

1 **Laboratory surrogate markers of residual HIV replication among distinct groups of**
2 **individuals under antiretroviral therapy**

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7 **Running Title:** Residual HIV replication and ART

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15 **Abstract**

16 **Background:** Residual HIV-1 replication among individuals under antiretroviral therapy
17 (ART) relates to HIV micro-inflammation.

18 **Objectives:** To determine levels of residual HIV replication markers among distinct
19 subgroups of antiretroviral-treated individuals.

20 **Methods:** 116 patients were distributed into 5 treatment groups: first-line suppressive ART
21 with non-nucleoside reverse-transcriptase inhibitor (NNRTI) (n=26), first-line suppressive
22 ART with boosted protease inhibitors (PI-r) (n=25), salvage therapy using PI-r (n=27),
23 salvage therapy with PI-r and raltegravir (n=22) and virologic failure (n=16). Episomal and
24 total DNA quantitation was evaluated. ELISA was used for HIV antibody and LPS
25 quantitation.

26 **Results:** Episomal DNA was positive in 26% to 38% of individuals under suppressive ART,
27 being higher among individuals experiencing virologic failure (p=0.04). HIV proviral load
28 was higher among patients with detectable episomal DNA (p=0.01). Individuals receiving
29 initial PI-r treatment presented lower HIV antibodies (p=0.027) and LPS (p=0.029) than
30 individuals receiving NNRTI. There was a negative correlation between episomal DNA
31 quantitation and suppressive ART duration (p=0.04), CD4+ T-cell count (p=0.08), and CD8+
32 T-cell count (p=0.07).

33 **Conclusions:** Residual HIV replication has been inferred among individuals under
34 suppressive ART according to episomal DNA detection. Residual replication may decrease
35 with longer periods of suppressive ART and higher levels of CD4+ and CD8+ T cells. The
36 relationship between episomal DNA and total DNA suggests a replenishment of the proviral
37 reservoir with impacts on HIV persistence. Lower antibody and LPS levels among patients
38 with initial PI-r ART suggest these regimens may more effectively suppress HIV with higher
39 capacity to decrease the HIV antigenic component.

40 **Introduction**

41 The deleterious effects of HIV are directly related to viral replication, which leads to
42 inflammatory processes, such as the activation of CD4+ and CD8+ T lymphocytes (1).
43 Maintaining viral replication at lower levels is critical for the reduction of cellular activation
44 and co-morbidities related to HIV-1 infection. However, the antiretroviral therapy (ART)
45 currently used does not completely suppress viral replication. Up to 80% of patients with
46 undetectable viral loads according to commercial tests show an average of 3.1 copies/mL of
47 residual viral load when ultrasensitive tests are used (2, 3). Although the stability of episomal
48 DNA is not completely understood, extrachromosomal DNA is useful as a surrogate marker
49 of HIV-1 replication when the HIV viral load is not detectable by currently available methods
50 (4). Other markers that relate to HIV-1 replication among individuals under ART include
51 proviral HIV DNA (5) and the quantitation of HIV antibody levels (6) or markers that relate
52 to bacterial translocation (7). ART regimens differ in potency as well as in the distinct genetic
53 barriers they create or effects they have in each step of the HIV replication cycle to alter viral
54 dynamics. For this reason, the evaluation of circular HIV DNA could be used as a tool to
55 indirectly compare the effectiveness of these distinct regimens on residual HIV replication.
56 Therefore, this study aimed to analyze surrogate markers of the residual replication rates of
57 HIV-1 among individuals receiving different antiretroviral regimens. We hypothesize that
58 drugs from different classes and previous ART virologic failure will affect surrogate markers
59 of HIV residual replication.

60 **Methods**

61 **Patients**

62 Patients were chosen between 2011 to 2013 in São Paulo Brazil according to their
63 current antiretroviral treatment (see Supplementary Table 1). Individuals were under ART
64 with undetectable plasma viral loads for at least one year, except for the virologic failure

65 group. This study was approved by the Ethics Committee in Research at the Federal
66 University of São Paulo (approval #0201/11), and informed consent was obtained from all
67 patients.

