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Permalink

<https://escholarship.org/uc/item/5dr9w159>

Journal

Circulation Heart Failure, 16(1)

ISSN

1941-3289

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[et al.](#)

Publication Date

2023

DOI

10.1161/circheartfailure.122.010119

Peer reviewed

Disclaimer: The manuscript and its contents are confidential, intended for journal review purposes only, and not to be further disclosed.

URL: <https://circ-submit.aha-journals.org/>

Manuscript Number: CIRCULATIONAHA/2022/061096

Title: Single-nuclear RNA sequencing of endomyocardial biopsies identifies persistence of donor-recipient chimerism with distinct signatures in severe cardiac allograft vasculopathy

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1 **Single-nuclear RNA sequencing of endomyocardial biopsies identifies persistence of donor-recipient**
2 **chimerism with distinct signatures in severe cardiac allograft vasculopathy**

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10 **Conflicts of interest disclosures:** Dr. Shah is supported in part by grants from the National Institutes of
11 Health and the American Heart Association. In the past 24 months, Dr. Shah has served as a consultant for
12 Myokardia, Cytokinetics, and Best Doctors, and has been on a scientific advisory board for Amgen. Dr. Shah is
13 a co-inventor on a patent for ex-RNAs signatures of cardiac remodeling. Dr. Moslehi has severed on advisory
14 boards for Bristol Myers Squibb, AstraZeneca, Myovant, Cytokinetics, Takeda, BeiGene, Kiniksa, Kurome
15 Therapeutics, Pfizer and is supported by National Institutes of Health grants (R01HL141466, R01HL155990,
16 and R01HL156021). The other authors have no conflicts of interest to disclose. All other authors declare no
17 relevant conflicts of interest.

18 **Funding:** R01HL141466, Team Phenomenal Hope Grant, K01HL140187

19 **Word count:** 789

20 **Figures:** 1

21 **References:** 5

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31 Cardiac allograft vasculopathy (CAV) is the leading cause of late allograft failure and mortality after heart
32 transplantation¹. Histologically, CAV is chronic vascular rejection characterized by diffuse intimal thickening of
33 macro- and microvasculature. While *in vitro* cellular models and *in vivo* histologic observations suggest
34 coordinated responses of endothelial, fibroblast, and smooth muscle cells in CAV pathology, cell-specific
35 transcriptional signatures among these in the transplanted human heart have not been studied. As current
36 standards of diagnosis and treatment of CAV have significant limitations, understanding cell-specific responses
37 may prove critical for developing improved detection strategies and novel therapeutics.

38 Here, we used single-nuclear RNA sequencing (snRNA-seq) to elucidate the transcriptomic landscape of CAV.
39 Importantly, we establish the feasibility of performing snRNA-seq from human endomyocardial biopsy (EMB)
40 specimens (3-10 mg in size) obtained at the time of right heart catheterization, enabling high-resolution
41 molecular profiling of samples collected during routine clinical practice. We compared tissue obtained at the
42 time of re-transplantation from 4 individuals with severe CAV to EMB specimens from 3 individuals post-
43 transplant without CAV (**Figure 1A**). For all 7 individuals, samples were obtained from the right ventricle (RV).
44 In 3 out of 4 patients with severe CAV, left ventricular (LV) samples were also obtained (for a total 10
45 samples).

46 After nuclear isolation with modifications to account for low tissue mass, libraries were generated, sequenced,
47 quality-controlled, and analyzed as previously described². Raw FASTQ files are deposited at the NIH NCBI
48 GEO data repository (GSE203548) and code used for these analyses are deposited at
49 <https://github.com/learning-MD/CAV>. This study was approved by the Vanderbilt University Medical Center's
50 Institutional Review Board.

