EXTERNAL VALIDATION OF BIOMARKER CLUSTERS BASED ON PROTEIN BIOMARKERS

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POPPY
Pharmacokinetic and clinical observations in people over 50

Background

Advances in antiretroviral therapy (ART) have dramatically improved life expectancy and reduced the risk of AIDS-related complications among people living with HIV (PLWH). However, PLWH exhibit chronic inflammation, and therefore, tend to have elevated levels of inflammatory biomarkers, which may contribute to comorbidities.

Aims

- To validate biomarker patterns identified in a previous independent smaller cohort of PLWH and HIV-negative controls[1].
- To assess associations between inflammatory biomarker patterns identified and demographic, lifestyle and clinical factors.

Methods

Participants

- The POPPY (Pharmacokinetic and clinical Observations in PeoPle over fiftY) Study cohort includes 3 groups (669 PLWH≥50 years, 374 PLWH<50 years, 304 HIV-negative controls ≥50 years) from the UK and Ireland[2]. Of these, the present analysis included 465 participants, with detailed information on demographic, lifestyle and clinical factors collected at the baseline POPPY visit.

Protein Biomarkers

- Thirty-one biomarkers across several inflammatory pathways were tested in plasma samples from the 465 participants included:

Coagulation		**Axonal injury**		**Microbial translocation**	
sCD40L	D-dimer	NFL		IL-18	I-FABP
sP-sel		S100B		IL-12	LBP
Endothelial function		**Systemic inflammation**		**Immune regulation**	
sICAM-1	E-selectin	hsCRP	IL-2	IL1RA	PD-L1
sVCAM-1	vWF	IL-6	TNF-alpha	IL-4	
Innate immune activation		IFN-gamma	IL1 beta		
sCD14	MCP1	TNF R I	TNF R II		
sCD163	MIP1 alpha	**Atherosclerosis**			
IL-10		Lp-PLA2	MPO		

Statistical analysis

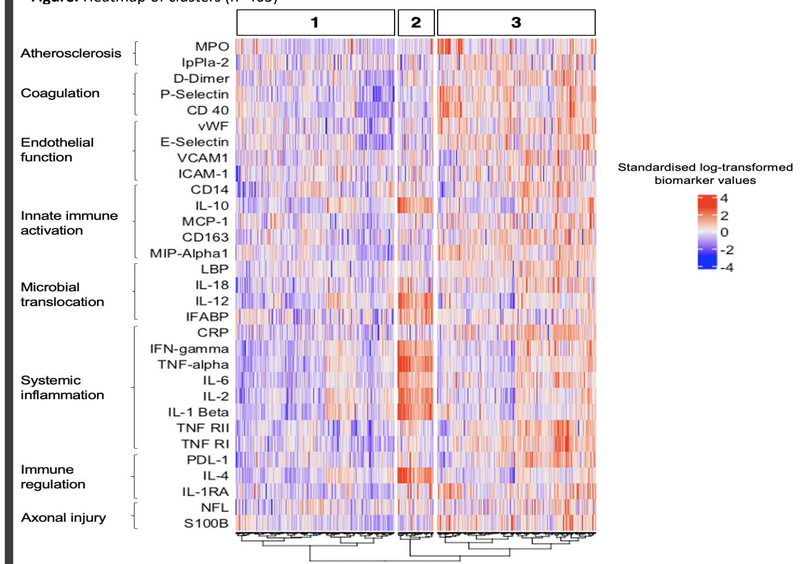
- Principal Component (PC) Analysis was conducted on the log-transformed biomarkers. The Elbow method was used to determine the number of PCs to retain.
- Agglomerative hierarchical clustering was used to group participants based on PC scores. The Average Silhouette Width method was used to determine the optimal number of clusters.
- Between-cluster demographic, lifestyle and clinical factor differences were assessed for significance using Kruskal-Wallis/Chi-squared tests:

  Demographic factors: Gender, age, ethnicity.
  Lifestyle factors: Men having sex with men (MSM) sexuality/route of HIV acquisition, current alcohol use.
  Clinical factors: Living with HIV status, obese (BMI ≥30 kg/m^2), systolic blood pressure, history of cardiovascular disease, history of arthritis of knee/hip.

