Heliyon 10 (2024) e29842

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Case report

HIV-1 RNA monitoring with a dual-target diagnostic assay: A case report

Giuseppe Sberna [a], Roberta Gagliardini [b], Gabriella Rozera [a], Federica Forbici [a], Stefania Cicalini [b], Andrea Antinori [b], Fabrizio Maggi [a], Alessandra Amendola [a,*]

[a] Laboratory of Virology and Biosafety Laboratories, National Institute for Infectious Diseases Lazzaro Spallanzani, IRCCS, 00149, Rome, Italy
[b] Clinical Department, National Institute for Infectious Diseases Lazzaro Spallanzani, IRCCS, 00149, Rome, Italy

ARTICLE INFO

Keywords:
HIV-1 RNA monitoring
Dual-target assay
Antiretroviral therapy
Case report
LTR

ABSTRACT

In a restricted subset of people living with HIV-1 (PLWH) on antiretroviral therapy (ART) with persistent suppressed viral load (i.e., *pol*-based HIV-RNA repeatedly undetected), a dual-target (*pol* and *LTR*) diagnostic assay for HIV-RNA monitoring can measure quantifiable levels of viral loads (VL) above 30 copies/mL exclusively through the amplification of the *LTR* region, while the *pol* target results undetected.

We report a patient who shows high levels of HIV-RNA detected exclusively through amplification of the *LTR* region while undetected by the *pol* region, during a long monitoring period, from 2018 to date. In this follow-up, the ART was modified without reaching *LTR*-based undetected HIV-RNA values. Immunological and virological parameters remained optimal with a progressive and steady gain of the CD4/CD8 ratio.

The clinical history of this patient, shows that *LTR*-based viremia above 50 copies/mL can be found occasionally or persistently in the plasma of PLWH under suppressive ART, even at high levels. Based on previous studies, VL detected and quantified exclusively through the amplification of the *LTR* region corresponds to partial or incomplete HIV-RNA transcripts, which cannot trigger new infections. Interestingly, changes in ART do not eliminate repeated findings of these unusual viral elements.

1. Introduction

Aptima HIV-1 Quant Dx (Aptima; Hologic, Inc., San Diego, CA; [1]) diagnostic assay is based on the dual-target (i.e., *pol* and *LTR)* and dual-probe technology and shows both the viral load (VL) measured with each target region of the HIV-RNA [1]. The VL value is always calculated on the basis of the *pol* target amplification; however, in some cases, the VL is quantified only through the *LTR* target amplification (i.e., when the *pol* region is not detected and *LTR* target is quantified).

We report the case of a Caucasian man in his fifties who was diagnosed with HIV-1 infection, showing persistent suppressed viral load, i.e., HIV-RNA undetected based on the *pol*-target amplification (using the single-target assay Abbott m2000 RealTime HIV), from 2010 to 2018. Interestingly, with the introduction of the Aptima dual-target assay, this patient showed high VL based only on the *LTR* target repeatedly (i.e., *pol* region not detected), from 2018 to the present.

* Corresponding author. Laboratory of Virology and Biosafety Laboratories, National Institute for Infectious Diseases "Lazzaro Spallanzani" IRCCS Via Portuense n. 292 00149, Rome, Italy.
E-mail address: alessandra.amendola@inmi.it (A. Amendola).

https://doi.org/10.1016/j.heliyon.2024.e29842
Received 6 February 2024; Received in revised form 16 April 2024; Accepted 16 April 2024
Available online 19 April 2024

2. Case presentation

At diagnosis of HIV infection in March 2010, the patients reported heterosexual intercourses as risk factor, and showed a VL of 23,583 copies/mL (using the *pol*-single-target assay Abbott m2000 RealTime HIV), a $CD4^+$ cells count of 439/mm^3 and a CD4/CD8 ratio of 0.487; he was classified as stage B according to the Centers for Disease Control and Prevention (CDC) [2]. HIV genotype resistance testing revealed a subtype B wild type, with only secondary mutations in protease (L63P, V77I, I93L) and T69A/T mutation in reverse transcriptase (RT). He started ART with EFV one pill a day + TDF/FTC one pill a day with a VL of 17,214 copies/ml and a $CD4^+$ cell count of 353/mm^3. After five months, he was simplified to EFV/TDF/FTC one pill a day which lead to a virological suppression (VL < 50 copies/mL) in eight months. From April 2014 to May 2015, therapeutic drug monitoring was measured and drug plasma concentrations resulted adequate. In February 2017, ART was changed to RPV/TDF/FTC one pill a day as RVP induces fewer side effects than EFV.

