Comparison of qPCR and dPCR methods to investigate the latent HIV reservoir in a paediatric population with long viral suppression on therapy

Judith Heaney1, Eloise Busby2, Kathleen Gänster3, Paul Grant4,5, Moira J. Spyer1, Denise M. O’Sullivan2, Triantafylia Gkouleli1, Anne Genevieve Marcelin1, Deenan Pillay6,7,8, Caroline Foster2, Pablo Rojo9, Paolo Palma9, Maria Angeles Muñoz-Fernández10, Anita deRossi10, Jim F. Hugett11 and Eleni Nastoul12 on behalf of the EPICAL consortium

1Advanced Pathogen Diagnostic Unit, UCLH, London UK; 2Molecular and Cell Biology Team, NML, Teddington, UK; 3UCL, GOS Institute of Child Health, London, UK; 4Molecular Pathology, Health Services Laboratories, London, UK; 5Epicentre and Marie Curie University, Paris, France; 6Epidemiology of Infection and Immunity, UCL, London, UK; 7Africa Health Research Institute, Durban, South Africa; 8Molecular Pathology, Health Services Laboratories, London, UK; 9Department of Pediatrics, University of Southern Denmark, Odense, Denmark; 10Department of Pediatrics, University of Medicine and Dentistry of New Jersey, Newark, USA; 11Section of Oncology and Immunology-DISC, University of Padova, Padova, Italy.

Background

Despite effective antiretroviral therapy (ART), HIV persists as integrated provirus generating latent viral reservoirs even in the absence of detectable plasma viremia.

Latently infected cells, primarily CD4+ T cells, have the potential to release progeny virus and contribute to viral rebound after treatment interruption or HIV-1 remission.

Methods

Inclusion criteria

Children and adolescents (40) were recruited into the CARMA study from 7 European sites / cohorts (3 UK, 2 Spain, 2 Italy)

- aged ≥5 years
- viral suppression ≤50 c/ml maintained for a minimum of 5 years

Methods

HIV-1 DNA was measured from purified total PBMCs (5 x 10⁶/ml), 120µL was lysed with 240µL ATL buffer and nucleic acid extracted on the QIAasymphony platform using the DSP Virus/Pathogen Mini Kit (Qiagen), eluted in 60µl.

Results (I)

Detection of total HIV-1 DNA by qPCR and dPCR assay methodologies

HIV-1 DNA was detected in 34 of 40 adolescents patients recruited into the CARMA study, 26 by qPCR and 28 by dPCR, overlap of 22 in paired extracts.

By qPCR, the calculated total HIV-1 DNA range was 11.4 – 409.9 copies per million cells; 14 patients had undetected HIV-1 DNA. In contrast, using qPCR, the calculated total HIV-1 DNA range from this patient cohort was 19.9 – 1420.2 copies per million cells with 12 patients having undetected HIV-1 DNA.

Results (II)

Expression of PDH human reference gene

Good correlation was observed between the two assays (R² = 0.9, p < 0.0001) however, qPCR showed higher values of PDH per µL of extract included in each PCR reaction, range 550.6 – 60709.2 copies (qPCR) and 36.3 – 49074 (dPCR) with one sample having undetected PDH in both systems.

Results (III)

Comparison of total HIV-1 DNA by qPCR and dPCR using reaction volumes

For direct comparison of assay sensitivity the total HIV-1 DNA was determined per PCR reaction (20µL), therefore not being normalized to a human genomic reference gene. As observed with the PDH real time PCR assays, the values measured by qPCR were higher than those measured by dPCR (5.3 – 237.8 copies per 20µL qPCR) and 0.9 – 24.2 (dPCR). Again, good correlation was observed between both assays (R² = 0.65, p = 0.0001).

Results (IV)

Comparison of total HIV-1 DNA by qPCR and dPCR assay methodologies

dPCR analysis was repeated on independent extracts from the total PBMC samples. The HIV-1 DNA range was 0.8 – 309.6 copies per million cells; 11 patient samples having undetected HIV-1 DNA (A). Further extraction and qPCR were performed and an additional 10 patient samples showed detectable HIV-1 DNA, albeit at very low levels, below 50 copies per million cells. The total HIV-1 DNA range (0.1 – 450 copies per 10⁶ cells, 4 undetected), determined by qPCR, was subsequently compared to results obtained by dPCR (B).

Conclusions

- At low levels of HIV-1 DNA, such as those measured in this early treated well suppressed cohort, the stochastic nature of molecules must be considered as it might challenge meaningful quantification.
- Multiple repeats should be included in order to minimise the risk of not identifying positive samples and to enable more accurate quantitation.
- Normalisation methods can determine absolute quantitation values and should therefore be appropriately reported and kept consistent when following up patients longitudinally.
- HIV-1 DNA quantitation is widely used as a marker in remission studies; attention should be drawn to the assays used when extrapolating and comparing data within and between studies as there can be variability between methods as illustrated here.
- Digital PCR is becoming a main tool in HIV cure research given its advantage of not requiring a standard curve for result analysis. More data are required however on how dPCR performance compares to qPCR and what its performance is between different laboratories.

Acknowledgements

The EPICAL project is funded through an independent grant by ViV Healthcare UK. This work was also supported by the European Metrology Programme for Innovation and Research joint research project [HIT7] "AntiMicroResist"