and is not expressed by lung tissue. Airway PGA4 is a marker of aspiration and predicts CLAD secondary to aspiration-induced lung injury.

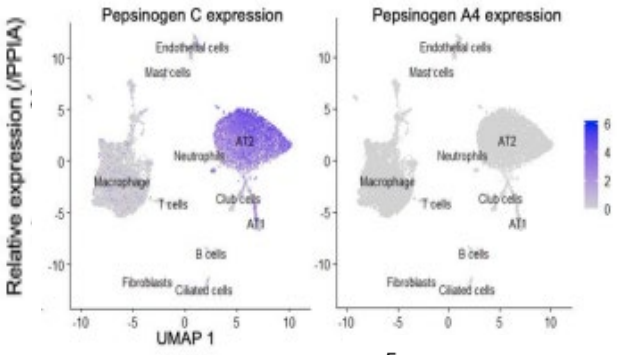

Figure 1. Comparison of pepsinogen C and pepsinogen A4 expression in lung tissue

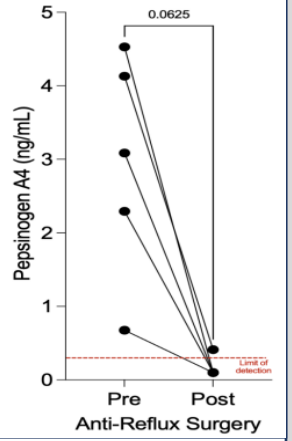

Figure 2. Airway pepsinogen A4 levels before and after anti-reflux surgery in lung transplant recipients

Reviewer's Comments

- Can help identify high-risk patients for anti-reflux surgery thereby improving longer-term lung transplant outcomes.
- Measuring this biomarker could be used to monitor response to current and future reflux therapies, and may help improve treatments in other reflux-affected lung conditions.
- Further evidence that anti-reflux surgery is effective at preventing aspiration and aspiration-induced lung injury.

Limitations

- The centre has a low threshold for starting lung transplant recipients on anti-reflux and pro-motility agents.
- The study does not identify if airway PGA4 is affected by medical therapies, such as PPIs – are results affected by confounding treatments?
- Single-center and relatively small cohorts – are the results reproducible in other centres with slightly different sampling techniques and patient populations?

ISHLT JOURNAL WATCH

Sanjeeb Sean Bhattacharya, MD
Cleveland Clinic, Cleveland OH USA

Forum Kamdar, MD, PhD
EDITOR
ISHLT.ORG

Advanced hemodynamic and cluster analysis for identifying novel RV subfunction phenotypes in patients with pulmonary hypertension

A. Janowski, et al. *JHLT* December 2023 | https://doi.org/10.1016/j.healun.2023.12.009

Study Highlights

Objective: Identify novel RV phenotypes using unsupervised clustering methods on advanced hemodynamic features of RV function.

Methods: Participants were identified using University of Arizona Pulmonary Hypertension Registry. RV-PA coupling (Ees/Ea), RV systolic (Ees) and diastolic function (Eed) were quantified from stored RV waveforms. Clustering analysis was performed identifying RV subphenotypes **(Figure 2D).**

Results: Five distinct RV clusters (C1-C5) with distinct RV subphenotypes were identified. These carried distinct RV physiology. No survival difference was observed between the distinct RV phenotypes **(Figure 5).**

Conclusions: RV function phenotyping offers possibilities of better utilization of precision medicine-based management approach.

