MASSIVE RELEASE OF PLATELET-DERIVED EXTRACELLULAR VESICLES DURING HIV INFECTION

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BACKGROUND:

Extracellular Vesicles (EVs) derived from different cell types by ectocytosis (microvesicles) or endocytosis (exosomes) may serve as intercellular messengers in pathogenic processes. Circulating mitochondrial DNAs (mtDNA) are potent danger-associated molecular patterns (DAMPs) found in inflammatory diseases including viral infections. Herein, we evaluate the EV profile and plasma mtDNA levels in a well-characterized cohort of HIV-infected patients and controls.

METHODS:

Plasma samples from HIV-infected patients from the HIV Biobank-Spanish HIV/AIDS Network and 2 hospitals in Galicia were selected. Five groups were defined: 1) ART-naïve; 2) ART-exposure with non-detectable viremia (ND) > 1 year; 3) elite controllers (EC) (<50 copies/mL without ART > 1 year); 4) viremic controllers-VC (HIV-RNA >50 and < 2000 copies/mL without ART for more than 1 year); and 5) a control group of HIV negatives. Plasma mtDNA was quantified using a quantitative real-time PCR assay (**Figure 1**). EVs (<1µm, CD9+) were quantified and characterized by flow cytometry using monoclonal antibodies targeting their source CD61/CD41 for platelets; CD16/CD11b/CD66b for neutrophils. MitoTrackerDeepRed identified EVs containing mitochondria (**Figure 2**).

Figure 1. Plasma sample processing for mtDNA isolation & quantification, and EV characterization.

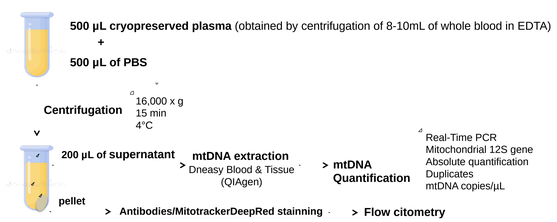
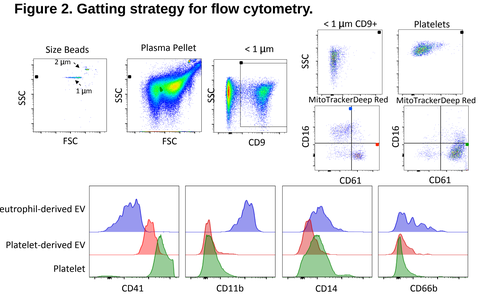


Figure 2. Gatting strategy for flow cytometry.



RESULTS:

A total of 120 HIV-infected patients (30 ART-naïve, 30 ART-exposed, 30 EC, and 30 VC) and 30 controls (HIV negative) were included (Table I).

Table I. Main characteristics of the study population.

	Control (N=30)	ART-naïve (N=30)	ART-exposed (N=30)	EC (N=30)	VC (N=30)	*p-value*
Age (years)	36.0 [29.0-41.0]	41.0 [32.0-52.0]	52.0 [43.0-56.0]	52.5 [43.5-57.2]	50.5 [38.5-54.2]	<0.001
Sex (male)	50.0 (15)	71.0 (22)	66.7 (20)	50.0 (15)	56.7 (17)	n.s
HIV diagnosis (year)	NA	2017 [2017-2018]	2004 [1997-2011]	1996 [1986-2004]	2003 [1990-2008]	<0.001
Days since HIV diagnosis	NA	7 [0-64]	4803 [2316-7373]	5246 [2048-8249]	2347 [787-7421]	<0.001
HIV viral load (copies/mL)	NA	27450 [9700-110000]	ND	50 [35-50]	361 [85-788]	<0.001
Anti-HCV+	0 (0)	12.9 (4)	23.3 (7)	62.1 (18)	36.7 (11)	<0.001
CD4 (cells/µL)	842 [712-1018]	456 [267-657]	827 [609-1071]	830 [519-1077]	670 [540-1100]	<0.001
CD8 (cells/µL)	474 [404-653]	983 [730-1284]	824 [512-933]	855 [637-1133]	925 [718-1177]	<0.001
CD4/CD8 ratio	1.6 [1.4-2.1]	0.4 [0.2-0.7]	1 [0.8-1.3]	0.9 [0.6-1]	0.8 [0.7-1]	<0.001

Data are expressed as median [interquartile range], %(n). NA: not applicable; ND: not detectable; n.s: not significant. Kruskal-Wallis H and Chi-squared tests were used for comparisons between groups.

FUNDING:

Fondo de Investigación Sanitaria (FI14/00557, PI16/02159, MV16/00054); Xunta Galicia-Fondo Social Europeo (IN606A-2016/023); Red Española de Investigación en SIDA (RD16/0025/0026); Fundación Biomédica Galicia Sur; HIV Biobank-Spanish HIV/AIDS Network; SEIMC. CWRU Center for AIDS Research

✓ Higher levels of plasma mtDNA were observed among HIV-infected patients, specially for the EC and VC groups (**Figure 3**).

Figure 3. Plasma mtDNA levels among study groups.

