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Daily Immunological/Virological Variations in Aviremic ART-treated HIV Participants

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BACKGROUND

Biological functions fluctuate in a circadian manner to align with environmental changes. In healthy uninfected individuals, variations in T-cell trafficking are documented in the blood, with nadir CD4 counts in the morning. Daily variations are also observed for plasma cortisol and melatonin, two regulators of immune functions. HIV infection is associated with profound alterations in CD4 T-cell homeostasis and chronic immune activation. HIV transcription is regulated by BMAL1, a circadian clock master regulator. However, daily variations in immunological/virological parameters during ART-treated HIV infection remain unknown.

METHODS

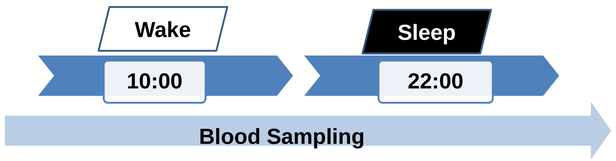
ART-treated people living with HIV (PLWH; median CD4 counts: 606 cells/ml; age: 57 years; time since infection: 242 months; aviremia under ART: 216 months) were hospitalized at the CRCHUM Phase I Clinic a Friday afternoon for 40 hours. Starting the next morning, blood was collected/processed every 4 hours for 24 hours before food intake. Polychromatic flow cytometry allowed cell counting/phenotypic analysis on fresh blood. Plasma levels of cortisol/melatonin and markers of mucosal barrier impairment (FABP2, LBP) were measured by ELISA. PBMC were frozen. HIV DNA/RNA were quantified by PCR on sorted CD4+ T-cells.

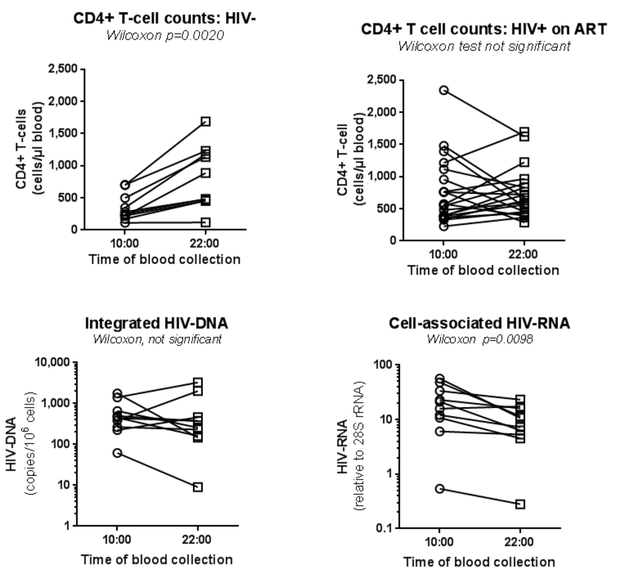
RESULTS

Study cohort I clinical parameters

Group	Sex (M/F)	Age (years)[a]	CD4 T (cells/µl) [a]	CD8 T (cells/µl) [a]	CD4/CD8 ratio[a]	Plasma viral load	Time since infection (months)	Time since ART initiation (months)
HIV- (n=10)	6/4	50 [36-60]	556 [418-790.5]	247.5 [184.8-343.8]	2.17 [1.34-3.32]	---	---	---
HIV+ on ART (n=20)	20/0	53 [45-56]	621 [459-739]	582 [407-700] ****	0.71 [0.37-0.83] ****	<40 HIV-RNA copies/ml	156 [99-225]	126 [84-204]

Experiment protocol





TAKE HOME MESSAGE

Melatonin, cortisol, markers of microbial translocation, HIV-RNA and CD4 T-cell counts undergo daily variations in ART-treated PLWH and should be considered for clinical monitoring during HIV cure/remission interventions