Central Figures

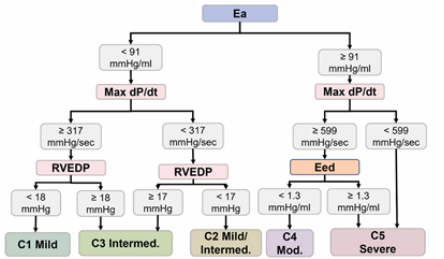

Figure 2D. Simplified clustering based on all clustering variables

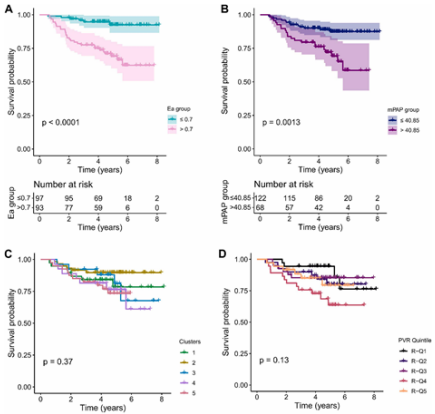

Figure 5: All cause mortality survival analysis across RV function clusters

Reviewers Comments

- This study provides a more in depth analysis of RV function in pulmonary hypertension.
- These RV phenotypes can be assessed and provide a better understanding of distinct RV physiology at time of presentation.
- Further investigations are required to assess if RV centric assessment vs classical WHO Groups.

Limitations

- Single center-based study with small number of patients leads to limitations in data analysis.
- No follow up was provided in this study which would be helpful to understand treatment response to specific RV phenotypes.

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Sanjeeb Sean Bhattacharya, MD
Cleveland Clinic, Cleveland OH USA

Forum Kamdar, MD, PhD
EDITOR
ISHLT.ORG

Defining cardiac functional recovery in end stage heart failure at single cell resolution

J. Amrute, et al. *Nature Cardiovasc Res.* Aug 2023 | https://doi.org/10.1038/s44161-023-00260-8

Study Highlights

Objective: Recovery in cardiac function remains goal of heart failure therapy. However, it is still a rare phenomena and poorly understood. This study was designed to identify cell specific transcriptional signatures of recovery, prominently in macrophages and fibroblasts.

Methods: Utilizing single nucleus RNA sequencing (snRNA-seq) on paired transmural LV specimens from apical anterior wall, comparisons were performed on non diseased control patients to patients with heart failure with experienced recovery and those who had persistent heart failure post LVAD **(Figure 1).**

Results: Cell-specific transcriptional signatures of recovery were identified. Unique signatures were seen within macrophages and fibroblasts. Inflammatory signatures were negative predictors of recovery and downregulation of RUNX1 was associated with recovery. In addition, a distinction was made with gene expression in HF recovered state and non-diseased donors.

Conclusions: A comprehensive single-cell transcriptomic map of human myocardial recovery was created while establishing a biological distinction from healthy and persistent diseased states. Finally, possible therapeutic interventions involving disruption of RUNX1 were identified.

Central Figures

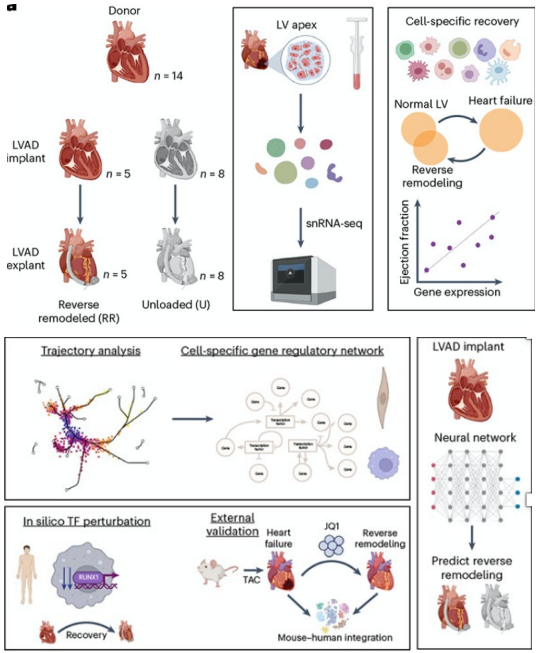

Figure 1: Study design

Reviewers Comments

- This is a unique study assess unique transcriptional profiles in cardiac recovery. Previous studies focused on differences in diseased and non-diseased states.
- Unique signatures were seen in myocardial recovery with cardiac macrophages and fibroblasts.
- Further investigation is needed to assess possible therapeutic interventions in disruption of RUNX1.