68 One hundred sixteen patients were allocated to five treatment subgroups as follows:
69 (1) patients treated with two nucleoside-analogue reverse-transcriptase inhibitors (NRTI)
70 associated with the non-nucleoside analog reverse-transcriptase inhibitors (NNRTI) Efavirenz
71 or Nevirapine as the first ART regimen; (2) patients treated with two NRTIs and a protease
72 inhibitor boosted with ritonavir (PI-r) as the first ART regimen; (3) patients on salvage
73 therapy with two NRTIs and a PI-r; (4) patients under salvage therapy containing two NRTIs,
74 PI-r and the integrase inhibitor raltegravir; and (5) patients under antiretroviral virologic
75 failure with the confirmed presence of HIV ART resistant strains. Peripheral blood samples
76 were collected, and clinical data on the patients were analyzed, including CD4+ and CD8+ T-
77 cell counts, the duration of treatment with undetectable viral loads and the number of ART
78 schemes previously used by the patient.

79 **HIV-1 Episomal DNA Detection and Quantitation**

80 To obtain HIV episomal DNA, 400 μ L of peripheral blood mononuclear cells
81 (PBMC) isolated using density gradient centrifugation were extracted using a QIAprep Spin
82 Miniprep commercial kit (Qiagen, Valencia, California, USA). After extraction, qPCR
83 amplification was performed in a single round of 45 qPCR cycles to amplify
84 extrachromosomal DNA as previously described (8, 9). The qPCR quantitation values were
85 normalized based on cell numbers estimated by CCR5 quantitation and are expressed as the
86 number of DNA copies per 10^6 PBMC.

87 **Total HIV DNA Quantitation**

88 Total viral DNA was extracted from 50 μ L of PBMC using a Blood QIAamp DNA
89 Mini Kit Mini Kit (Qiagen, Valencia, California, USA) according to the manufacturer's

90 instructions. Total HIV DNA was qPCR amplified using a mix containing 1x TaqMan
91 Universal PCR Master Mix (Applied Biosystems), 0.4 μ M primers/probe(10) (F522-43
92 GCCTCAATAAAGCTTGCCTTGA, R626-43 GGGCGCCACTGCTAGAGA and Probe
93 CCAGAGTCACACAACAGACGGGCACA) and 5 μ l of extracted DNA. CCR5 was also
94 used to quantify genomes to express the measurements as copies per 10^6 PBMC.

95 **Quantitation of anti-HIV-1 Antibodies**

96 HIV-1 specific antibodies were measured using the capture enzyme immunoassay kit
97 Aware BED Incident HIV-1 EIA Test (Calypte Biomedical Corporation, Portland, Oregon,
98 USA) according to the manufacturer's instructions. The optical density values of the samples
99 were normalized based on the controls (negative, calibrator, lower positive and higher
100 positive) using the spreadsheet available at http://www.calypte.com/aware_BED.html.
101 Specimens with an ODn > 0.8 are considered positive.

102 **Levels of LPS in Plasma**

103 The quantitation of endotoxin was performed using a Limulus Amebocyte Lysate
104 (LAL) QCL-1000 (Lonza, Walkersville, MD) kit according to the manufacturer's
105 instructions. The absorbance was determined spectrophotometrically at 405–410 nm. Since
106 this absorbance is in direct proportion to the amount of endotoxin present, the concentration
107 of endotoxin was calculated from a standard curve. The background color of the sample was
108 subtracted.

109 **Statistical analysis**

110 The Statistical Program for the Social Sciences, version 18.0 (SPSS 18.0) was used
111 for data analysis. Descriptive analyses, ANOVA using z-score normalized data, and chi-
112 squared tests, at a confidence level of 5%, were performed.

113 **Results**

114 **Episomal DNA**

115 The general patient data including age, gender, CD4+ and CD8+ T-lymphocyte
116 counts, treatment time, number of regimens and number of medications used were compiled
117 and are shown in Tables 1 and 2, grouped according to the type of ART received.

118 2-LTR circles were detected in 39 (34%) of the patients in the study. Table 3
119 summarizes the measurements obtained according to treatment group. The treatment group
120 had no effect on the quantitation of episomal HIV DNA ($F(115,4) = 1.263$, $p = 0.289$). The
121 prevalence of detectable 2-LTR ($n=39$) was not different between the groups ($F(38,4)=1.014$,
122 $p=0.414$).

