T-cell Exhaustion and Senescence in PWH With and Without Progressive Multifocal Leukoencephalopathy

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BACKGROUND

- Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease caused by the reactivation of the JC virus (JCV), which often has devastating consequences, even among people with HIV (PWH) who are profoundly immunosuppressed.
- As no effective treatment is currently available, identifying predictors of therapeutic response is crucial.

In this study, we analysed CD4 and CD8 T cell profiles associated with JCV-specific responses in people with HIV (PWH) with and without PML, in order to explore potential predictive markers.

METHODS

- We enrolled 13 PWH with PML and 13 without PML [8 with HIV-related neurological involvement (NI-HIV) and 5 without neurological complications (nNI-HIV)], admitted to two major clinical centers in Rome between 2022 and 2025.
- Clinical and laboratory data were collected, including analysis of PD-1, CD57, CD27/CD28, and T-cell differentiation markers by flow cytometry in whole blood (WB) and cerebrospinal fluid (CSF).
- HIV-1 RNA and JCV DNA were quantified in CSF/plasma pairs with the Aptima® HIV-1 Quant Dx assay (Hologic) with a LoQ of 30 cps/ml and by an in house Real-Time PCR method on LightCycler (Roche), targeting VP1, with LoD of 150 cps/ml, respectively.
- JCV-specific T-cell responses were assessed at baseline via IFN-γ production using the ELISpot assay.

RESULTS-1

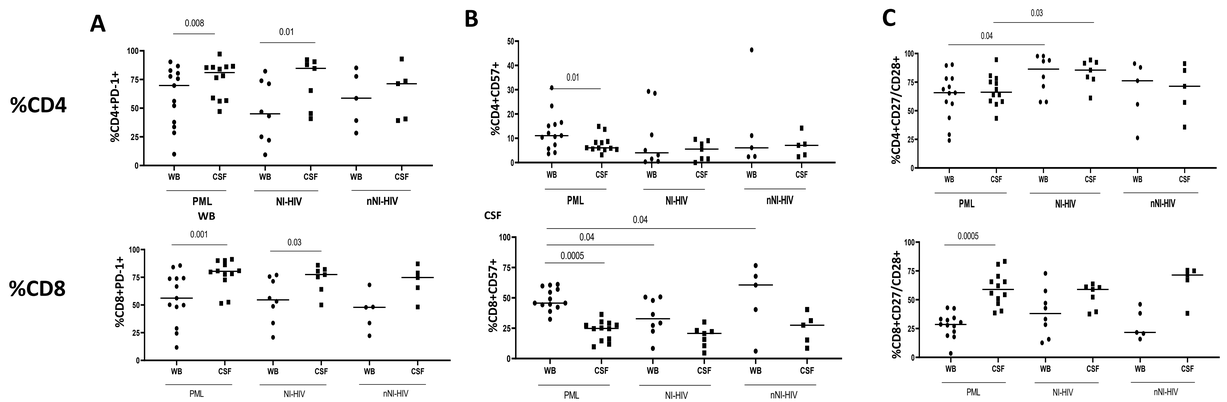
Table 1. General characteristics of PWH

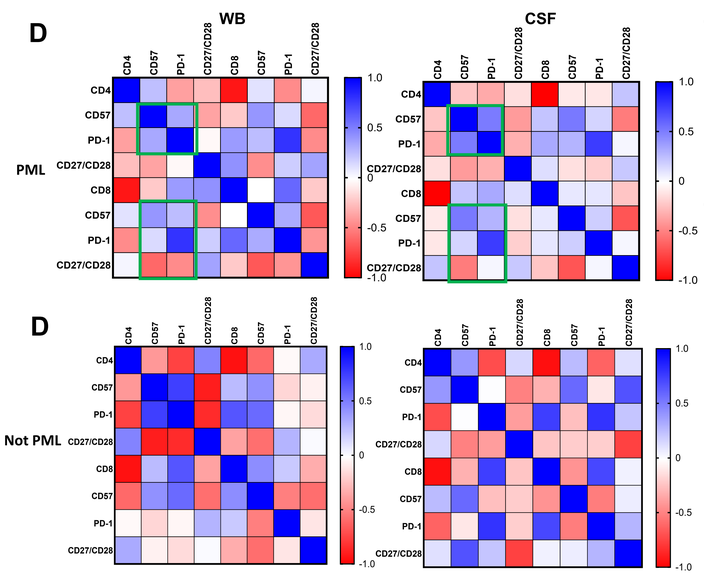
	Overall population (N=26)	PWH with PML (N=13)	PWH without PML (N=13)
Clinical characteristics at LP			
Male gender, n (%)	21 (77)	9 (69)	10 (76)
Age, median, years (IQR)	54 (27-68)	55 (35-68)	51 (27-66)
Mode of HIV transmission, n (%)			
Heterosexual	10 (38.4)	5 (38.5)	5 (38.5)
MSM	12 (46.2)	5 (38.5)	7 (53.8)
IVDU	4 (15.4)	3 (23)	1 (7.7)
Stage C, n (%)	24 (92.3)	13 (100)	11 (84.6)
Neurological symptoms, n (%)	21 (80)	13 (100)	8 (61.5)
Naive, n (%)	4 (15.3)	---	4 (30.7)
On ART, n (%)	15 (57.7)	10 (77)	5 (38.5)
Viro-immunological characteristics at LP			
CD4 count, cell/mmc, median (IQR)	184 (84-992)	99 (8-992)	250 (5-950)
CD8 count, cell/mmc, median (IQR)	686 (429-1,680)	667 (43-1,571)	866 (224-1680)
Plasma JCV DNA, cp/ml, median (IQR)	Ril <100	Ril <100	Not detectable
CSF JCV DNA, cp/ml, median (IQR)	5,060 (179-9,905)	5,060 (179-9905)	Not detectable
Plasma HIV RNA, cp/ml, median (IQR)	44,189 (439-332,951)	Ril<30	54,695 (7,231-332,951)
CSF HIV RNA, cp/ml, median (IQR)	1,306 (77-11,000,000)	4,178 (77-8,278)	1,306 (406-11,000,000)

