ISHLT JOURNAL WATCH
March 2023 | Research & Immunology

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Metabolomic Profiling of Cardiac Allografts after Controlled Circulatory Death

T. Hautbergue et al. *J Heart Lung Transplant* Feb 2023 https://doi.org/10.1016/j.healun.2023.02.1492

Study Highlights

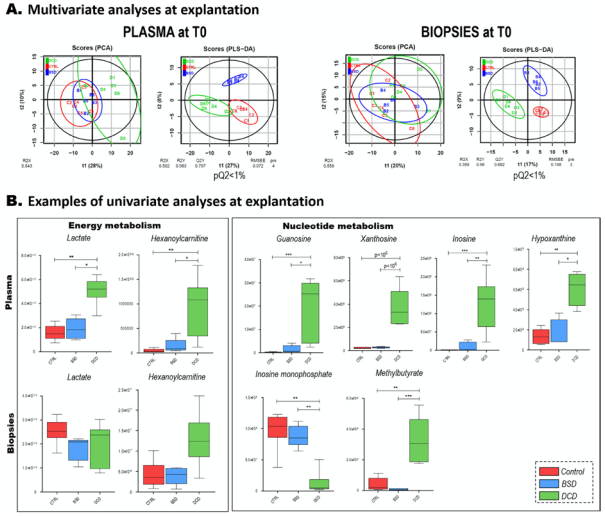
Objective: Due to the limitations of using lactate concentrations to assess myocardial viability during ex-situ heart perfusion (ESHP), this study investigates the metabolic signature associated with donation after circulatory death (DCD) and the impact of ESHP on the myocardial metabolome.

Methods: Porcine hearts were procured utilizing warm ischemia (DCD) or after brain stem death (DSB). Hearts were perfused using normothermic oxygenated blood. Plasma and myocardial samples were collected every 30 and 60 minutes and analyzed by an untargeted metabolomic approach using liquid chromatography coupled to high-resolution mass spectrometry.

Results: More than 300 metabolites were detected in plasma and heart biopsy samples. Compared to DSB, metabolomic changes involving energy and nucleotide metabolisms were observed in plasma samples of DCD animals. Normalization of DCD metabolic profile was remarkable after 4 hours of ESHP.

Conclusions: A specific metabolic profile was observed in DCD hearts, mainly characterized by an increased nucleotide catabolism. DCD and BSD metabolomes proved normalized during ESHP. Complementary investigations are needed to correlate these findings to cardiac performances.

Central Figures



Legend: Statistical analyses on 250 and 256 metabolites identified in myocardial tissues and plasma at T0 respectively. (A) Multivariate analyses on plasma and biopsies samples. (B) Examples of univariate analyses on metabolites from nucleotide metabolism and energy metabolism.

Reviewer's Comments

- DCD proves a potential for increasing cardiac transplant donor pool
- Currently, lactate provides limited insight into donor heart metabolic status.
- Investigating specific metabolic profiles can provide a greater insight into graft viability and provide insight on optimal duration of ESHP.

Limitations

- Metabolomic findings were not correlated with cardiac performance nor post transplant outcomes.
- Donor blood was used to prime ESHP circuit. Metabolites were present in perfusate precluding direct comparison of circulating metabolites during ESHP.

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Circulating Markers of Inflammation and Angiogenesis and Clinical Outcomes Across Subtypes of Pulmonary Arterial Hypertension

K. Hirsh et al. *J Heart Lung Transplantation* Nov 2022 https://doi.org/10.1016/j.healun.2022.10.026

Study Highlights

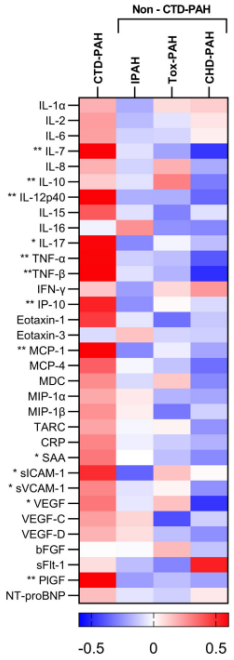
Objective: Compare inflammatory and angiogenic biomarker profiles across four pulmonary arterial hypertension (PAH) subtypes and relate them to clinical outcomes.