123 There was no difference in the quantitation of 2-LTR circles among groups with first
124 treatment ($F(49,1) = 1.429$, $p = 0.23$, Figure 1A). Additionally, there was no difference (F
125 $(47,1) = 1.692$, $p = 0.20$) when comparing the 2 distinct salvage therapy groups. We also
126 observed no difference between the two groups receiving PI-r ($F(50,1)=0.197$, $p=0.65$).
127 Furthermore, there was no significant difference between the first treatment groups together
128 and the salvage groups together, ($F(98,1) = 1.229$, $p = 0.27$) nor when comparing the groups
129 with virologic success to that of virologic failure ($F(114,1) = 0.601$, $p = 0.44$).

130 We then transformed episomal DNA quantitation into a categorical variable for
131 detection and named samples LTR positive when detection was possible and LTR negative
132 when there was no detection. Based on this categorization, we performed a chi-square test.
133 The results showed no statistically significant association between the received treatment and
134 the detection of circular DNA ($\chi^2(3) = 5.412$, $p = 0.248$). Comparing the number of positive
135 episomal DNA samples between the subjects with virologic suppression and individuals
136 experiencing virologic failure, there was an increase in the number of episomal DNA-positive

137 samples in the failure group ($\chi^2 (3) = 4.259, p = 0.039$, Figure 2A). In addition, the mean of
138 total DNA was higher among individuals with positive episomal DNA (ANOVA, $F (109,1) =$
139 $2.794, p = 0.09$; Figure 2B).

140 **Total HIV DNA**

141 Total HIV DNA was detected in 111 (96%) of the patients included in the study
142 (Table 3). An ANOVA tests showed no differences between the treatment groups for
143 quantitation of total HIV DNA ($F (115,4) = 2.015, p = 0.098$; Table 3 and Figure 1B).
144 Additionally, there was no difference in total HIV DNA quantitation between the groups with
145 a first-line regimen (Groups 1 and 2, $F(47,1) = 0.010, p = 0.922$), nor between the two groups
146 on salvage therapy (Groups 3 and 4, $F (44,1) = 1.230, p = 0.273$), nor between the groups on
147 a first-line regimen and salvage therapy ($F (93,1) = 0.007, p = 0.935$). Finally, there was a
148 difference between the groups with virologic success and virologic failure ($F (109,1) = 7.528,$
149 $p = 0.007$) in which virological failure group shows higher total HIV DNA mean.

150 There was no statistical significance between total HIV DNA and the other tested
151 variables.

152 **Quantitation of anti-HIV-1 antibodies**

153 In this test, we considered samples with normalized optical densities (ODn) higher or
154 equal to 0.8 as positive. Table 3 summarizes the measurements obtained according to
155 treatment group. ANOVA showed no differences in the HIV antibody levels between the
156 groups ($F (115,4) = 1.675, p = 0.161$, Figure 1C). However, the antibody levels were higher
157 among patients given first treatment with NNRTI compared to first treatment with PI-r
158 (ANOVA; $F (49,1) = 5.189, p = 0.027$). There was no difference when comparing the two
159 types of salvage therapy schemes ($F(47,1)=0.189, p = 0.66$) nor between the first-line
160 treatment and salvage therapy groups ($F(98,1)=0.146, p = 0.70$). In addition, there was no

161 difference when comparing the groups with virologic successful and virologic failure
162 ($F(114,1)=1.289$, $p = 0.25$).

163 Considering ODn as a categorical variable in which positive samples had an ODn \geq
164 0.8, there was a decreased number of positive samples in the first treatment group using PI-r
165 ($\chi^2(1) = 9.600$, $p = 0.007$) compared to the first treatment group using NNRTI as well as an
166 increase in positivity when compared to salvage therapy with PI ($\chi^2(1) = 4.038$, $p = 0.044$)
167 (Figure 3). There was no significant difference between the first-line regimen groups and the
168 salvage therapy groups, ($\chi^2(1)=0.360$, $p=0.34$) nor any difference when comparing groups
169 with or without virologic failure ($\chi^2(1)=1.945$, $p=0.13$).

170 Positive antibody quantitation was not associated with the positivity of episomal
171 DNA ($\chi^2(1) = 1.889$, $p = 0.119$) or with the episomal DNA quantitation ($F(114,1)=0.112$,
172 $P=0.738$). Patients with positive antibody quantitation showed slightly higher HIV total DNA
173 ($F(109,1)=2.787$, $p=0.09$).