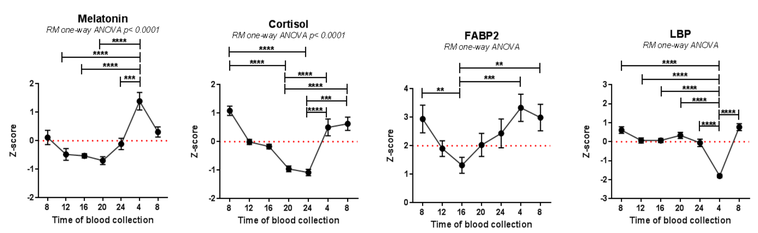
Study cohort II clinical parameters

Participant ID	Sex	Age (years)	CD3 T (cells/µl)	CD4 T (cells/µl)	CD8 T (cells/µl)	CD4/CD8 ratio	Plasma viral load	Time since infection (months)	Time since ART initiation (months)	ART Regimen
CLOCK #1	Male	60	1,839	602	1,321	0.5	<40 HIV-RNA copies/ml	228	108	Truvada/Isentress
CLOCK #2	Male	52	1,114	491	613	0.8	<40 HIV-RNA copies/ml	242	240	Valacyclovir/Raltegravir Effexor/Telmisartan/ Acebutol/Truvada
CLOCK #3	Male	57	1,481	606	855	0.7	<40 HIV-RNA copies/ml	212	132	Temazepam/Truvada, Valtrex/Isentress
CLOCK #4	Male	57	1,793	846	901	0.9	<40 HIV-RNA copies/ml	257	252	Ventolin/D/C/F/TAF
CLOCK #5	Male	57	1,421	410	924	0.4	<40 HIV-RNA copies/ml	393	360	Genvoya/ Prezista
CLOCK #6	Male	63	1,237	867	553	1.2	<40 HIV-RNA copies/ml	171	168	Triumeq
CLOCK #7	Male	50	880	379	498	0.8	<40 HIV-RNA copies/ml	260	216	Tivicay/ Apo-emtricitabine-Teno
CLOCK #8	Male	58	649	311	331	0.9	<40 HIV-RNA copies/ml	272	270	Genvoya
CLOCK #9	Male	57	1,439	800	597	1.3	<40 HIV-RNA copies/ml	381	335	Edurant/Tivicay/Descovy
CLOCK #10	Male	58	1,228	675	494	1.4	<40 HIV-RNA copies/ml	157	155	Triumeq plus
CLOCK #11	Male	54	2,478	1,082	1,425	0.8	<40 HIV-RNA copies/ml	192	188	TEVA ABACAVIR/ Lamivudine/ Prezista/Norvir

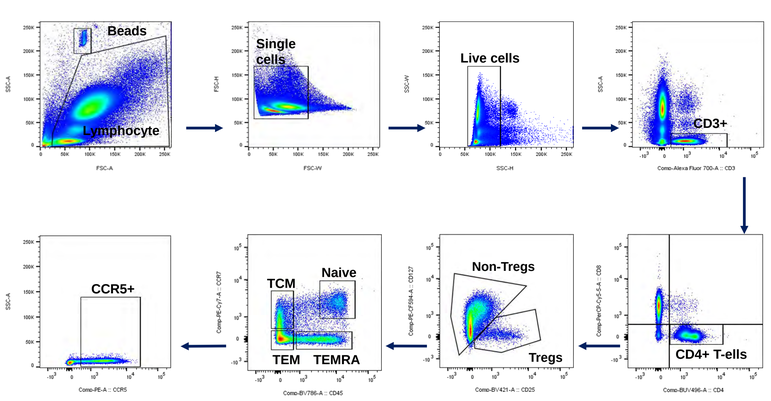
Experiment protocol

1. Plasma soluble factor quantification
2. PBMC immunological/virological measurements

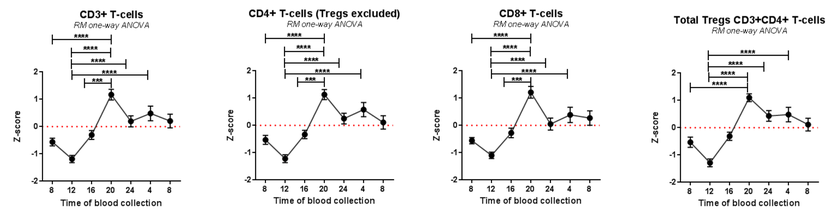
Circadian Variations in Plasma Markers



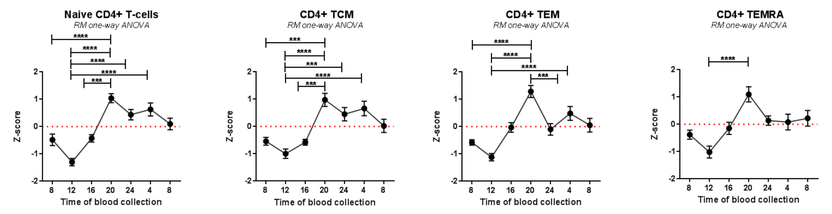
Flow Cytometry Analysis - *gating strategy*



