Host Transcriptional Signatures Associated with Distinct Inflammatory Bioprofiles in People with HIV

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All-Ireland Infectious Diseases cohort

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Background

- Some people with HIV (PWH) exhibit high levels of inflammation despite effective treatment.
- In two independent cohorts of treated PWH, we previously demonstrated that PWH fall into three distinct inflammatory clusters: cluster 1 is defined by lower inflammation; cluster 2 is characterised by high innate immune activation, gut microbial translocation, and vascular inflammation; and cluster 3 is characterised by gut epithelial dysfunction, T-cell activation, and systemic inflammation. The cluster 3 phenotype is associated with a seven-fold increased risk of prevalent cardiovascular disease (CVD)(1).
- We aimed to determine the transcriptional pathways associated with these distinct inflammatory phenotypes in PWH compared to a demographically similar control group without HIV.

Methods

- This project is based on a combined cohort from the Understanding the Pathology of Bone Diseases in HIV Positive Persons' Coronary Artery Disease (HIV UPBEAT CAD) and the All-Ireland Infectious Disease (AIID) cohorts. The UPBEAT CAD cohort includes PWH and demographically matched controls, while the AIID samples consisted only of PWH.
- We first conducted a principal component analysis (PCA), followed by unsupervised hierarchical clustering, on treated PWH and controls within the combined cohort (N = 372) (Figure 1A).
- A representative subset (N=143) was then selected for transcriptional profiling comprising samples with the shortest Euclidean distance to the cluster centroids (Figure 1B). This subset included three distinct PWH groups, each corresponding to a cluster, along with a control group of individuals without HIV, matched for age ±5 years, smoking status, sex at birth, BMI, and ethnicity.
- Gene expression in Peripheral Blood Mononuclear Cells (PBMCs) was analysed using the NanoString Immune Exhaustion 800-gene panel.
- Differential expression (DE) analysis was performed using the Rosalind Cloud Analysis Software, with a fold-change threshold of ±1.5 and a significance cutoff of P<0.05. The control group served as the baseline for DE analyses. We used the Benjamini-Hochberg method to estimate the false discovery rate and adjust for multiple comparisons.
- The Reactome Voronoi tool was utilised to explore the overrepresented pathway topologies of DE genes.

Results

- The Combined cohort PCA identified three clusters with increasing levels of inflammatory biomarkers. Cluster 1 (n=175, 94% PWH) had lower systemic, innate, and vascular inflammation. Cluster 2 (n=155, 80% PWH) exhibited higher systemic and vascular inflammation but lower T-helper 1 responses and microbial translocation. Cluster 3 (n=42, 88% PWH) showed the highest levels of T-helper 1 responses, systemic inflammation, and microbial translocation (Figure 1A).

Table 1. Transcriptional Profiling Participant Demographics Stratified by Group

	Total N=143	PWH Cluster 1 n=45	PWH Cluster 2 n=45	PWH Cluster 3 n=35	Controls n=18	*P*
Age - Median (IQR) Years	45 (40, 52)	45 (35, 50)	47 (41, 52)	45 (40, 48)	47 (44, 52)	0.04
BMI - Median (IQR) kg/m²	26 (23, 30)	26 (23, 31)	26 (24, 30)	27 (22, 31)	28 (25, 32)	0.60
Sex at Birth - n (%)						
Female	63 (44%)	21 (47%)	18 (40%)	14 (40%)	10 (56%)	0.81
Male	80 (56%)	24 (53%)	27 (60%)	21 (60%)	8 (44%)	
Caucasian Ethnicity - n (%)	70 (49%)	18 (40%)	24 (53%)	18 (51%)	10 (56%)	0.17
Current Smoker n (%)	43 (30%)	10 (22%)	18 (40%)	8 (23%)	7 (39%)	0.45
CD4 Count - Median (IQR) Cells/cm³	619 (433, 824)	588 (497, 821)	707 (528, 958)	673 (554, 934)		0.17
CD8 Count - Median (IQR) Cells/cm³	669 (481, 903)	777 (619, 930)	758 (541, 950)	617 (512, 868)		0.36
CD4:CD8 Cell Ratio - Median (IQR)	0.8 (0.6, 1.16)	0.8 (0.6, 1.15)	1.0 (0.8, 1.35)	1.0 (0.7, 1.29)		0.13
Duration of ART - Median (IQR) Years	5 (2, 10)	4 (2, 10)	7 (4, 11)	5 (2, 11)		0.18

- 143 (87.4% PWH) participants were then included in the transcriptional profiling analysis. No significant differences existed between the groups in PWH-specific parameters (Table 1). After adjusting for multiple comparisons, 206 genes were DE in PWH compared to the people without HIV control group, with 103 genes shared across the three inflammatory clusters (Figure 1C).

