Longitudinal High-Dimensional Proteomics Reveals Evolving CNS Immune Dysfunction during Early HIV

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BACKGROUND

- Antiretroviral therapy (ART) has greatly contributed to the effective management of HIV causing a significant improvement of lifespan and quality of life of people with HIV (PWH). That being said, PWH typically initiate ART years after HIV acquisition, contributing to perturbed brain health trajectories later in life despite suppressive ART.[1]
- The pathobiology of HIV in the CNS during untreated early infection stage remains poorly characterized. To explore the patterns of HIV in the CNS during these early stages, we longitudinally analyzed the CSF proteomic profile of people with untreated early HIV infection.

METHODS

- We used CSF samples collected in the Primary Infection Stage CNS Events Study (PISCES, conducted in San Franciso, CA from 2005-2014) which recruited men who have sex with men during primary HIV infection (PHI, <1 year since HIV acquisition) prior to ART initiation.
- Among the 33 males with PHI, 15 had ≥ 3 follow-up visits.
- CSF samples collected only once from 20 age and sex matched people without HIV (PWoH) from San Francisco, CA were also included.

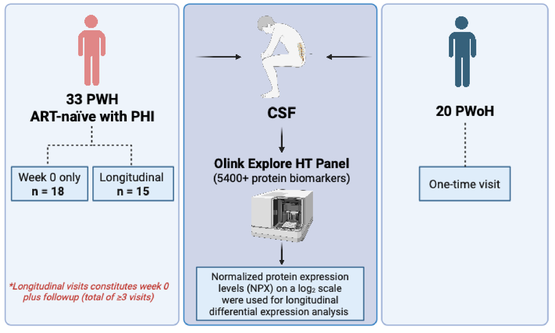


Figure 1: Study design.

RESULTS

Table 1: Baseline Participants Characteristics for the PWH (n =33) vs PWoH (n = 20)

	PHI (n=33) Median (IQR) or n (%)	PWoH (n=20) Median (IQR) or n (%)	p-value
Age, years	40 (31-46)	40 (33-46)	0.65
Male	33 (100)	20 (100)	>0.99
CD4+ count, cells/μL	601 (418 -743)	821 (711-1004)	<0.001
CD4:CD8 ratio	0.57 (0.44-0.90)	1.8 (1.4-2.3)	<0.001
CSF WBC, cells/μL	6.0 (3–8)	1.0 (1.0-1.0)	<0.001
Estimated days of infection	133 (75-177)	-	-
Plasma HIV RNA, log_{10} copies/mL	4.5 (3.9-5.0)		
CSF HIV RNA, log_{10} copies/mL	2.6 (1.8-3.0)	-	-
Plasma – CSF HIV RNA Ratio, log_{10} copies/mL	1.81 (1.3-2.2)	-	-

We observed an early upregulation of CSF proteins in men with PHI compared to matched PWoH. Longitudinal differential expression analysis revealed temporal changes in the CSF proteome and pathways that are linked to inflammation were enriched. This highlights the importance of early HIV diagnosis and treatment strategies to prevent progressive CNS inflammation that could later impact cognitive ability.

RESULTS

30 differentially expressed proteins were observed as early as 133 days of HIV acquisition in men with PHI compared to PWoH

CSF protein expression is either increasing or decreasing from 0 weeks to 12 months of untreated PHI

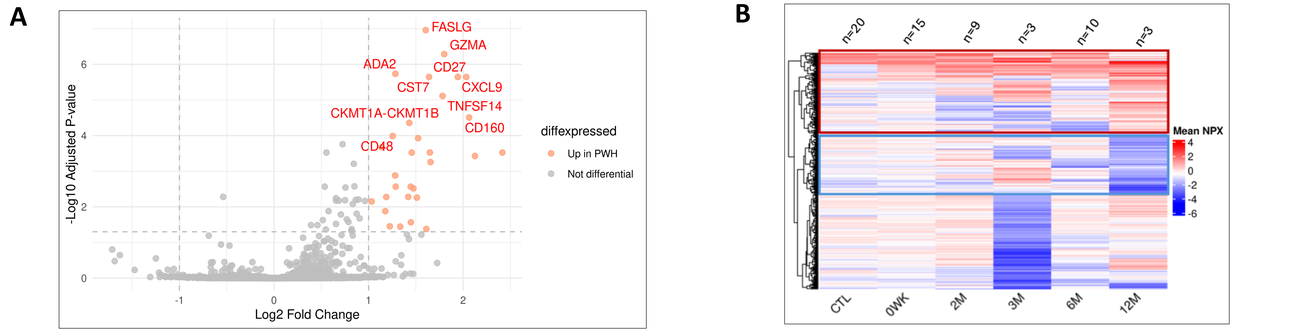


Figure 2: A) Volcano plot of differentially expressed proteins between people with PHI (n = 33) and PWoH (n =20) at first visit. We found 30 proteins that were already upregulated in PWH compared to PWoH at this initial time-point. **B)** A heatmap of the 500 most variable proteins for timepoint with ≥ 3 measurement (40 total measurements). Average protein expression is given for control group (CTL), PHI group at week 0 (0WK) and 2 to 12 months (2M, 3M, 6M and 12M). Two different clusters were identified; the first cluster shown in red show an increasing trajectory over the course of 12 months, while in another cluster (in blue) exhibits a progressive downregulation in the CSF proteome over time.