174 **LPS quantitation**

175 Due to the unavailability of samples, LPS quantitation was performed for only 55
176 patients (Table 3). An ANOVA test showed a significant difference between the first-line
177 regimen groups, with LPS higher among individuals treated with NNRTI compared to PI-r
178 ($F(55,4)=2.947$, $p= 0.029$, Figure 1D), as well as between the NNRTI and salvage therapy
179 groups and the PI-r group ($p=0.019$, Bonferroni Test).

180 **Correlations**

181 Spearman correlation tests were performed only with samples in which episomal
182 DNA was detected. There was a negative correlation between the quantitation of episomal
183 DNA and the CD8+ T-cell count ($\rho = -0.426$, $p = 0.007$) and the CD4+ T-cell count ($\rho = -$

184 0.276, $p = 0.08$), LPS quantitation in plasma ($\rho = -0.500$, $p = 0.041$) and treatment time with
185 an undetectable viral load ($\rho = -0.358$, $p = 0.044$).

186 Spearman correlation between total HIV DNA showed positive correlation with
187 episomal DNA quantitation ($\rho = 0.256$, $p = 0.007$), antibody levels ($\rho = 0.181$, $p = 0.05$) and
188 also a negative correlation with CD8⁺ T-cell count ($\rho = -0.243$, $p = 0.01$) and trend to
189 correlate with CD4⁺ T-cell count ($\rho = -0.16$, $p = 0.09$).

190 **Discussion**

191 As mentioned before, antiretroviral treatment is not fully suppressive in all
192 individuals, as shown by the detection of viremia in individuals evaluated with ultrasensitive
193 viral load assays (3) or with tests for cell-associated RNA (11). Interestingly, this residual
194 viremia may come from so-called sanctuaries, such as the gastrointestinal tract (12). As such,
195 they form an obstacle for achieving a sterilizing cure. Furthermore, specific HIV
196 inflammation inferred by the levels of T-cell lymphocyte activation persists among
197 antiretroviral treated individuals in spite of undetected viral loads (13). Efforts and strategies
198 to mitigate HIV-related inflammation is currently a major task. One effective way to
199 decrease this inflammation would be to maximize the antiretroviral suppressive effect, thus
200 reducing residual replication.

201 Furthermore, continuous suppressive therapy is able to decrease the number of latent
202 HIV infected cells over time (14), bringing the individual close to a sterilizing cure when the
203 right strategies become available. On the other hand, residual viremia is conceivably able to
204 replenish latent HIV reservoirs.

205 To learn more about residual HIV-1 replication among individuals under ART, we
206 used different surrogate markers of HIV replication. The presence and quantitation of
207 episomal HIV DNA has been considered one accurate marker to infer active HIV replication
208 and its entrance into the cell environment (9, 15). Total or integrated HIV DNA also

209 indicates the size the HIV infected cell pool. It is well known that early treatment initiation
210 affects the number of latently infected cells (16), and over time, cells will exit latency and
211 die, decreasing the proviral DNA pool in ART treated individuals. The levels of HIV-1
212 antibodies detected using less sensitive assays also relate to the levels of HIV-1 replication
213 (6). As HIV-1 residual replication may come from the gastrointestinal tract (12), it is also
214 conceivable that less effective antiretroviral treatment could be associated with higher levels
215 of bacterial translocation (7) and therefore increasing laboratory translocation markers such
216 as LPS or sCD14 levels.

217 We also wanted to investigate the relationship between different HIV ART schemes
218 or strategies. The main questions were: is initial treatment more suppressive when two
219 distinct steps of reverse transcription are inhibited, such as schemes using NRTIs with an
220 NNRTI, or is the inhibition of pre- and post-integration more effective, such as schemes
221 using NRTIs and boosted PI? A number of clinical trials comparing NNRTI with boosted PIs
222 as the second antiretroviral class show one advantage of NNRT, which relies mainly on
223 tolerance and adherence issues, since boosted PI schemes do not present antiretroviral
224 resistance upon failure (17). The other question is whether salvage therapy is associated with
225 more residual HIV replication than initial antiretroviral therapy. Usually, salvage therapy
226 relies on a boosted PI-based regimen with or without the use of a new antiretroviral class.
227 Therefore, a further question would be whether the association of a third antiretroviral class
228 would more suppressive than salvage therapy schemes containing 2 NRTIs and a boosted PI
229 only. We therefore performed a cross-sectional evaluation of a distinct group of individuals
230 under “suppressive” antiretroviral treatment with good treatment adherence using 2 NRTIs
231 and either efavirenz/nevirapine or PI-r as the first-line treatment. We also evaluated
232 individuals who previously experienced antiretroviral virologic failure and had their HIV
233 viremia subsequently suppressed with 2 NRTIs and a PI-r only or PI-r associated to