51 We successfully isolated 62,465 nuclei and identified 17 major cell types with heterogenous distribution across
52 the ten different samples (**Figures 1B, 1C**). When comparing RV samples, endothelial cells and fibroblasts in
53 CAV exhibited increased expression of *SERPINE1*, which promotes neointimal hyperplasia and fibrosis³.
54 Endothelial cells were enriched for pathways involved in angiogenesis, cell migration, and extracellular matrix
55 (ECM) organization (**Figures 1D, 1E**). Fibroblasts in CAV exhibited increased expression of genes involved in
56 ECM deposition and fibrosis (e.g., *MMP2*, *CCN1*, *THBS1*) while also highly expressing *IL6ST*, involved in IL-6
57 signaling. As expected, macrophages in CAV showed increased expression of genes associated with
58 inflammation (e.g., *TLR2*, *IFNAR2*). While no significant differences in T cells were noted between conditions,

59 subclusters included CD4 central memory T cells (*IL7R*, *TCF7*), CD4 T regulatory cells (*FOXP3*, *CTLA4*,
60 *IL2RA*), and CD4 T cells exhibiting markers of exhaustion (*LAG3*, *CTLA4*, *PDCD1*), along with CD8 memory T
61 cells (*CCL5*). No major differences in gene expression were noted between RV and LV CAV samples.

62 We repurposed a genotype-free demultiplexing tool to infer donor- and recipient-derived nuclei from each
63 individual CAV sample. Using 5 of the 7 combined CAV samples (including both LV and RV tissue), 2,827
64 nuclei were confidently called as donor- or recipient-derived in the absence of genotyping (**Figure 1F**).

65 Endothelial cells exhibited significant donor-recipient chimerism (21.8% recipient-derived). Donor-derived
66 endothelial cells were enriched for markers of endothelial-to-mesenchymal transition (EndoMT; *SERPINE1*,
67 *VIM*, *COL3A1*; **Figure 1G**). In contrast, immune cells were largely replaced by those originating from the
68 recipient (91.1% of macrophages/monocytes, 92.6% of NK cells, 88% of T cells). Recipient-derived
69 macrophages included both *CCR2*⁺ monocyte-derived macrophages and *CCR2*⁻ *MRC1*⁺ tissue resident
70 macrophages, traditionally thought to be involved in cardiac repair⁴ (**Figure 1H**). Macrophages exhibited
71 markers of activation, including *HLA-DRA* and *CD74*, and increased expression of *TGFB1*, a potential driver
72 for the EndoMT observed in donor-derived endothelial cells.

73 This study is the first to successfully utilize human EMB samples to isolate large numbers of intact nuclei for
74 single-nuclear transcriptomics. As expected from an ischemic allograft, we see enrichment for genes and
75 pathways involved in inflammation, fibrosis, and tissue healing. We highlight several unique findings enabled
76 by this approach: 1) cell composition amongst EMB samples is highly heterogeneous, suggesting that bulk
77 RNA-seq approaches may exhibit high levels of variability due to sampling bias; 2) there are unique
78 transcriptomic signatures of donor- versus recipient-derived cells, particularly endothelial cells, highlighting
79 putative novel avenues for investigation; and 3) the presence of recipient-derived *CCR2*⁻ macrophages
80 warrants further study, as only a small percentage would be expected to be recipient-derived⁴. However,
81 recent single-cell data have implicated partial replacement of MHC-II^{hi}*CCR2*⁻ cardiac macrophages by
82 monocytes, suggesting a still evolving understanding of macrophage subsets⁵.

83 Our study is limited by a small sample size and the use of samples derived from severe CAV. However, these
84 data demonstrate feasibility of performing snRNA-seq using frozen EMBs, presenting a unique opportunity that
85 may have broad ramifications on the fields of heart transplantation and cardio-oncology/immunology. These
86 data also lay the groundwork for ongoing experiments to study serial, routinely-collected EMB specimens after

87 heart transplantation to identify novel biomarkers and pathways through which early CAV pathogenesis can be
88 interrupted, thereby prolonging allograft survival.

