Non-conventional Pericardial Fluid T Cells in Dilated Cardiomyopathy

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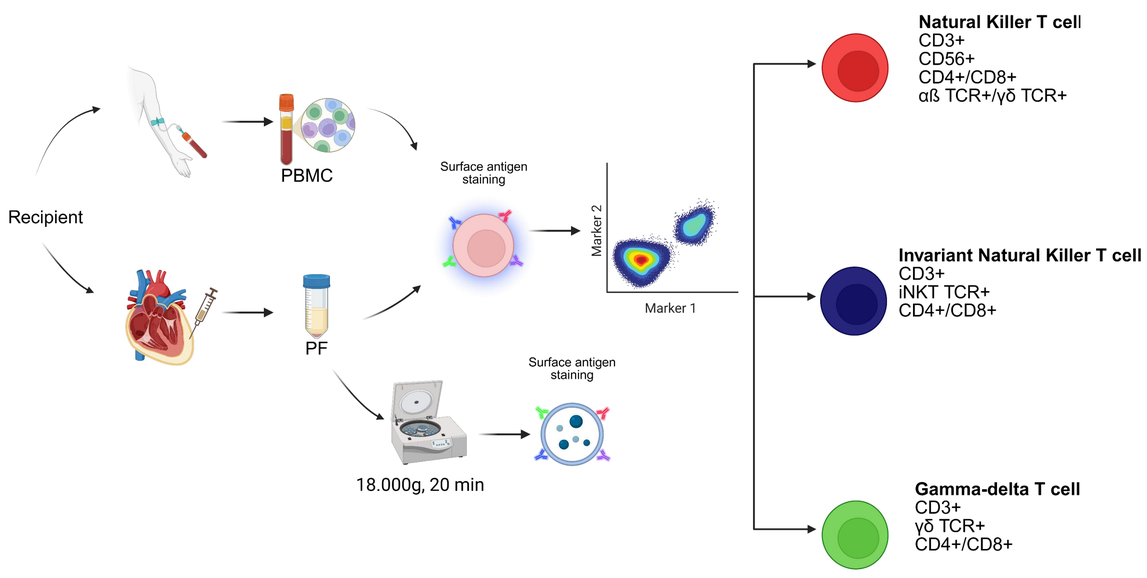
INTRODUCTION

Non-conventional T cell subsets typically express TCRs with limited diversity, recognize non-peptide antigens presented by non-polymorphic molecules without classical MHC restriction, and are capable of rapid responses with less confinement to lymphoid tissues.
Their presence and function in pericardial fluid (PF) versus peripheral blood mononuclear cells (PBMC) remains underexplored.

AIMS

This study aimed to compare the frequencies and cytotoxic activity of non-conventional T cell populations in PF and PBMC samples from heart transplant recipients with dilated cardiomyopathy to assess their potential local immunological role. We also analyzed immune markers on the extracellular vesicles isolated from the pericardial fluid samples.

