00812

Cell-Associated HIV-1 DNA/RNA in Children: Performance of Real-Time and Digital PCR

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Background and Objective

Despite suppressive antiretroviral treatment (ART) HIV-1 persists in host cells in a latent stage, integrated in the host-cell DNA. This is defined as the HIV-1 reservoir. Some of those cells might harbour the potential to express viral RNA, which could lead to HIV-1 protein expression and subsequent replication and/or immune activation.

Two methods, quantitative real-time and digital PCR (qPCR, dPCR), were used to quantify cell-associated DNA (CA-DNA) and RNA (CA-RNA) in PBMCs in a cohort of 40 well-suppressed, early treated (ET), perinatally infected children (CARMA – **C**hild and **A**dolescent **R**eservoir **M**easurements on early suppressive **A**RT).

Methods

Nucleic acid extraction was done from 5x10^6 PBMCs with DSP Virus/Pathogen Mini Kit (Qiagen) on the QIAsymphony platform (Qiagen). Extracts were eluted in 120µl elution buffer. For RNA analysis samples were treated with DNaseI.

qPCR for CA-DNA and CA-RNA was performed including reference genes PDH [1], or IPO8 (Bio-Rad) and TBP1 (Bio-Rad), respectively, in a multiplex assay. Serial dilutions of HIV-1 DNA or RNA with known copy numbers were included as standard curves. 10µl of DNA was analysed with the Quantitect Multiplex PCR kit (Qiagen) [1]. 10µl of RNA was analysed either with LTR-primers for total HIV-1 RNA (spliced and unspliced) [1] or pol-primers for HIV-1 genomic RNA (unspliced only, US RNA) [2] in a one-step quantitative reverse transcription PCR (qRT-PCR) with TaqMan Fast Virus (Thermo Fisher). Cycling conditions were 5 min at 50˚C, 20 sec at 95˚C followed by 45 cycles of 15 sec at 94˚C and 90 sec at 60˚C. All qPCR results were analysed with CFX Maestro Software (Bio-Rad) and copy numbers were calculated per 10^6 PBMCs.

dPCR was performed on CA-DNA or CA-RNA with the same primers/probes as for qPCR [1,2]. To analyse DNA the QX200 droplet (Bio-Rad) system was used as published [1]. CA-RNA (10µl) was analysed with the One-Step RT ddPCR Supermix (Bio-Rad) using the QX200 droplet system (Bio-Rad). Cycling conditions for RNA analysis were 60 sec at 50˚C, 10 min at 95˚C, followed by 40 cycles of 95˚C for 30 sec, 56˚C for 1 min, and a final denaturation step at 98˚C for 10 min. Copy numbers were calculated per 10^6 PBMCs.

Statistical comparison between qPCR and dPCR was performed using the Bland-Altman agreement test. The Wilcoxon-Rank test was used to compare CA-RNA and CA-DNA expression. For the prediction model the Hosmer-Lemeshow test was used.

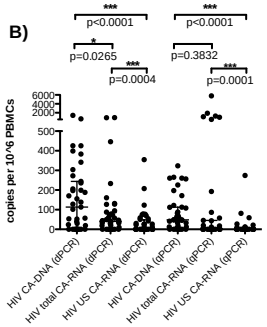
Results (I)

Comparison of qPCR and dPCR. A) Detection of CA-DNA and CA-RNA in 40 long-term suppressed ET; **B)** Comparison of detection of CA-DNA and CA-RNA species with dPCR and qPCR

A)

	qPCR (copy no. per 10^6 PBMCs)	dPCR (copy no. per 10^6 PBMCs)	negative with both methods	positive with both methods
CA-DNA	81 (0.1-323)	174 (19-1420)	3	27
total RNA	23 (1-5789)	29 (11-857)	9	21
US RNA	19 (0.04-274)	18 (11-325)	17	14

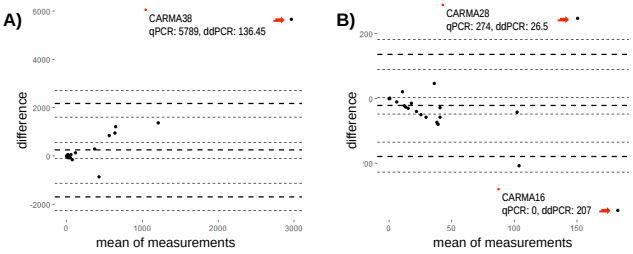
B)



- HIV-1 CA-DNA reservoir is very low (dPCR and qPCR are comparable)
- different HIV-1 CA-RNA species can be detected with both methods at low levels
- median copy numbers of CA-DNA are higher than total and US HIV-1 RNA
- median copy numbers of US HIV-1 RNA are lower than total HIV-1 RNA

Results (II)

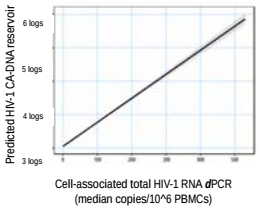
Bland-Altman test for CA-RNA assays (concordance between qPCR and dPCR); **A)** total CA-RNA per 10^6 PBMCs; **B)** US CA-RNA per 10^6 PBMCs



- high agreement (97.4% for total and 94.7% for US CA-RNA) of both methods can be observed
- 1/38 in Fig. 2A falls out of the 95% confidence interval
- 2/38 in Fig. 2B fall out of the 95% confidence interval

Results (III)

Test whether total CA-RNA could be a predictor of the CA-DNA reservoir (dPCR data)



	Exp (coef) CI 95%	P-value
Cell-associated total HIV-1 RNA dPCR (cp/ 10^6 PBMCs)	1.11 [1.10; 1.11]	<0.001
Early treated (<6 months at ART)	0.93 [0.87;1.0]	0.067
Baseline %CD4	0.98 [0.98;0.99]	<0.001
Baseline VL (log10 HIV-1 RNA)	3.3 [3.16;3.51]	<0.001
Interactions		
Cell-associated total HIV-1 RNA * Early treated	0.98 [0.98;0.98]	<0.001
Cell-associated total HIV-1 RNA * %CD4	1.001 [1.0;1.001]	<0.001
Cell-associated total HIV-1 RNA * VL	0.98 [0.97;0.98]	<0.001
P-value Hosmer-Lemeshow Test= 1.00		

- for each unit of total CA-RNA an increase of 11% in CA-DNA can be observed
- age at ART, baseline CD4, and viral load seem to play a significant role as confounding factors

Conclusions

- Bland-Altman concordance suggests comparability of qPCR and dPCR for low HIV-1 copy-number detection, due to high agreement
- both, qPCR and dPCR possess equivalent sensitivity for detection of very low levels of CA-DNA and CA-RNA in long-term suppressed children
- due to high sensitivity of qPCR and especially dPCR, sufficient repeats of negative controls must be included for each experiment to ensure validity of data at low copy numbers
- detection of US CA-RNA was lower than total CA-RNA, suggesting expression of different species of HIV-1 RNA and on-going replication even under ART
- total CA-RNA could be a predictor of the CA-DNA reservoir

➢ single-genome sequencing studies are on-going to determine the proportion of intact proviruses in ET well-suppressed children (see poster 00813)

References

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[2] BREATHER (PENTA 16) Trial Group. **Weekends-off efavirenz-based antiretroviral therapy in HIV-infected children, adolescents, and young adults (BREATHER): a randomised, open-label, non-inferiority, phase 2/3 trial.** Lancet HIV. 2016 Sep;3(9):e421-e430.

Acknowledgements

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