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- 90 1. Pober JS, Jane-wit D, Qin L, Tellides G. Interacting mechanisms in the pathogenesis of cardiac allograft
91 vasculopathy. *Arterioscler Thromb Vasc Biol.* 2014;34(8):1609-1614.
- 92 2. Tucker NR, Chaffin M, Fleming SJ, et al. Transcriptional and Cellular Diversity of the Human Heart. *Circulation.*
93 2020;142(5):466-482.
- 94 3. Ji Y, Weng Z, Fish P, et al. Pharmacological Targeting of Plasminogen Activator Inhibitor-1 Decreases Vascular
95 Smooth Muscle Cell Migration and Neointima Formation. *Arterioscler Thromb Vasc Biol.* 2016;36(11):2167-2175.
- 96 4. Bajpai G, Schneider C, Wong N, et al. The human heart contains distinct macrophage subsets with divergent
97 origins and functions. *Nat Med.* 2018;24(8):1234-1245.
- 98 5. Dick SA, Macklin JA, Nejat S, et al. Self-renewing resident cardiac macrophages limit adverse remodeling
99 following myocardial infarction. *Nat Immunol.* 2019;20(1):29-39.

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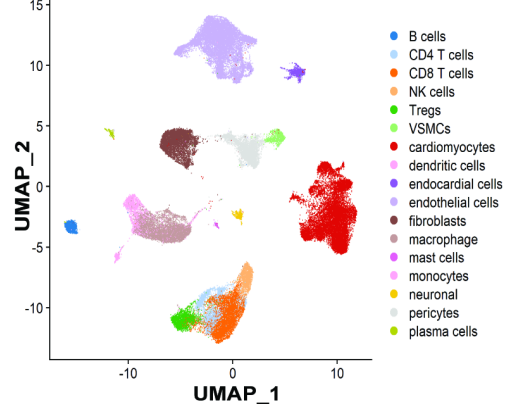
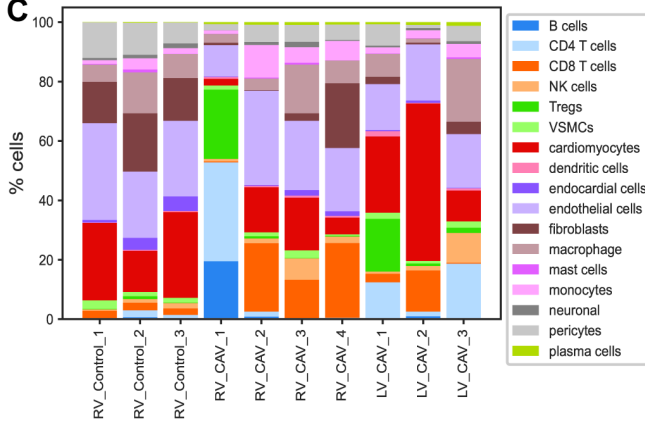
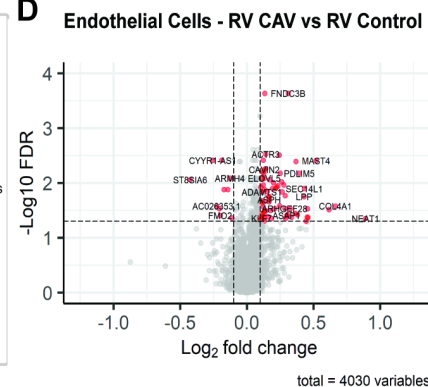
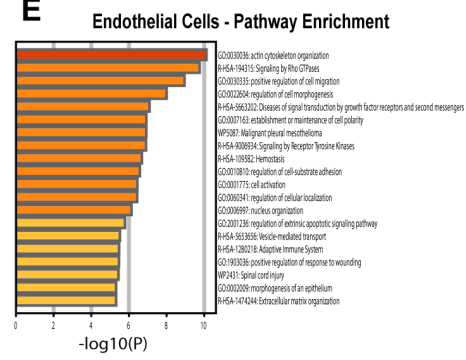
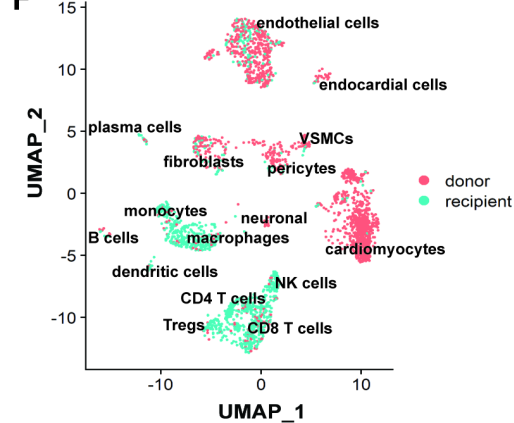
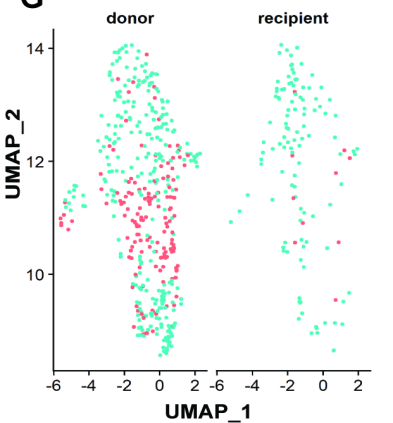
115 **Figure 1. A)** Clinical characteristics of all seven patients studied. **B)** Uniform Manifold Approximation and
116 Projection (UMAP) of 62,465 nuclei identified 17 major cell types using canonical marker genes. **C)** Cell
117 compositional analyses were performed using scCODA v0.1.6. Labels correspond to patients described in
118 *Figure 1A*. No significant difference in cell composition was noted using an automated reference cluster. **D)**
119 Differential gene expression was performed using MAST. Volcano plot representing right ventricular CAV vs.
120 control samples for the endothelial cell cluster. **E)** Biological pathway enrichment analysis of differentially
121 upregulated genes in RV CAV endothelial cells using Metascape. **F)** Genotype-free inference of donor- versus
122 recipient-derived nuclei was performed using souporcell v2.0. UMAP of donor- vs. recipient-derived nuclei. The
123 clusters correspond to the same clusters annotated in *Figure 1B*. **G)** Donor-derived endothelial cells are
124 enriched for markers of endothelial-to-mesenchymal transition. **H)** The monocyte/macrophage cluster is largely
125 recipient-derived. Presence of distinct $CCR2^+$ monocytes and $CCR2^+MCR1^+$ macrophages is highlighted using
126 Nebulosa. *LVEF = left ventricular ejection fraction; HTN = hypertension; DM2 = type 2 diabetes mellitus; CKD*
127 *= chronic kidney disease; DSA = donor-specific antibodies; ACR = acute cellular rejection; AMR = antibody-*
128 *mediated rejection; ER = extended release; MMF = mycophenolate mofetil; RV = samples from right ventricle;*
129 *LV = samples from left ventricle; CAV = cardiac allograft vasculopathy; EndoMT = endothelial-to-mesenchymal*
130 *transition.*