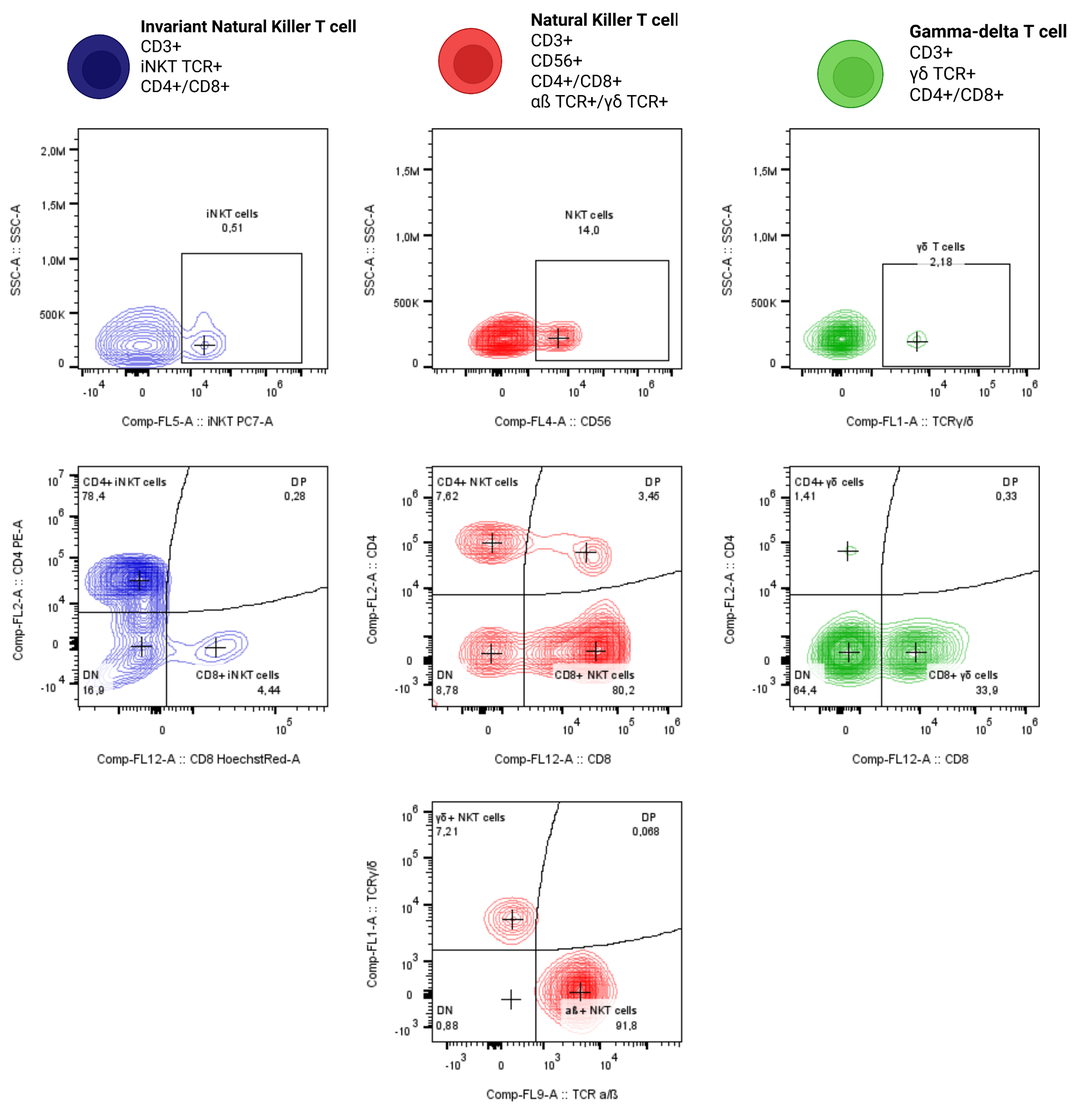
MATERIALS AND METHODS



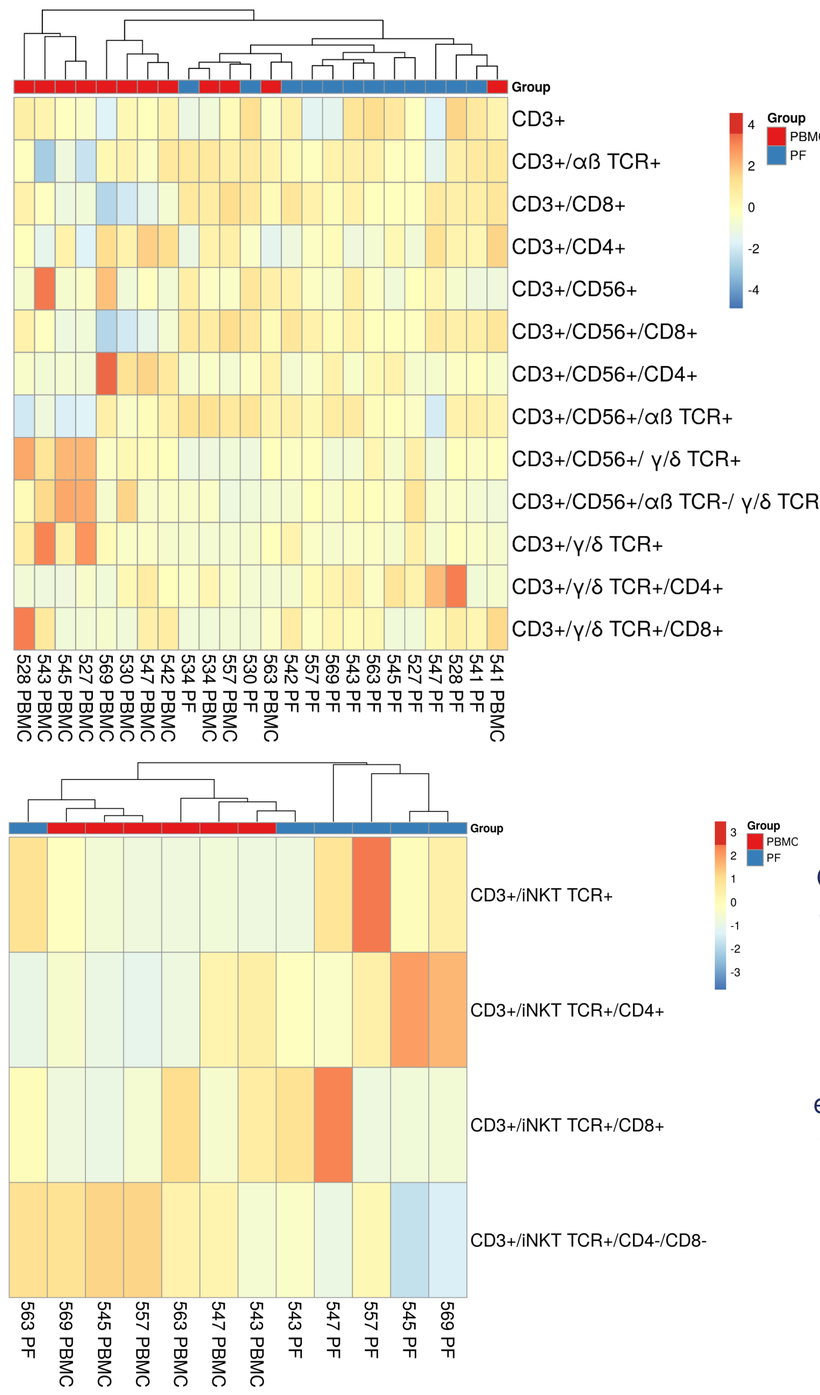
The recipient's peripheral blood is collected and the Peripheral Blood Mononuclear Cells (PBMCs) are isolated by density gradient centrifugation. In parallel, pericardial fluid (PF) is collected and undergoes a two-step centrifugation process: First, it is spun at 400 g for 10 minutes (repeated twice) to isolate the cells. Then, it is spun at 18,000 g for 20 minutes to concentrate the large extracellular vesicle (IEV) fraction. The isolated cells and concentrated IEV fraction are then stained with surface antigens and analysed by flow cytometry.

Flow cytometry gating strategy:

Lymphocytes/Single cells/Live cells/CD3+ cells:



Results I.