Methods: Serum concentrations of 33 biomarkers were measure in n=112 patients with connective tissue disease-associated PAH (CTD-PAH), congenital heart disease-associated PAH (CHD-PAH), toxin-associated PAH (Tox-PAH), and idiopathic PAH (IPAH)

Results: Angiogenic and inflammatory biomarkers were higher in the CTD-PAH compared with the other three subtypes. Mortality was associated with Interleukin-6 (IL-6), Soluble fms-like tyrosine kinase 1 (sFLt-1) placental growth factor (P1GF) Interferon gamma-induced protein 10 (IP-10), tumor necrosis factor-beta (TNF-ß) and N-terminal pro-brain natriuretic peptide (NT-proBNP).

Conclusion: Inflammatory and angiogenic biomarkers were elevated in CTD-PAH when compared to other PAH subtypes, along with distinct associations with survival within CTA-PAH versus non-CTD-PAH subtypes.

Central Figure



Legend: Biomarkers concentration by PAH subtype. Asterisks denote biomarkers that significantly differ across subtypes.

Reviewer's Comment

- Granular distinctions about protein concentrations, and protein outcomes associations by PAH subtype remains underreported, studies have generally focused on prognostication or treatment for PAH in aggregate.
- This study provide in-depth comparison of multiple biomarkers across multiple subtypes of PAH that might warrant consideration for understanding heterogeneity in PAH and identifying more precise approaches to PAH prognostication and treatment.

Limitations

- The limitations of this study includes the low mortality and sample size.
- Statistical analysis with multiple comparisons that increase the probability of identifying differences by chance.
- Patient heterogeneity including lack of treatment data including imunnosuppression, which may bias interpretation of biomarkers concentrations.

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Single-Nuclear RNA Sequencing of Endomyocardial Biopsies Identifies Persistence of Donor-Recipient Chimerism With Distinct Signatures in Severe Cardiac Allograft Vasculopathy

K. Amancherla et al. *Circ Heart Fail.* Jan 2023 https://doi.org/10.1161/CIRCHEARTFAILURE.122.010119

Study Highlights

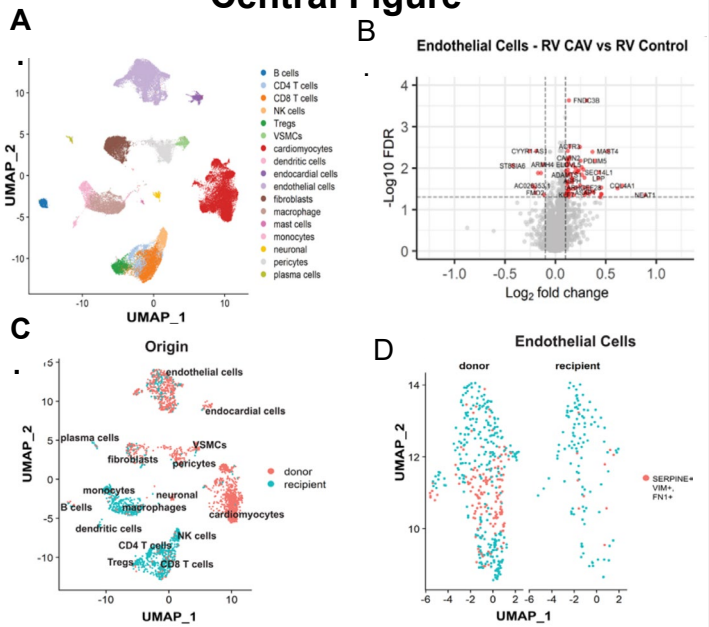
Objective: Demonstrate the feasibility of performing single-nuclear RNA sequencing of human endomyocardial biopsies (EMBs) obtained during routine clinical practice. Compare EMBs from patients with and without severe cardiac allograft vasculopathy (CAV)

Methods: Nuclei were isolated from EMBs from cardiac transplant patients with CAV (n=4) and without CAV (n=3) underwent RNA sequencing to identify cellular composition.

Results: 17 major cell types with heterogenous distribution were identified across all samples. Endothelial cells (EC) and fibroblast from right ventricle samples in the CAV group exhibit increased expression of SERPINE1 compared to non-CAV. The EC from the CAV samples exhibit donor-recipient chimerism (21.8 % recipient derived). Donor derived EC cells were enriched for markers of endothelial-to-mesenchymal transition. Immune cells were largely replaced by those originating from the recipient. Macrophages exhibit markers of activation and increased expression of TGFB1.

Conclusion: This is the first study to successfully demonstrate feasibility of using human EBMs to perform single nuclear RNA sequencing. There are unique transcriptomic signatures of donor versus recipient-derived cells highlighting putative novel avenues for investigation.