234 raltegravir. We also used as a “control group”, individuals experiencing virologic failure in
235 which antiretroviral resistance had been detected. We attempted to avoid individuals not
236 using or adhering to ART at the time of the study.

237 We were able to confirm the relationship between HIV-1 replication and the detection
238 of episomal DNA, which was higher among individuals experiencing virologic failure
239 compared to individuals with viral loads below detection, even with the smaller sample size
240 of the virologic failure group.

241 We also detected a negative correlation between episomal DNA quantitation and the
242 time of treatment with undetectable viral loads as well as a negative correlation between
243 episomal DNA and CD8+ T-cell counts. It is conceivable that lower CD8 levels enable HIV-
244 1 viral replication, as has been seen in animal models; the elimination of CD8+ T cells using
245 monoclonal antibodies was associated with the return of detectable viremia in SIV-infected
246 monkeys in spite of the use of suppressive ART (18). Likewise, we hypothesize that longer
247 durations of effective antiretroviral treatment will progressively strengthen the immune
248 system, by increasing the number of naïve CD4+ T cells and thus further decreasing residual
249 HIV-1 replication. This speculation is further supported by the observation of a negative
250 correlation between the levels of episomal DNA and CD4+ T-cell counts. However, we were
251 not able to explain the negative correlation between episomal DNA levels and LPS levels.

252 Interestingly, the levels of total HIV DNA were found to be higher among individuals
253 with evidence of residual HIV replication as inferred by the presence of episomal DNA. This
254 association suggests that the pool of infected cells is being replenished or maintained in
255 association with residual HIV replication.

256 We were not able to detect any differences between episomal or total DNA levels
257 between first-line regimens and successful salvage therapy regimens, nor between NNRTI
258 versus PI-r regimens or salvage therapy using two or three classes (NRTI + PI-r versus NRTI

259 + PI-r and raltegravir). However, the levels of antibodies were lower in first-line PI-r ART
260 compared to the NNRTI group as the number of negative antibody results were higher among
261 the initial PI-r treatment group. Furthermore, the levels of LPS were higher among the
262 NNRTI first-line treatment group compared to the first-line PI-r or other salvage therapy
263 groups that also have a PI-r in the treatment scheme. Notably, the proportion of patients
264 taking tenofovir, abacavir or zidovudine was similar in the PI-r and NNRTI groups (Table
265 S1). Although clinical trials have noted that NNRTI-based regimens are usually more durable
266 and effective than PI-r-based regimens despite a basal viral load and higher CD4+ T-cell
267 levels, these results are mainly due to better performance of intention to treat analyses, which
268 are influenced by tolerance and adherence issues. Importantly, 14 individuals in the PI-r
269 group were treated with boosted atazanavir, whereas 11 were treated with boosted lopinavir
270 [Table S1]. However, this study analyzed patients on stable ART without adherence or
271 tolerability issues. We can therefore hypothesize that the effective inhibition of two different
272 steps of the HIV replicative cycle is more effective than inhibiting only one step.

273 We recognize that the retrospective cross-sectional nature of this study may preclude
274 more definite conclusions. The evaluation of only one time point in this group prevents us
275 from understanding the dynamics of these surrogate markers for HIV replication.
276 Furthermore, other sensitive assays measuring residual HIV replication, such as cell-
277 associated RNA or inflammatory markers, have not been evaluated here.

278 However, we were able to clearly demonstrate that episomal DNA was present in 26%
279 to 38% of individuals with “successful” antiretroviral treatment, thus suggesting that residual
280 HIV replication is occurring despite the scheme analyzed here. We were also able to
281 demonstrate the association of PI-r schemes with lower antibody and LPS levels, which
282 deserves further confirmation to better understand the related mechanisms involved that can
283 explain these findings.