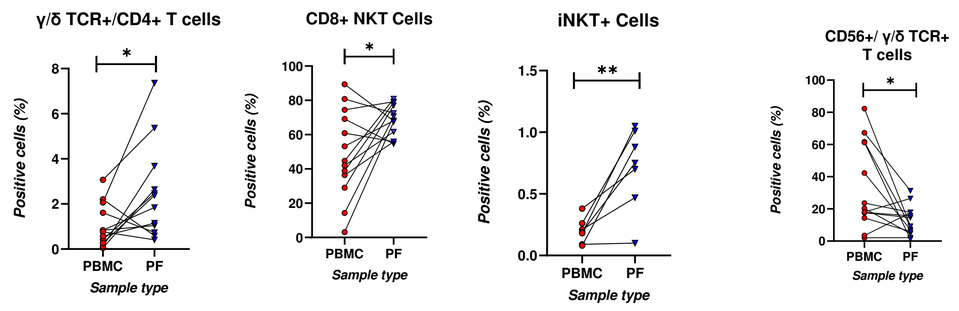


Based on T cell subset distribution, most samples cluster by origin (PF vs PBMC), with **CD3⁺/CD8⁺, CD3⁺/γδ TCR⁺/CD4+, CD3+/CD56+/γδ TCR⁺** and **CD3⁺/CD56⁺/CD8⁺** subsets contributing most to the separation.

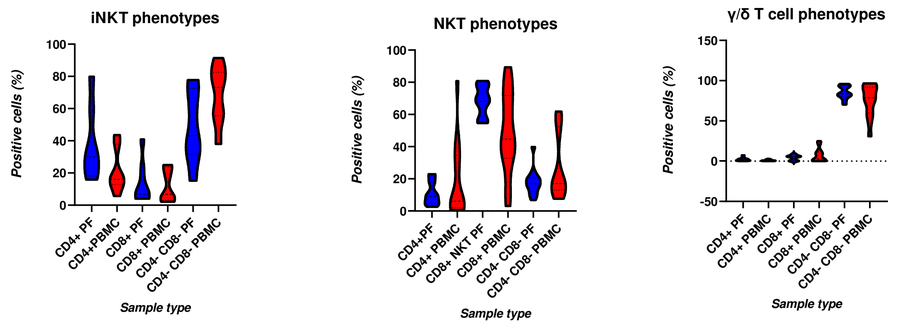
Clustering reveals that a subset of pericardial fluid (PF) samples separates from peripheral blood (PBMC) samples, primarily driven by elevated proportions of **CD3⁺/iNKT TCR⁺** and **CD3⁺/iNKT TCR⁺/CD4⁺**

Clustering distance for rows:: no clustering, Clustering distance for columns: Manhattan; Clustering method for columns:complete

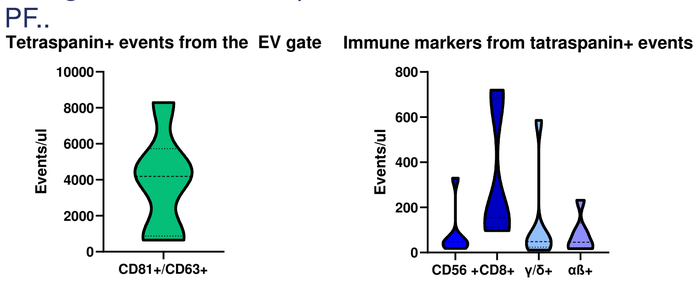
Results II.



Quantification of T cell subsets reveals a significantly higher proportion of **CD3+/CD8+, CD3⁺/γδ TCR⁺/CD4+, CD3+/CD56+/CD8+ and CD3⁺/iNKT TCR⁺** cells in PF compared to PBMC samples In contrast, **CD3⁺/CD56+/γδ TCR⁺** cells are more abundant in PBMC samples. Paired t-test, * p<;0.05



The data reveal a distinct tissue-specific distribution of T cell phenotypes. Notably, **CD8+ NKT** cells are enriched and dominate in the PF. In contrast**, iNKT and γδ T cells are largely double-negative** in both compartments, with **iNKT** cells showing an increase in **CD4+** cells in PF..



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