Limitations

- Small number of patients with myocardial recovery (rare phenomena). Difficult to expand to global population with varying etiologies of heart failure.
- Differences in utilizing snRNA-seq vs bulk RNA sequencing (used in previous studies).

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Lourdes Chacon Alberty, MD, MCTM
Texas Heart Institute, Houston TX USA

Forum Kamdar, MD, PhD
EDITOR
ISHLT.ORG

Molecular States Associated with Dysfunction and Graft Loss in Heart Transplants

P. Halloran, et al. *JHLT* Nov 2023 | https://doi.org/10.1016/j.healun.2023.11.013

Study Highlights

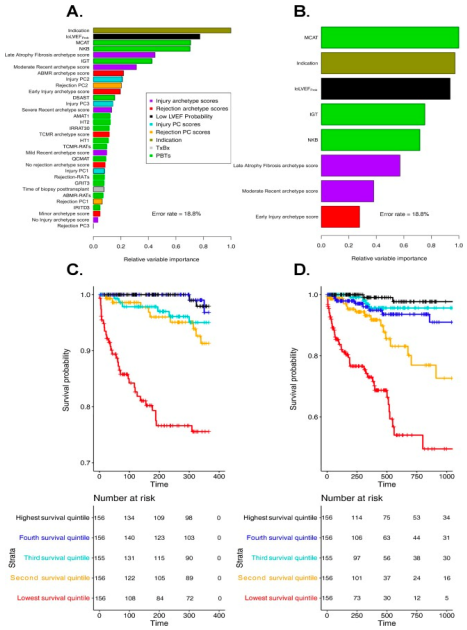
Objective: Investigate the molecular basis of heart dysfunction and risk of failure, by exploring the changes in gene expression and pathways that correlate with LVEF ≤ 55 and graft failure three years post-biopsy.

Methods: Genome-wide microarrays were used to define mRNA changes correlating with dysfunction and risk of graft loss within 3years postbiopsy. LVEF data was available for 1,013 biopsies and survival data for 779 patients. Molecular classifiers were built for predicting dysfunction and post-biopsy 3-year survival.

Results: Dysfunction correlated with decreased expression of matrix-related genes, and loss of normal heart transcripts. Survival analysis found that short-term failure shared features with dysfunction genes but also increased expression of genes related to response to hypoxia and glycolysis and reduced expression of genes related to VEGF pathways and angiogenesis. Expression of *NPPB* was associated with dysfunction and graft loss.

Conclusion: Dysfunction in transplanted hearts reflects dedifferentiation, decreased matrix genes, injury, and inflammation. The risk of short-term loss includes these changes but is also associated with microcirculation abnormalities, glycolysis, and response to hypoxia.

Central Figure



Models predicting the survival of heart transplants after biopsy. (A) 33-variable random forest (RF) model. (B) The trimmed RF model using only 8 top variables. (C-D) Kaplan-Meier plots for the survival scores using the survival probability score: the mean of the RF and glmnet classifier scores.

Limitations

- The small number of events in the survival analysis may affect the generalizability and robustness of the survival predictions.
- The choice of LVEF ≤ 55 as the cutoff for dysfunction is acknowledged as somewhat arbitrary.
- Some demographic and clinical data, especially from service biopsies, were noted to be minimal limiting the depth of the analysis.
- The study involves a median follow-up time post-biopsy of 313 days, and the time dependency of the identified molecular features and their correlation with long-term outcomes needs exploration.

Reviewer's Comments

While the study contributes to the understanding of heart transplant outcomes and molecular features associated with dysfunction and survival, its findings should be interpreted within the context of these limitations, and further research is warranted to validate and extend the results. Validation in larger, independent cohorts is essential to confirm the reliability of the identified classifiers and molecular features.