284

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289 **Competing interests**

290 The authors have no competing interests to declare.

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364

365 **Table 1: Demographic characteristics and mean T-cell counts between the different treatment groups.**

	N	Gender		Age (years)			CD4+ T-cell count (cells/mm ³)				CD8+ T-cell count (cells/mm ³)			
		Male	Mean	Maximum	Minimum	Median	Mean	Maximum	Minimum	Median	Mean	Maximum	Minimum	Median
1st Treatment NNRTI	26	21	47.17	63.40	32.20	50.55	791.3	1705.0	183.0	704.0	1033	1723	465	938
1st Treatment PI-r	25	20	48.20	64.60	32.00	49.10	609.4	1062.0	330.0	549.0	990	2738	476	882
PI-r Salvage Therapy	27	17	50.01	82.60	35.90	46.60	623.3	1592.0	67.0	560.0	924	1941	300	782
PI-r and RAL Salvage Therapy	22	18	51.69	70.80	33.12	49.68	485.5	1308.0	34.0	420.0	996	1757	413	884
Virologic Failure	16	9	40.35	67.00	19.60	38.65	185.3	566.0	1.0	126.0	828	2444	51	720
Total	116	85	47.97	82.60	19.60	48.33	571.4	1705.0	1.0	519.0	963	2738	51	873

366 NNRTI= non-nucleoside analog reverse-transcriptase inhibitor, PI-r = ritonavir boosted protease inhibitor, and RAL = Raltegravir.

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374 **Table 2: Treatment response and characteristics between the distinct treatment groups.**

	Time (years) with undetectable viral load				Viral load (log ₁₀ HIV copies/mL)				Number of treatment schemes				Number of drugs			
	Mean	Maximum	Minimum	Median	Mean	Maximum	Minimum	Median	Mean	Minimum	Maximum	Median	Mean	Minimum	Maximum	Median
1st Treatment NNRTI	6.43	14.60	2.30	5.60
1st Treatment PI-r	7.14	12.70	1.00	6.60
PI-r Salvage Therapy	6.81	13.60	1.00	7.60	6	1	11	6	9	4	14	10
PI-r and RAL Salvage Therapy	3.70	7.50	1.10	3.60	8	1	13	7	12	5	19	13
Virologic Failure	4.66	5.45	2.16	3.68	5	2	14	4	9	6	15	7
Total	6.13	14.60	1.00	5.60	4.66	5.45	2.16	3.68

375 NNRTI= non-nucleoside analog reverse-transcriptase inhibitor, PI-r = ritonavir boosted protease inhibitor, and RAL = Raltegravir

