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HIV-1 infection disrupts circadian patterns of extracellular vesicle abundance and microRNA contents

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Introduction

- During last decade, extracellular vesicles (EVs) have gained importance as novel vehicle of cell to cell communicators.
- EVs and their microRNA contents are involved in modulating immune responses and consequently the pathogenesis of HIV infection.
- In plasma, EV abundance reflects immune activation states associated with infection, making them and their contents biomarkers of disease progression.
- However there is a current lack of information on the physiologically shedding of EVs and their miRNA content in health condition.
- Here, we investigated day and night variation of plasma EVs abundance and microRNA contents in a cohort of 10 HIV uninfected control and 10 HIV infected receiving viral suppressive antiretroviral therapy (ART) venous blood sampled at 10AM and 10PM on the same day.

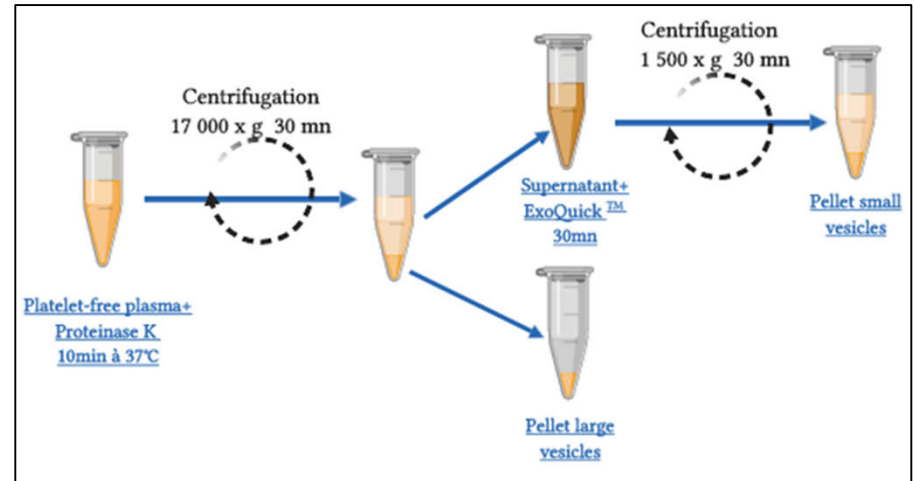
Methods

1- Patients characteristics

Characteristics	HIV infected (n=10)	HIV uninfected (Control) (n=10)	p -value
Years with HIV	16.10 ± 6.03	NA	NA
Years on HAART	11.80 ± 7.38	NA	NA
Age (years)	52.80 ± 4.78	50.00 ± 11.77	0.9685
CD4 T cell (/μL)	578.90 ± 155.80	601.40 ± 199.16	0.9705
CD8 T cell (/μL)	788.78 ± 214.06	288.33 ± 150.76	0.0003
CD4/CD8 ratio	0.74 ± 0.24	2.47 ± 1.28	<0.0001