In May 2018, in our lab the diagnostic system for HIV-RNA monitoring based on a single-target (*pol* region) assay was replaced by Aptima (a dual-target assay). In June 2018, in this patient, Aptima detected 3550 copies/mL only through the *LTR* target, after eight years of viral suppression. The VL remained detectable with only the *LTR* target from 2018 to the present, ranging from 912 to 11,220 copies/mL (Fig. 1). During this period, the $CD4^+$ cell count was always adequate, over 500/mm^3 (range: $CD4^+$ 634 – 1406/mm^3). At the same time, in addition to the mutations already described, genotypes showed two other RT mutations (E138E/K and D67D/G/N/S). In September 2019, ART was changed to BIC/TAF/FTC one pill a day, without impact on the VL that continued to be detected only on the amplification of the *LTR*-target.

He always reported a good level of adherence to the prescribed ART, no drug-drug interactions were present, and there was no suspicion of malabsorption. In 2005, he was diagnosed with syphilis and treated accordingly. No other coinfections with hepatitis or other sexually transmitted infections or/and comorbidities were present. Renal and liver function were always in the normal range. Vaccinations for SARS-CoV-2 in April 2021 and January 2022 had no impact on HIV viremia.

3. Discussion

We report the case of a chronic PLWH for a long time (>14 years) under suppressive antiretroviral therapy (i.e., *pol*-based undetected HIV-RNA) showing persistent plasma HIV-RNA detected and quantified only through the *LTR*-target by the Aptima dual-target assay. Interestingly, other patients show, occasionally or repeatedly over a limited time, VL undetected with the *pol*-target, but detected with the *LTR* region above 50 copies/mL by the Aptima dual-target assay [3–5]. In *in-vitro* setting [5], it has been shown