We identified ***three distinct inflammatory clusters***, associated with differences in important cardiometabolic features suggesting the presence of biological phenotypes that ***may contribute to clinical outcomes.***

Whether this personalized approach can inform disease prevention and improve treatment for PLWH with multimorbidity requires further study.

Figure. Heatmap of clusters (n=465)



Note, log-transformed standardized biomarker values are used for the heatmap. Red represents relatively high biomarker values; blue represents relatively low biomarker values.

Results

- The 465 included participants (236 PLWH≥50 years, 107 PLWH<50 years, 122 HIV-negative) had a median (interquartile range [IQR]) age 54 [50-60] years, 80% were male, 88% white and 71% MSM sexuality/route of HIV acquisition (**Table**).
- Among PLWH, most (98%) were on ART, 92% had a viral load ≤50 copies/mL and median [IQR] CD4+ T-cell count was 610 [470-785] cells/mm^3.

Table. Selected Cluster Demographic, Lifestyle and Clinical Factors

n (%) or median (IQR), unless otherwise noted	Total (n=465)	Cluster 1 (n=209)	Cluster 2 (n=47)	Cluster 3 (n=209)	p-value
Demographic Factors					
Age in years	54 (50, 60)	54 (48, 60)	56 (51.5, 61)	55 (50, 60)	0.08
Male	374 (80.4%)	172 (82.3%)	32 (68.1%)	170 (81.3%)	0.08
White	408 (87.7%)	189 (90.4%)	41 (87.2%)	178 (85.2%)	0.26
Lifestyle Factors					
MSM sexuality/route of HIV acquisition	332 (71.4%)	155 (74.2%)	30 (63.8%)	147 (70.3%)	0.33
Current alcohol use	384 (82.6%)	175 (83.7%)	41 (87.2%)	168 (80.4%)	0.45
Clinical Factors					
Living with HIV	343 (73.8%)	152 (72.7%)	28 (59.6%)	163 (78.0%)	0.03
Obese (BMI ≥30 kg/m^2)	81 (17.6%)	22 (10.6%)	10 (21.3%)	49 (23.8%)	0.002
Systolic Blood Pressure (mmHg)	126 (117, 140)	126 (116, 138)	135 (125, 154)	126 (116, 138)	0.002
History of cardiovascular disease	206 (44.3%)	82 (39.2%)	13 (27.7%)	111 (53.1%)	0.001
History of arthritis of knee/ hip	54 (11.6%)	16 (7.7%)	4 (8.5%)	34 (16.3%)	0.02

Three clusters displaying distinct patterns of inflammatory biomarkers were identified (**Figure**):

Cluster 1 (n=209, 45% of subjects) included individuals with generally low levels of inflammation; ***Cluster 2*** (n=47, 10% of subjects) included those with increased markers associated with T-cell and B-cell activation and proliferation; and ***Cluster 3*** (n=209, 45% of subjects) identified those with elevated levels of biomarkers across a range of inflammatory pathways.

- Individuals in each cluster were similar for most demographic/lifestyle factors: median age (p=0.08); male (p=0.08); white (p=0.26); MSM sexuality/route of HIV acquisition (p=0.33); and current alcohol use (p=0.45) (**Table**).
- However, there were significant differences in the proportion of PLWH included (p=0.03); proportion who were obese (BMI ≥30 kg/m^2) (p=0.002); median systolic blood pressure (p=0.002); and proportion with a history of cardiovascular disease (p=0.001) and arthritis of knee/hip (p=0.02) (**Table**).

Summary and Discussion

We have identified three clusters with inflammatory patterns similar to those identified in a previous smaller study. These three clusters of distinct inflammatory patterns were identified, associated with differences in important cardiometabolic features suggesting the presence of biological phenotypes that may contribute to clinical outcomes. Future studies are needed to clinically validate the ability of biomarker [clusters] to inform disease progression and improve the treatment of PWH with multimorbidity.

References:
[1]McGettrick P, *et al. CROI* 2021.
[2]Bagkeris E, *et al. Int J Epidemiol* 2018; **47(5):** 1391-1392e.