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Highly precise measurements of HIV DNA in CSF and blood by droplet digital PCR.

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Background

- Cerebrospinal fluid (CSF) can be used to access the immunological and virological milieu in the central nervous system (CNS).
- The development of new tools to measure the HIV DNA reservoirs will help to better understand the dynamics of HIV DNA in the CNS.

Objective

To use for the first time droplet digital (dd)PCR technology to measure levels of HIV DNA in CSF and blood cells.

Methods

Samples:
- Paired peripheral mononuclear blood cells (PBMC) and CSF from HIV-infected adults (see table 1)
- N=20: undetectable HIV RNA in blood and CSF (<50 copies/mL)
- N=9: detectable HIV RNA in blood plasma and CSF

Data generated:
- PBMC: genomic DNA extracted by QIAamp DNA Midi Kit (Qiagen)
- CSF cellular pellets lysed by direct lysis
- HIV DNA levels quantified by ddPCR

Statistical analysis:
- Differences between groups (detectable versus undetectable HIV RNA levels) by Mann-Whitney U test
- Associations across and within anatomic compartments by non-parametric univariate regression analysis (Spearman)

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Results

- HIV DNA was detected in 29 (100%) PBMC samples and 19 (66%) CSF cell pellets, including 10 (52%) samples in which HIV RNA was undetectable in CSF supernatant.

TABLE 1. Population characteristics and levels of HIV RNA and DNA in PBMC and CSF.

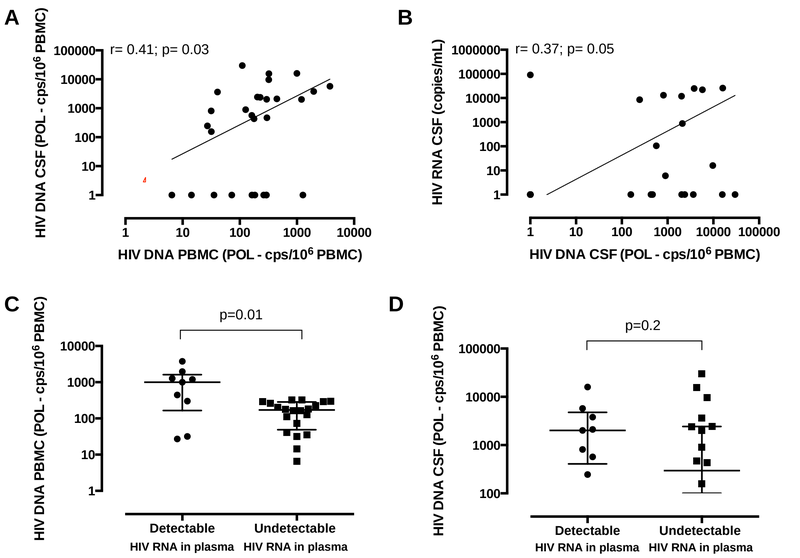
Characteristic (N=29)	Undetectable HIV RNA group (N=20)	Detectable HIV RNA group (N=9)	Mann-Whitney Test p
EDI (years)[a]	12.7 [7.8 – 15.8]	18.4 [17.8 – 21.2]	0.09
CD4 count[a]	499 [365.3 – 788.3]	458.5 [383.3 – 553.3]	0.5
CPE[a]	7 [7 – 7.8]	9 [8 – 10]	0.5
Current ART, n (%)	20 [100]	2 [22]	<0.05
Time in ART (years)[a]	2.3 [1.1 – 3.1]	2.8 [1.9 – 3.6]	0.9
HIV RNA (cps/mL; log_{10})			
blood plasma[a]	-	3.8 [3.5 – 4.8]	-
CSF[a]	-	4.1 [3.9 – 4.4]	-
HIV DNA (cps/million of PBMC; log_{10})			
blood plasma[a]	2.2 [1.8 – 2.4]	3 [2.5 – 3.1]	0.01
CSF[a]	3.4 [2.8 – 3.8]	3.4 [2.9 – 3.6]	0.72

[a]Data shown as median [interquartile range, IQR]
EDI: estimated duration of infection; ART: antiretroviral therapy; CPE: CNS Penetration Effectiveness score.

- Samples with detectable HIV DNA:
  - RPP30 was detected from 44 – 110,600 copies/cell pellet.
  - Levels of HIV DNA were not significantly associated to the levels of RPP30 (r=0.20; p=0.29).

- Levels of HIV DNA in CSF cell pellet was positively associated with:
  - levels of HIV DNA in PBMC (r=0.4; p=0.03). **Figure 1A.**
  - HIV RNA viral load in CSF supernatant (P=0.05). **Figure 1B.**
- A subset of subjects (34.5%) presented undetectable HIV DNA levels in CSF despite detectable HIV DNA in blood. **Figure 1A; red circle.**
- Levels of HIV DNA in PBMC were significantly higher in individuals with detectable levels of cell free HIV RNA in blood plasma than those with undetectable HIV RNA (p=0.01). **Figure 1C**.
- There was no statistical difference in HIV DNA levels in CSF cell pellet between individuals with detectable or undetectable cell free HIV RNA in CSF supernatant (p=0.2). **Figure 1D**.

Figure 1: Levels of HIV DNA and RNA in blood and CSF



- Levels of HIV DNA in PBMC were also positively associated with cell free HIV RNA levels in blood plasma (p=0.001), as expected.
- Levels of cell free HIV RNA significantly correlated between both compartments (r=0.73; p<0.0001), as expected.

Conclusions

- **Levels of HIV DNA in CSF pellets can be measured by ddPCR.**
- **HIV DNA and RNA levels correlate within and across compartments.**
- **The discordance between detectable HIV DNA in CSF cells and PBMC in a subset of subjects (red circle in figure 1A) suggests that HIV-infected cells can be actively excluded from the CNS.**
- **The association between suppressive ART and lower HIV DNA levels in blood, but not in CSF cells, is consistent with poorer ART penetration into the CNS, or slower decay of CNS HIV DNA compared to blood.**