Data are presented as the means ± standard deviation. NA not applicable

2- Purification of two EVs subpopulation from plasma



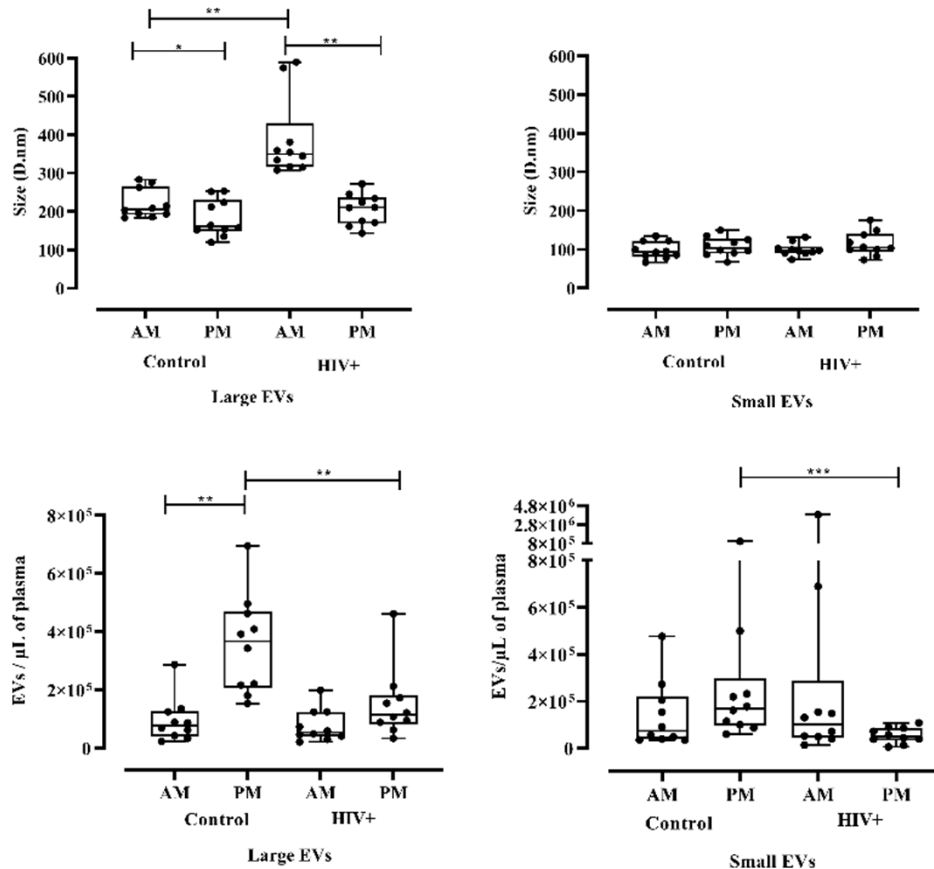
3- Characterization of purified EVs

- Vesicles hydrodynamic size was estimated with dynamic light scattering
- EVs abundance was assessed by flow cytometry upon staining with DiD and CFSE
- Total RNA was extracted from EVs and qRT-PCR was used to quantify a panel of 5 miRNAs including miR-29a et b, miR-92, miR-155 and miR-223

4-Statistical Analysis

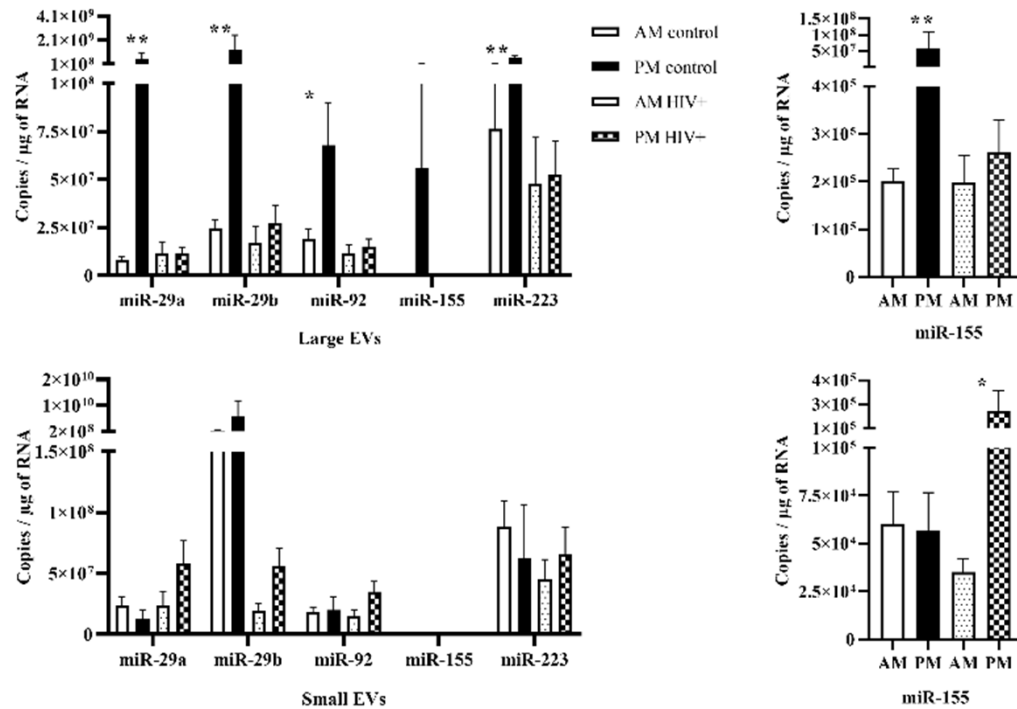
Wilcoxon matched-pairs signed rank test and Kruskal-Wallis test were used for comparison

Results1: EVs size and abundance



- Plasma large extracellular vesicles are more larger at AM in control and HIV infected
- Days *versus* night variation of large EVs abundance in control was disrupted in HIV infected patients
- Large and small EVs were more abundant in control patients at PM

Results 2: Diurnal variation of EVs microRNA content



- The amounts of all 5 microRNAs were increased significantly in control large EVs at night *versus* morning collected samples and this trend was lost in HIV infected.
- In small EVs, only miR-155 level varied between AM and PM in HIV infected.

Conclusion: Our results indicate that EVs in plasma are dynamic structures, with their size, abundance and miRNA content varies depending on the time of day and this diurnal variation was disturb by HIV infection. These variations must be taken account in the validation of EVs and their content are biomarkers of HIV infection disease.

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