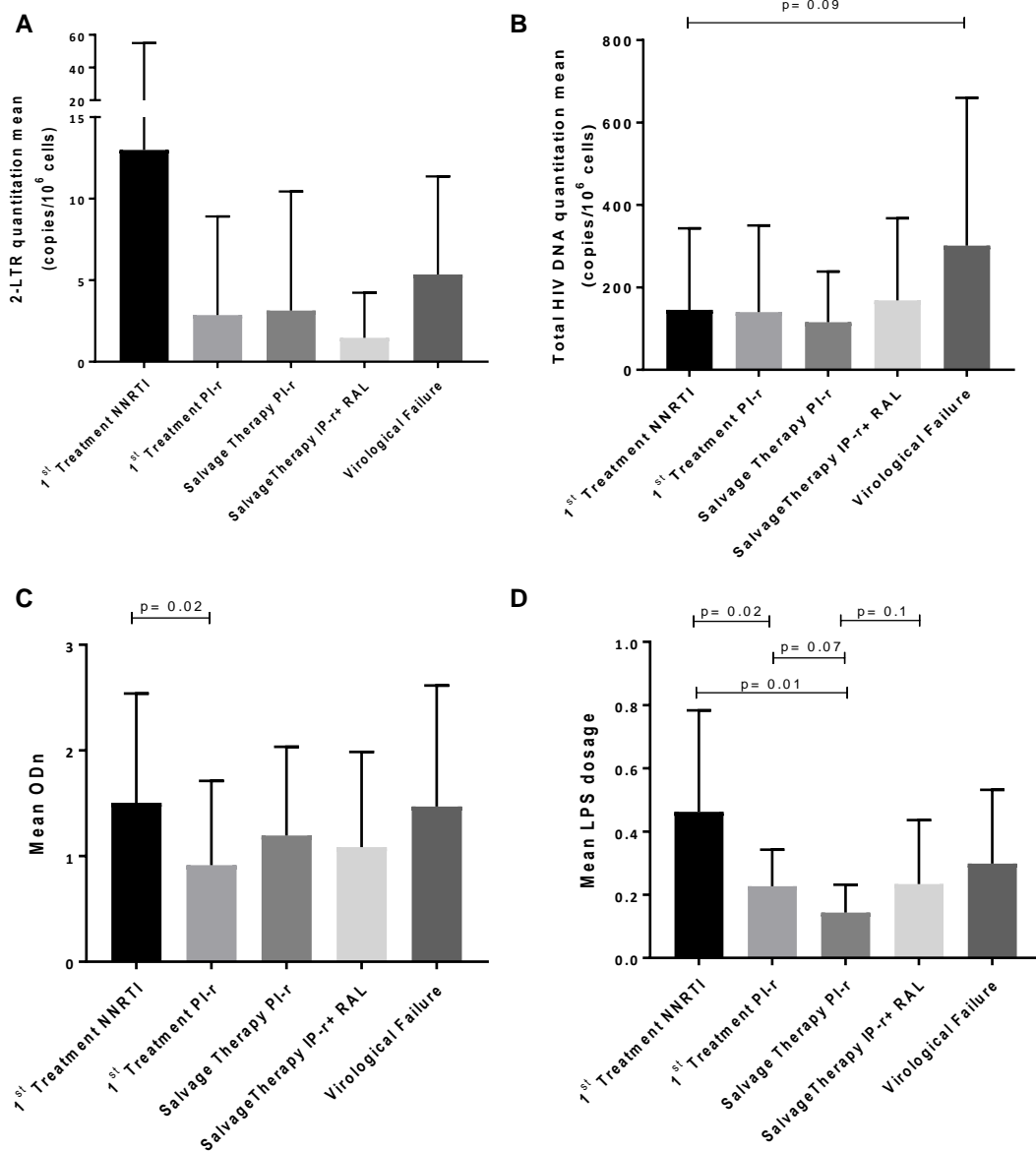
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378 **Table 3: Prevalence of 2-LTR and HIV total DNA and EIA Optical density (ODn) and plasma LPS for the different treatment groups**

	2-LTR (copies/10 ⁶ cells)			Total HIV DNA (copies/10 ⁶ cells)			ODn			LPS		
	n positives (%)	Mean	Median	n positives (%)	Mean	Median	n positives (%)	Mean	Median	Missing	Mean	Median
1st Treatment NNRTI	10 (38)	12.99	0.00	25 (96)	145.65	101.15	17 (65)	1.505	1.201	20	0.462	0.448
1st Treatment PI-r	7 (28)	2.86	0.00	24 (96)	139.87	81.14	7 (28)	0.914	0.653	17	0.227	0.206
PI-r Salvage Therapy	7 (26)	3.14	0.00	25 (93)	115.42	84.32	15 (56)	1.194	1.252	14	0.143	0.140
PI-r and RAL Salvage Therapy	6 (27)	1.46	0.00	21 (95)	168.65	123.23	11 (50)	1.086	0.782	6	0.234	0.162
Virologic Failure	9 (56)	5.35	2.54	16 (100)	301.30	196.62	11 (69)	1.470	1.412	3	0.299	0.226
Total	39 (34)	5.27	0	111 (96)	164.37	101.78	61 (53)	1.221	0.887			