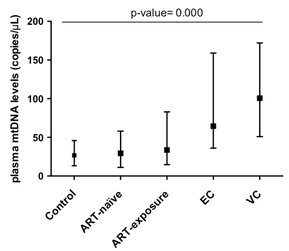
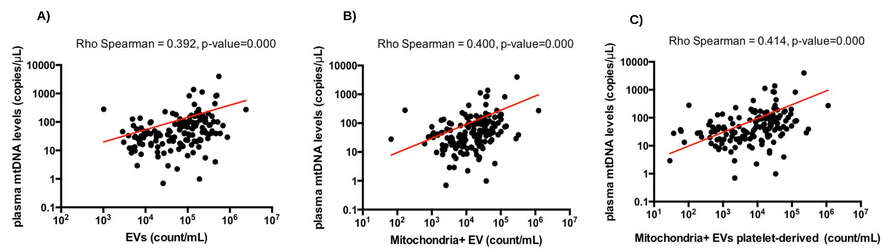


Figure 4. Correlation between plasma mtDNA levels: A) EV count; B) EV mito+ count; C) EV platelet-derivedMito+ count.



✓ EVs and EV Mito+ counts showed a correlation with circulating plasma mtDNA levels (Figures **4A** & **4B**).
✓ A positive moderate correlation was found between the number of platelet-derived mitochondria+ EVs (CD61/CD41+, MitotrackerDeepRed+) and plasma mtDNA levels (rho=0.414; p<0.001) (**Figure 4C**) but not for neutrophil-derived mitochondria+ EVs (CD16/CD11b+, MitotrackerDeepRed+) (**Table II**).

✓ Higher EV (< 1µm, CD9+) and EV Mito+ (< 1µm, CD9+, MitotrackerDeepRed+) counts were associated with HIV-infection regardless of HIV status.
✓ A different profile of EVs according to cellular origin was shown between HIV-infected patients and controls: HIV patients had more EVs with platelet markers (CD61/CD41+) (80% vs 40%, respectively) and lower proportions of EVs with neutrophil markers (CD16/CD11b+) (2% vs 7%, respectively).

Table II. EV profile and mitochondial MFI among study population.

	Control (N=30)	ART-naïve (N=30)	ART-exposure (N=30)	EC (N=30)	VC (N=30)	p-value
Total EVs (count/mL)	6982 [5185-8971]	68468 [27299-145591]	116385 [66713-220466]	120009 [29266-377811]	139602 [81729-259127]	<0.001
Total EVs mito+ (count/mL)	2661 [1893-4196]	20432 [7687-34460]	19710 [8262-30118]	26451 [7650-51141]	29783 [12165-45518]	<0.001
EVs platelet-derived (%)	40.6 [25.6-51.1]	84.8 [71.9-89.9]	91.8 [85.5-94.5]	79.7 [64.7-91.4]	81.9 [72.0-89.6]	<0.001
EVs neutrophil-derived (%)	6.2 [2.7-24.2]	1.1 [0.3-2.4]	0.1 [0.1-0.5]	1.04 [0.3-6.6]	1.88 [0.3-4.5]	<0.001
EVs platelet-derivedMito+ (%)	29.4 [19.3-37.2]	22.1 [16.6-25.1]	13.4 [11.1-21.7]	20.9 [10.6-36.7]	21.3 [10.8-29.6]	<0.001
EVs neutrophil-derivedMito+ (%)	88.9 [72.5-93.1]	88.6 [57.9-98.1]	48.2 [34.8-81.7]	65.9 [31.4-84.3]	64.1 [30.1-85.9]	<0.001
Mitochondrial MFI	1346 [1101-1799]	720 [691-1260]	628 [584-754]	764 [567-1038]	711 [632-796]	<0.001

Data are expressed as median [interquartile range].

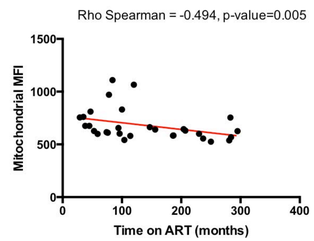
✓ Within the ART-exposed population, lower proportions of platelet and neutrophil-derived EVs that were Mito+ and lower MFIs were observed Moreover, a negative correlation between mitochondrial MFI and the time under ART (median time of exposure to ART 109 months [IQR: 70-215]) was found (**Figure 5**).
✓ A lower proportion of Mito+ platelet-derived EVs was found for patients receiving Abacavir (3TC+ABC) than for those receiving Tenofovir or tenofovir alafenamide (TDF/TAF+ETC/FTC) **(Table III)**.

Table III. NRTI backbone and Mito+ EV populations plasma mtDNA and mitochondiral MFI in ART-exposed patients.

	EVs platelet-derivedMito+ (%) *p=0.032*	EVs neutrophil-derivedMito+ (%)	Mitochondrial MFI
3TC + ABC (N=9)	12.8 [8.1-18.5]	48.5 [35.7-81.7]	644 [599-676]
TDF/TAF + 3TC/FTC or without NRTI (N=21)	18.5 [12.7-24.7]	48.0 [33.3-72.7]	630 [602-754]

Data are expressed as median [interquartile range]. 3TC: Lamivudine; ABC: Abacavir; TDF: Tenofovir fumarate; TAF: Tenofovir alafenamida; FTC: Emtricitabine; NRTI: Nucleoside reverse transcriptase inhibitor.

Figure 5. Correlation between mitochondrial MFI and time on ART.



CONCLUSIONS:

A massive release of platelet-derived EVs occurs during HIV infection regardless of HIV status. EV count correlates with plasma mtDNA levels. HIV infection and ART both appear to diminish mitochondrial density in EVs yet as EV numbers are expanded, total mitochondrial levels in plasma are preserved in HIV infection or increased. The mechanisms underlying these perturbations in EV levels and the mitochondria within them in HIV infection are not known.