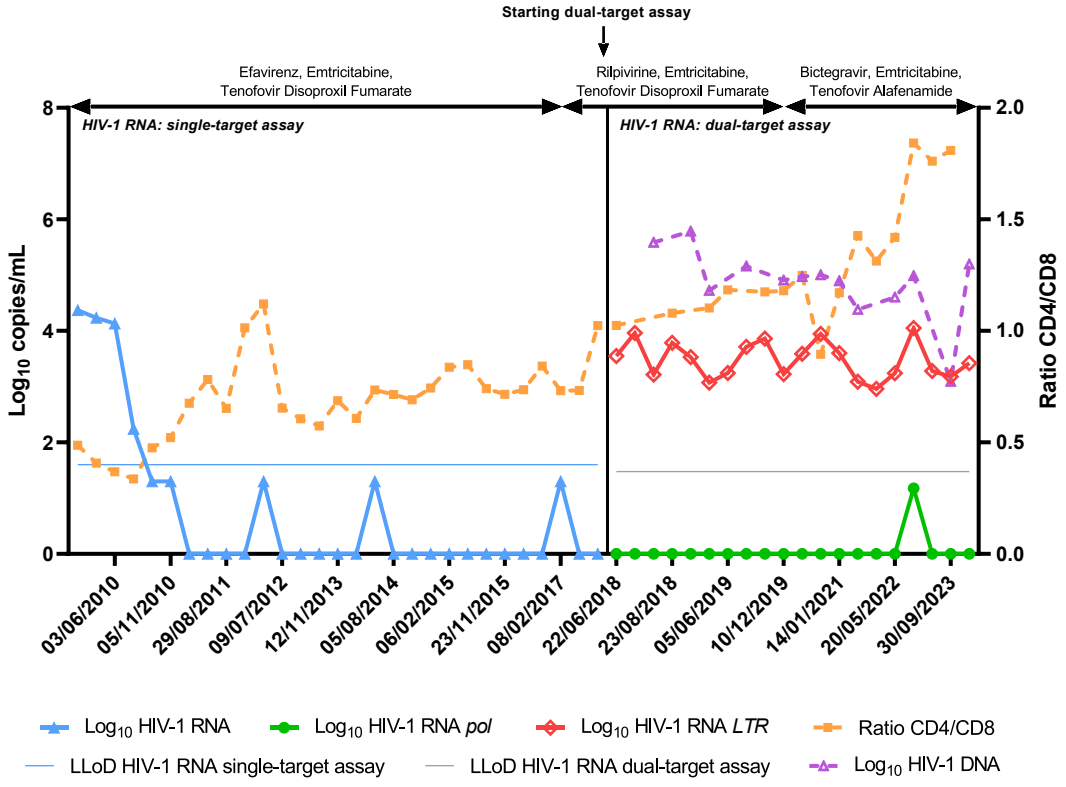

Fig. 1. Trends of virological and immunological parameters. The treatment regimens taken by the patient during the follow-up are shown at the top of the graph. The black arrows describe the period of intake of the above-mentioned drug regimens.

that viral genomes detected in plasma samples of PLWH exclusively through the *LTR* target consist of partial or incomplete genetic elements, unable to encode intact or replication-competent viruses; therefore, these elements cannot trigger new cycles of viral replication nor transmit the infection. These elements, released as virus-like particles showing smaller diameters than the canonical one [5], probably result from biologically active defective proviruses [6–11] produced following the clonal expansion of the HIV reservoir [12,13]. Accordingly, during HIV-RNA monitoring of ART-treated PLWH with Aptima, it would be appropriate to specifically notify the VL quantified above 50 copies/mL exclusively through the *LTR* target as "*LTR* detection only". Aptima ensures the detection of the *pol* and *LTR* regions by utilizing multiple specific primers for each target region. This approach guarantees the detection of one or both HIV targets even when mutations are present within these regions. VLs that are based solely on the *LTR* target are typically indicative of a highly mutated or absent *pol* region. To further confirm the nature of the *LTR*-viremia, it is recommended that VL testing be repeated on subsequent blood draws or using different diagnostics. However, it should be noted that other diagnostic tests for HIV-1 RNA monitoring, even those that are dual-target, do not allow for the recognition of this category of VL. In conclusion, it is crucial to inform clinicians when VL is detected solely with the *LTR* region. This is because such viremias are unlikely to indicate ART failure, and therefore, the changes in treatment regimen usually recommended in instances of virologic rebound [2,14,15] would not be necessary [3–5].

A limitation of this study is that we have not been able to sequence the transcripts detected with the LTR region by Next Generation Sequencing, but a protocol based on RNA-seq methodology is being set up that should allow us to identify the nature and origin of these particular HIV-RNA elements.

Funding

This study was supported by Ministry of Health: Ricerca Corrente – Linea 2, Progetto 1.

Ethics statement

This study was approved by the Institutional Ethics Committee of the National Institute for Infectious Diseases, Lazzaro Spallanzani, IRCCS (Opinion n.2/2019 of Ethics Committee trials register), and an informed consent was signed by the patient.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Giuseppe Sberna: Writing – original draft, Formal analysis, Data curation, Conceptualization. **Roberta Gagliardini:** Formal analysis. **Gabriella Rozera:** Methodology. **Federica Forbici:** Formal analysis. **Stefania Cicalini:** Methodology. **Andrea Antinori:** Resources. **Fabrizio Maggi:** Writing – review & editing, Supervision, Resources. **Alessandra Amendola:** Writing – original draft, Supervision, Project administration, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors sincerely thank Giulia Berno, Lavinia Fabeni and Isabella Abbate for their support.