We identified 97 proteins in the PHI group that are different from controls and exhibits a significant temporal change over 12 months period

Table 2: Twenty top ranked differentially expressed proteins out of 97 total proteins in PWH that were evolving and different from PWoH (adjusted p-value < 0.05).

Rank	Name	Rank	Name
1	CD27	11	MMP9
2	TNFSF14	12	CD79B
3	GZMA	13	CRTAM
4	CST7	14	CXCL9
5	FASLG	15	CD48
6	PDCD1	16	SLAMF7
7	ADA2	17	IGLC2
8	CXCL10	18	GZMH
9	MZB1	19	CD80
10	CD160	20	FCRL5

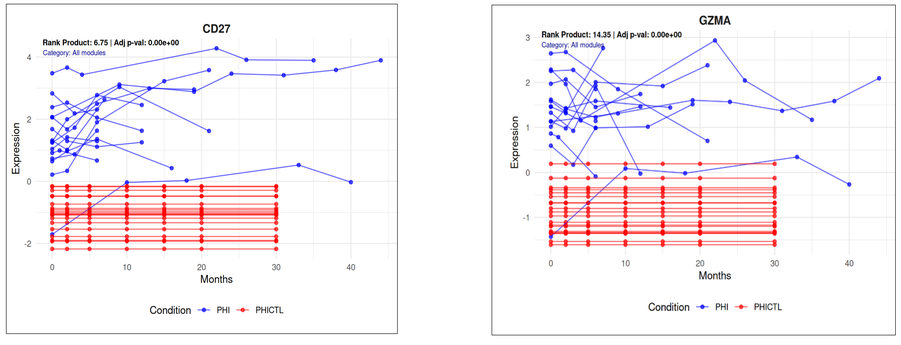


Figure 3: Spaghetti plots of top differentially expressed proteins among the 97 in the PHI group that were different compared to PWoH and evolved over time (here, the PWoH are treated as a stable reference since they did not have longitudinal timepoints). A changing trajectory was seen in both CD27 and GZMA proteins over the 12 months period of untreated HIV infection (adjusted p-value < 0.05).

Pathways related to inflammation are seen during untreated PHI

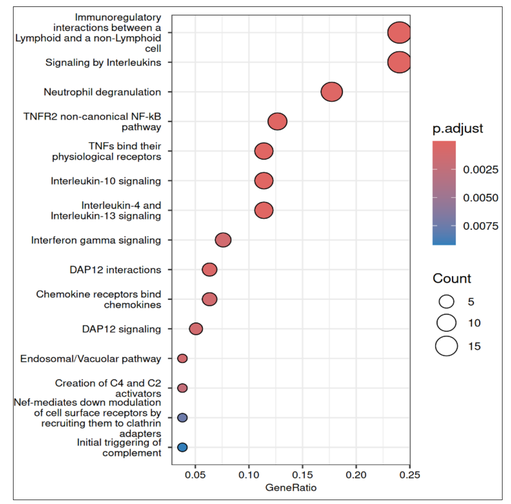


Figure 4: Reactome pathway analysis plot of the 97 evolving proteins showing significantly enriched CSF proteins over 12 months. Interaction between lymphoid and non-lymphoid cells as well as interleukin signaling, and neutrophil degranulation were the top ranked pathways. These are pathways signal inflammation.

CONCLUSIONS

- Our study show an immediate alteration of the CSF proteome in untreated men with PHI relative to matched uninfected controls. We also observed a temporal shift in the CSF proteins during early HIV infection, and pathways linked to inflammation were the most highlighted by these proteins. This suggests that immune dysregulation occur very early in HIV infection and also persists in the absence of treatment.
- Taken together, our study highlights the need for strategies to improve early HIV diagnosis and treatment, thereby preventing progressive CNS inflammation that could impair cognitive function in the future.

REFERENCES

1. Edén A, Rydberg F, Yilmaz A, Hagberg L, Gostner J, Nilsson S, et al. Residual Central Nervous System Immune Activation Is Not Prevented by Antiretroviral Therapy Initiated During Early Chronic HIV Infection. Open Forum Infectious Diseases. 2023;10(2).

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