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	Control 1	Control 2	Control 3	CAV 1	CAV 2	CAV 3	CAV 4
Age	56	44	59	52	54	45	54
Gender	M	M	F	F	F	F	M
Days After Transplant	304	188	146	2045	615	2531	2450
Dual-Organ Transplant	No	No	Yes	No	No	Yes	Yes
LVEF	65	80	55	50	55	55	40
HTN	Yes	Yes	No	Yes	No	No	Yes
DM2	Yes	Yes	Yes	Yes	No	No	Yes
Dyslipidemia	Yes	Yes	Yes	Yes	Yes	Yes	Yes
CKD	Yes	Yes	Yes	Yes	Yes	Yes	Yes
DSAs	No	No	No	No	Yes	Yes	Yes
Prior ACR $\geq 2R$	Yes	Yes	No	No	No	Yes	Yes
Prior AMR	No	No	No	No	Yes	Yes	Yes
Current ACR	1R	1R	0R	Yes	0R	0R	Yes
Current AMR	No	No	No	No	No	No	No
Stanford Classification	III	IV	n/a	n/a	n/a	n/a	n/a
Specimen Source	Biopsy	Biopsy	Biopsy	Explant	Explant	Explant	Explant
Immunosuppression Regimen	Tacrolimus, Sirolimus	Tacrolimus ER, azathioprine	Tacrolimus, MMF, Prednisone	Cyclosporine, MMF, Prednisone	Tacrolimus, MMF	Tacrolimus, Prednisone	Tacrolimus, MMF

B**C****D****E****F****G****H**