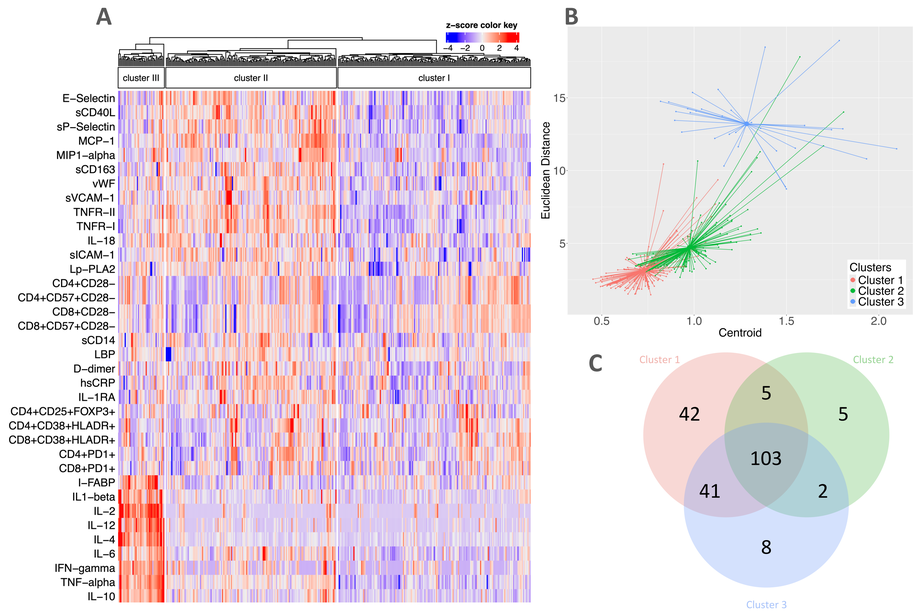


Figure 1. (A) Heatmap of 38 Biomarkers (27 Inflammatory Plasma Markers and 11 Flow-Cytometric T-Cell Markers in PBMCs) Contributing to Cluster Formation. **(B)** Cluster Centroids and Euclidean Distances of Samples Viewed on a 1-Dimensional Surface. **(C)** Venn Diagram of Shared and Unique Differentially Expressed Genes Compared to People Without HIV.

- The overrepresented pathway topologies of the adaptive immune system, cytokine signalling, and innate immune system were shown in cluster 1; programmed cell death topology was overrepresented in cluster 2, while the CVD associated cluster 3 unique gene list mapped to regulation of lipid metabolism, pyroptosis, and cellular immune senescence (Figure 2).

Distinct inflammatory profiles in PWH that are linked to altered risk of CVD, map to specific transcriptional pathways involved in lipid metabolism, pyroptosis and immune senescence. These insights suggest distinct pathways that may offer therapeutic targets to further reduce the impact of CVD in PWH

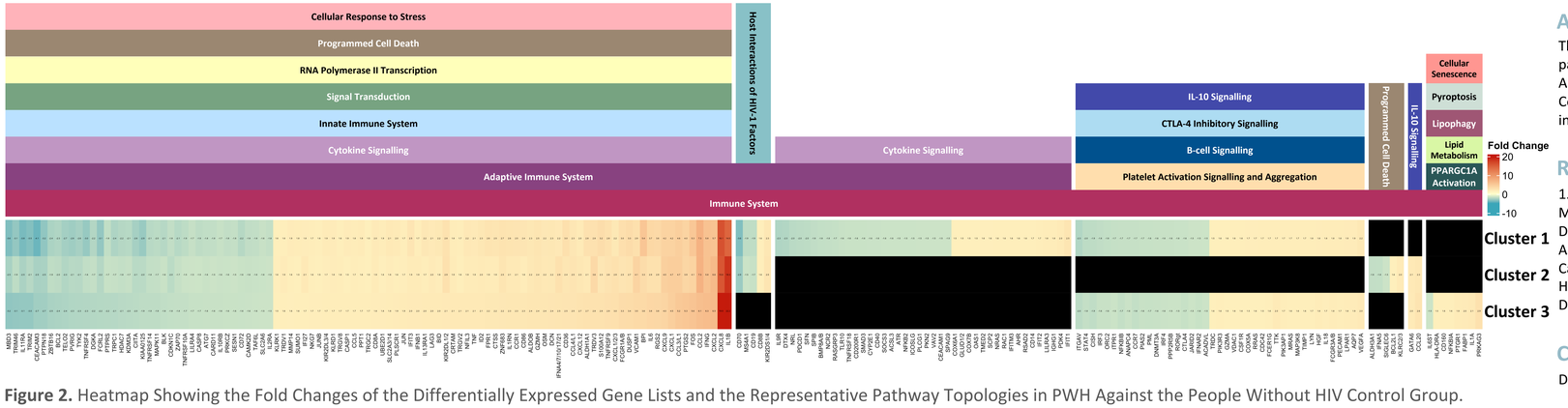


Figure 2. Heatmap Showing the Fold Changes of the Differentially Expressed Gene Lists and the Representative Pathway Topologies in PWH Against the People Without HIV Control Group.

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References

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