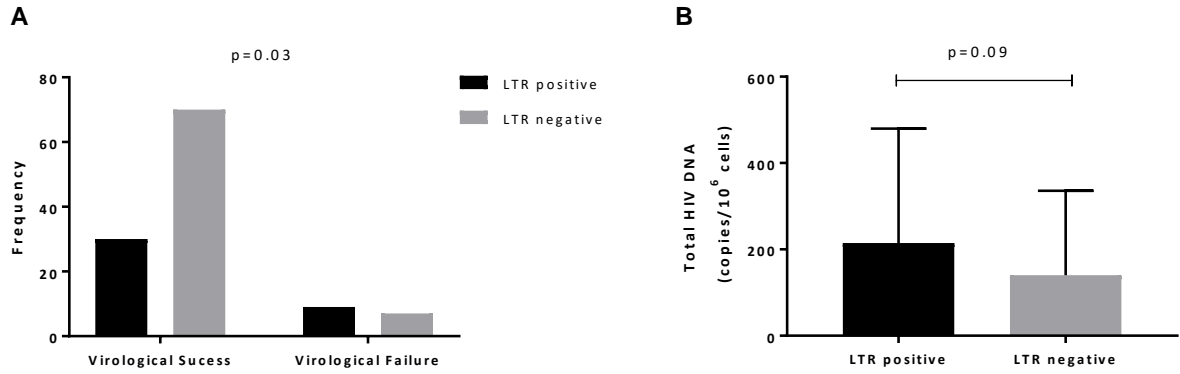
379 NNRTI= non-nucleoside analog reverse-transcriptase inhibitor, PI-r = ritonavir boosted protease inhibitor, and RAL = Raltegravir



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381 **Figure 1:** Mean levels of 2-LTR HIV DNA (Panel A), total HIV DNA (Panel B), EIA optical
 382 density (ODn; Panel C) and LPS levels (panel D) among the different treatment groups. Bars
 383 show standard deviation. P values lower than 0.1 are indicated.

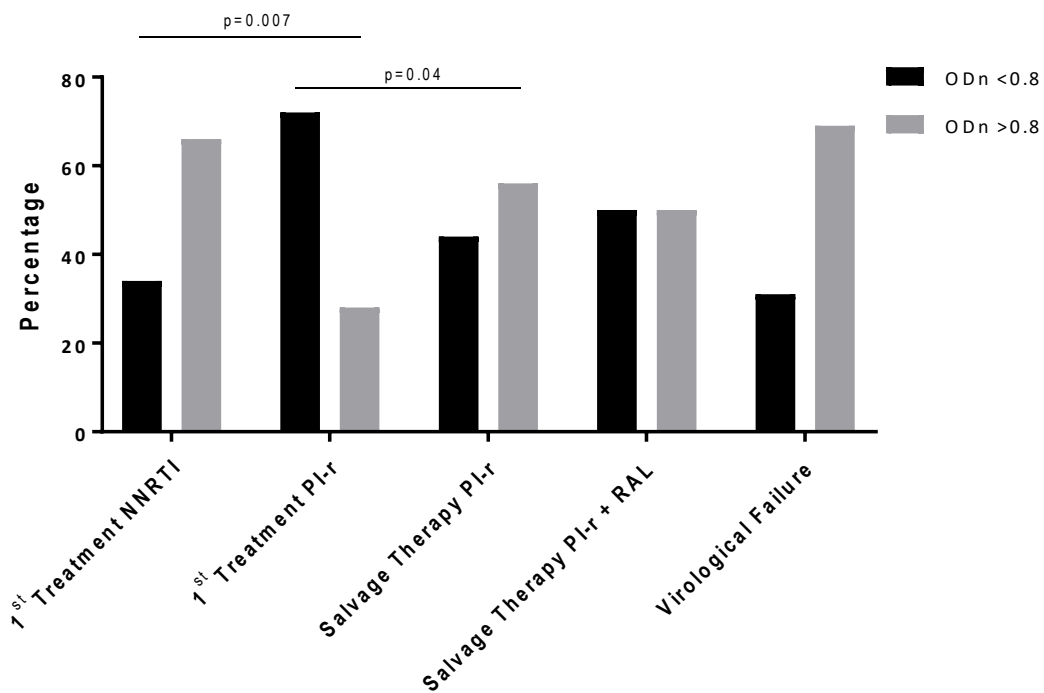
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387 **Figure 2:** (A) Frequency of 2-LTR positive samples among patients with virologic success and
388 virologic failure. (B) Mean levels of total HIV DNA in 2-LTR positive and negative samples. Bars
389 show standard deviation.

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393 **Figure 3:** Frequency of samples in which the less sensitive anti-HIV EIA optical density (ODn)
was higher or lower than 0.8, indicating a positive and negative result, respectively