- Distinct immunological patterns may characterize circulating and CSF CD4⁺ and CD8⁺ T cells in PWH with versus without PML.
- In our cohort, PML was associated with increased T-cell exhaustion and senescence, particularly in the CSF compartment.
- These findings suggest compartment-specific immune dysregulation and identify potential biomarkers of therapeutic response in PWH with PML.

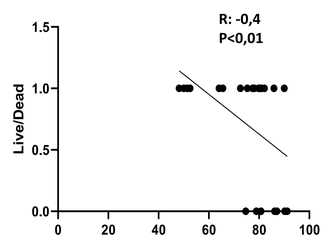
RESULTS-2

- Circulating and CSF CD4 and CD8 T cells from PML subjects showed an effector phenotype, with higher expression of CD57 (p=0.01), PD-1 (p=0.008), and CD27/CD28 (p=0.005) compared to NI-HIV and nNI-HIV (Fig.1, A-C).

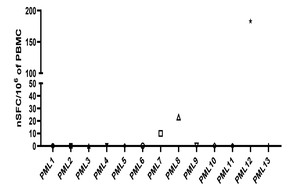




- Heatmap analysis revealed significant differences in exhaustion/senescence markers in WB (CD4/CD57 p<0.01; CD4/PD-1 p<0.002; CD8/PD-1 p<0.03; CD8/CD57/CD27 p<0.01) and CSF (CD4/PD-1 p<0.006; CD8/PD-1 p<0.006; CD8/CD57/CD27 p<0.02) in PML (Fig.1, D-E).
- Across the overall population, higher frequencies of CSF CD8⁺PD-1⁺ cells were associated with lower survival (Fig.1, F), suggesting their potential prognostic value as markers of immune exhaustion.



- Low IFN-γ production was observed in all PML subjects at baseline, though no significant associations were found with any of the analyzed markers.



DISCUSSION

- PML PWH show a marked effector T-cell phenotype in both blood and CSF, characterized by increased expression of exhaustion and senescence markers (PD-1, CD57, CD27/CD28 alterations), consistent with chronic antigenic stimulation and progressive functional impairment. This immune dysregulation appears compartmentally mirrored in the CNS.
- Notably, higher frequencies of CSF CD8⁺PD-1⁺ T cells are associated with reduced survival, suggesting a clinically relevant role of CNS immune exhaustion. Despite uniformly low IFN-γ production at baseline, no direct correlation with exhaustion markers was observed, indicating a broader functional impairment.

CONCLUSIONS

- This study highlights distinct immunological profiles of circulating and CSF CD4 and CD8 T cells in PWH with and without PML, associated with T cell exhaustion and senescence.
- T-cell exhaustion in both peripheral blood and CSF represents a key immunopathogenic feature of PML. Increased CSF CD8⁺PD-1⁺ T cells may serve as a potential prognostic marker, supporting the relevance of CNS immune dysfunction in disease outcome.
- Further studies are warranted to investigate the role of additional inhibitory markers as potential predictors of JCV response.

ADDITIONAL KEY INFORMATION

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PLAIN LANGUAGE SUMMARY

People with PML show signs that their immune cells are overworked and exhausted, particularly in the cerebrospinal fluid, which mirror the situation in the brain. Higher levels of these cells indicate that immune fatigue in the brain could affect disease outcomes.