References

[1] Food and Drug Administration (2023). https://www.fda.gov/media/102425/download. (Accessed 24 January 2023).
[2] Centers for Disease Control and prevention. https://www.cdc.gov/hiv/default.html, 2023. (Accessed 5 January 2024).
[3] A. Amendola, G. Sberna, F. Forbici, I. Abbate, P. Lorenzini, C. Pinnetti, A. Antinori, M.R. Capobianchi, The dual-target approach in viral HIV-1 viremia testing: an added value to virological monitoring? PLoS One 15 (2) (2020 Feb 5) e0228192 https://doi.org/10.1371/journal.pone.0228192.
[4] G. Sberna, S. Sarti, S. Cicalini, A. Antinori, A.R. Garbuglia, A. Amendola, Evaluating the dual-target Aptima HIV-1 quant Dx assay: comparison between viral loads measured with pol and LTR targets in the same samples, Microbiol. Spectr. 10 (5) (2022 Oct 26) e0136122, https://doi.org/10.1128/spectrum.01361-22.
[5] G. Sberna, R. Nardacci, G. Berno, G. Rozera, E. Giombini, L. Fabeni, E. Specchiarello, F. Maggi, A. Amendola, Virological characterization of HIV-1 RNA elements detected exclusively through the LTR region by the dual-target Aptima HIV-1 Quant Dx assay in a subset of positive patients, J. Clin. Virol. 167 (2023 Oct) 105575, https://doi.org/10.1016/j.jcv.2023.105575.
[6] H. Imamichi, R.L. Dewar, J.W. Adelsberger, C.A. Rehm, U. O'Doherty, E.E. Paxinos, A.S. Fauci, H.C. Lane, Defective HIV-1 proviruses produce novel protein-coding RNA species in HIV-infected patients on combination antiretroviral therapy, Proc. Natl. Acad. Sci. U.S.A. 113 (31) (2016 Aug 2) 8783–8788, https://doi.org/10.1073/pnas.1609057113.
[7] H. Imamichi, M. Smith, J.W. Adelsberger, T. Izumi, F. Scrimieri, B.T. Sherman, C.A. Rehm, T. Imamichi, A. Pau, M. Catalfamo, A.S. Fauci, H.C. Lane, Defective HIV-1 proviruses produce viral proteins, Proc. Natl. Acad. Sci. U.S.A. 117 (7) (2020 Feb 18) 3704–3710, https://doi.org/10.1073/pnas.1917876117.

[8] K.M. Bruner, A.J. Murray, R.A. Pollack, M.G. Soliman, S.B. Laskey, A.A. Capoferri, J. Lai, M.C. Strain, S.M. Lada, R. Hoh, Y.C. Ho, D.D. Richman, S.G. Deeks, J. D. Siliciano, R.F. Siliciano, Defective proviruses rapidly accumulate during acute HIV-1 infection, Nat. Med. 22 (9) (2016 Sep) 1043–1049, https://doi.org/10.1038/nm.4156.

[9] T.B. Manzoni, C.B. López, Defective (interfering) viral genomes re-explored: impact on antiviral immunity and virus persistence, Future Virol. 13 (7) (2018 Jul) 493–503, https://doi.org/10.2217/fvl-2018-0021.

[10] J. Kuniholm, C. Coote, A.J. Henderson, Defective HIV-1 genomes and their potential impact on HIV pathogenesis, Retrovirology 19 (1) (2022 Jun 28) 13, https://doi.org/10.1186/s12977-022-00601-8.

[11] R.A. Pollack, R.B. Jones, M. Pertea, K.M. Bruner, A.R. Martin, A.S. Thomas, A.A. Capoferri, S.A. Beg, S.H. Huang, S. Karandish, H. Hao, E. Halper-Stromberg, P. C. Yong, C. Kovacs, E. Benko, R.F. Siliciano, Y.C. Ho, Defective HIV-1 proviruses are expressed and can Be recognized by cytotoxic T lymphocytes, which shape the proviral landscape, Cell Host Microbe 21 (4) (2017 Apr 12) 494–506.e4, https://doi.org/10.1016/j.chom.2017.03.008.

[12] M.R. Pinzone, D.J. VanBelzen, S. Weissman, et al., Longitudinal HIV sequencing reveals reservoir expression leading to decay which is obscured by clonal expansion, Nat. Commun. 10 (2019) 728, https://doi.org/10.1038/s41467-019-08431-7.

[13] K.W. Joseph, E.K. Halvas, L.D. Brandt, S.C. Patro, J.W. Rausch, A. Chopra, S. Mallal, M.F. Kearney, J.M. Coffin, J.W. Mellors, Deep sequencing analysis of individual HIV-1 proviruses reveals frequent asymmetric long terminal repeats, J. Virol. 96 (13) (2022 Jul 13) e0012222, https://doi.org/10.1128/jvi.00122-22.

[14] WHO. Consolidated guidelines on HIV prevention, testing, treatment, service delivery and monitoring: recommendations for a public health approach. https://www.who.int/publications/i/item/9789240031593. Accessed on January 5, 2024]..

[15] European AIDS Clinical Society. Guidelines. https://www.eacsociety.org/guidelines/eacs-guidelines/. [accessed on January 5, 2024].[.