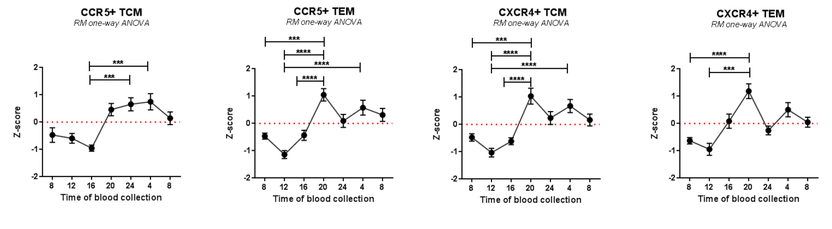
Daily Variations in T-Cell Counts



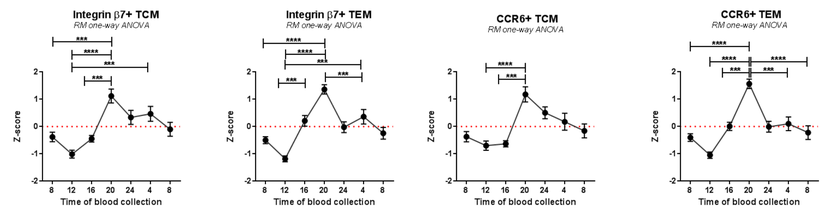
Daily Variation of Circulating CD4+ T-Cell Subsets



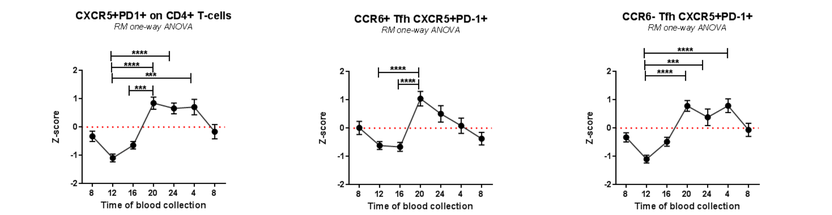
Daily Variations in HIV Co-Receptor Expression



Daily Variations in Gut-Homing Marker Expression



Daily Variations in Tfh Marker Expression



CONCLUSIONS

Daily variations in the blood T-cell/myeloid compartments, mucosal permeability markers, HIV transcription, and melatonin/cortisol levels, were observed in a cohort of aviremic ART-treated PLWH. These findings provide a rationale for studying the role of the circadian clock machinery in regulating residual HIV transcription under ART.

Acknowledgement

The authors thank Dr. Dominique Gauchat for the expert technical support in FACS analysis (the CHUM-Research Centre FACS Core Facility), Mario Legault (HIV/AIDS and Infectious Diseases Network of the Fonds de Recherche Québec-Santé (FRQ-S)) for their help with ethical approvals and informed consents. We thank Josee Girouard, Angie Massicotte and Maria Fraraccio (McGill University Health Centre, Glen) and Lucie Fuzeau (CRCHUM Phase I Clinical Research Unit) for their involvement in patient recruitment, follow-up during hospitalization and blood collection. We finally acknowledge the major contribution to this work of all human donors *via* their gift of blood samples. This study was funded by the Canadian Institutes of Health Research (CIHR) Project Grant #PJT-153052 to PA. This study was also supported by funding from FRQ-S HIV/AIDS and Infectious Diseases Network, Québec, Canada and The Canadian HIV Cure Enterprise Team Grant (CanCURE 1.0) funded by the CIHR in partnership with CANFAR and IAS (CanCURE 1.0; # HIG-133050 to PA and JPR).