Central Figure



Legend: snRNAseq in CAV EMB: A. Uniform manifold approximation and projection (UMAP) identified 17 major cell types **B**. Differential gene expression representing right ventricular CAV vs Non-CAV for the EC cluster. **C**. Genotype-free inference of donor- vs recipient-derived nuclei **D**. Donor-derived endothelial cells are enriched for markers of endothelial-to-mesenchymal transition

Reviewer's Commments

- The cell composition among EMB samples identified via single nuclear RNA sequencing is highly heterogenous demonstrating that bulk RNA-Seq approaches might exhibit high levels of variability.
- The use of single cell nuclear RNAseq of EBMs represent a unique opportunity that might contribute to identification of mechanistic pathways promoting CAV that can be used to treat CAV and identify markers of CAV to prolog allograft survival.

Limitations

- The limitations of this study includes the relatively small sample size and the use of samples from derived from patient with severe CAV. These data will need to be validated in other cohorts.
- The immunosuppression regimens were different across study population.

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Ex-Situ Oxygenated Hypothermic Machine Perfusion in Donation After Circulatory Death Heart Transplantation Following Either Direct Procurement or *in-situ* Normothermic Regional Perfusion

N. Moeslund et al *J Heart Lung Transplant* Feb 2023 https://doi.org/10.1016/j.healun.2023.01.014

Study Highlights

Objective: Donation after circulatory death (DCD) heart transplantation (HTx) uses *in situ* normothermic regional perfusion (NRP) or *ex situ* normothermic machine perfusion (NMP). This study compares NRP with static cold storage (SCS) to *ex situ* oxygenated hypothermic machine perfusion (HMP) in DCD HTx.

Methods: HTx after preservation by NRP-SCS, NRP-HMP or direct procurement (DPP)-HMP in a simulated DCD porcine model was evaluated 2h after wean from cardiopulmonary bypass (CPB) for biventricular (BiV) and diastolic function, and myocardial damage.

Results: BiV contractility was significantly increased in HTx after NRP-HMP or DPP-HMP vs NRP-SCS as noted by absolute BiV recovery with less dobutamine use. Troponin T was lower in the HMP groups with no primary graft dysfunction vs NRP-SCS, while diastolic function was similarly preserved.

Conclusion: BiV contractility was increased with lower inotropic support needs and less myocardial damage in the HMP groups vs NRP-SCS in a preclinical DCD HTx model.

Central Figure

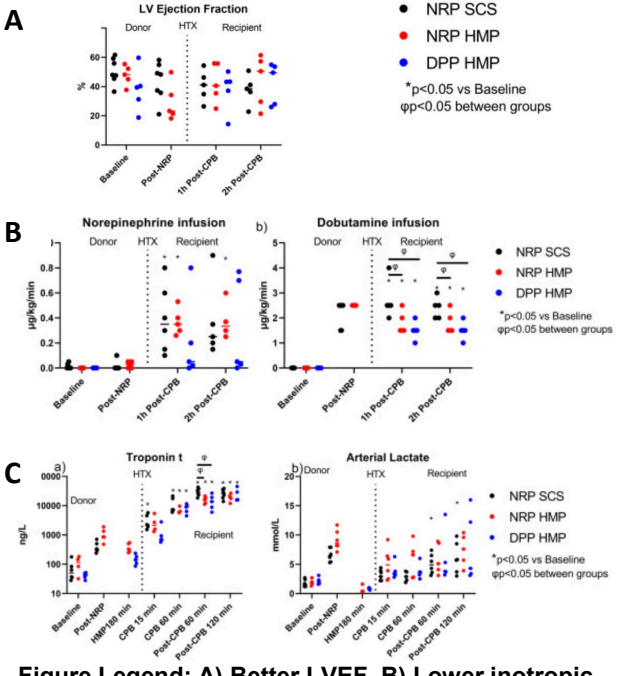

Figure Legend: A) Better LVEF, B) Lower inotropic support, and C) Less myocardial damage in HMP groups vs NRP-SCS DCD HTx recipients.

Reviewers' Comments

- The HMP groups had better BiV contractility, less inotropic support and reduced myocardial damage relative to a clinically relevant NRP-SCS protocol.
- No hearts with good baseline function had primary graft dysfunction after HMP HTx.
- DCD hearts may be procured and metabolically preserved using *ex situ* HMP, serving as a valuable alternative to warm *ex situ* perfusion.
- Human studies will need to evaluate the HMP technique for clinical translation of DCD HTx.

Limitations

- The preclinical young, healthy DCD porcine model may not reflect ischemia tolerance from the HMP HTx technique in humans.
- Post-procurement inotropic needs may not reflect solely heart graft health in the clinical setting.
- DPP-NRP, a common procurement method, and longer-term evaluation by TTE were not done.