

HIV-1 RNA quantification in CRF02_AG HIV-1 infection: too easy to make mistakes

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SUMMARY

The number of patients newly infected by HIV-1 non-B subtypes and circulating recombinant forms (CRFs) is increasing worldwide, including in the western countries. We report on a primary HIV-1 infection in a Caucasian patient. A routine quantitative assay (Nuclisens EasyQ HIV-1 2.0, BioMérieux SA) showed 6,700 HIV-1 RNA copies/ml. A combined antiretroviral therapy (cART) consistent with low baseline HIV-1 RNA was started. Few days later, the analysis performed with REGA HIV-1 Subtyping Tool - Version 3.0 attributed the HIV-1 sequence to the CRF02_AG recombinant form. Therefore, a second real-time PCR assay was performed, using the Versant HIV-1 RNA 1.0 Assay (kPCR) (Siemens HealthCare Diagnostics) which revealed a HIV-1 RNA of 230,000 copies/ml. Consequently, the ongoing cART was potentiated. This case suggests that the wide genetic variability of HIV-1 subtypes may affect the capability of the commonly used assays to detect and accurately quantify HIV-1 RNA in non-B subtypes and CRFs. In presence of CRFs different commercial HIV-1 RNA tests should be performed to find the most reliable for viral load quantification at the diagnosis, because it influences the choice of cART, and during the follow-up. Indeed, international guidelines for HIV-1 infection management suggest to monitor patient' HIV-RNA with the same assay over the course of treatment. As different commercial tests can be performed in the same laboratory with considerable difficulty, the laboratory should select an assay that is suitable not only for the more prevalent strain, but also for less frequent ones that, nevertheless, can occur. Then, knowing and investigating the spread of non-B strains has essential clinical and laboratory implications.

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INTRODUCTION

HIV-1 RNA quantitative evaluation is used worldwide as a prognostic marker, to predict the risk of clinical progression of HIV-1 infection, and as an efficacy marker, to monitor therapeutic response (Mellors *et al.*, 1997). Indeed, the aim of combined antiretroviral therapy (cART) is to achieve permanently undetectable HIV-1 RNA. Real-time nucleic acid amplification technologies are currently recommended to detect and measure HIV-1 RNA and gene sequencing techniques are used to perform HIV-1 genotype analysis (DHHS, 2014). Several automated assays are commercially available. Nevertheless, recent studies have shown discrepancies in viral load estimation among the existing commercial quantitative assays, especially for the quantification of non-B subtype strains (Bourlet *et al.*, 2011; Church *et al.*, 2011). In Europe and the United States

the number of patients newly infected by non-B subtypes is increasing (Santoro *et al.*, 2013; Siemieniuk *et al.*, 2013). In Italy, CRF02_AG is one of the most common non-B subtypes (Santoro *et al.*, 2012). Since HIV-1 RNA has major implications in therapeutic choices, clinicians should consider the possibility of discrepant HIV-1 RNA quantifications using different quantitative assays while approaching a patient infected by a non-B subtype. Here we report on a primary HIV-1 infection caused by a CRF02_AG in a Caucasian patient, in whom two different HIV-1 RNA amplification assays detected different values of viral load in the same plasma sample.

CASE REPORT

In July 2014, a 48 year-old man developed an acute febrile syndrome accompanied by sore throat, nausea, vomiting, diarrhea and fatigue. He reported a history of unprotected sexual intercourse in the last two months. He was given a two-week empirical antibiotic treatment by his family doctor without clinical improvement. Because of the persistence of symptoms, he was then admitted to our Infectious Diseases Unit in Genoa, Italy. On admission, he was afebrile, alert and cooperative. Physical examination

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showed enlargement of laterocervical, retronuclear and inguinal lymph nodes, which had tense-elastic consistency and were mobile. Abdominal examination revealed liver and spleen enlargement, with no other abnormality. Laboratory examinations showed normal blood cell counts and renal function, while liver enzymes were slightly increased (AST 41 U/L, normal value <50 U/L; ALT 96 U/L normal value <50 U/L). Serological tests for mononucleosis-like syndromes were performed. HIV test resulted positive using a fourth generation HIV-1/2 assay (Vironostika® HIVAg/Ab, BioMérieux). The result was confirmed by another fourth generation HIV-1/2 assay (Genscreen™ ULTRA HIV Ag-Ab, Bio-Rad). In order to confirm the clinical suspicion of primary HIV-1 infection, an anti-HIV-1 Western Blot (New Lav Blot I, Bio-Rad), avidity test and HIV-1 RNA (Nuclisens EasyQ® HIV-1 2.0, BioMérieux SA) (EQ) were also performed. The Western Blot revealed reactivity to gp160 and a very weak, uncertain positivity to gp120 band: according to the WHO criteria this test resulted indeterminate. The avidity test showed an avidity index of 0.57%; HIV-1 RNA was 6,700 copies/ml; CD4+ cell-count was 523/mm³ (17,1%) with a 0.2 CD4+/CD8+ T-cell ratio. Therefore, according to clinical history and immune-virological results, a primary HIV-1 infection was diagnosed (classified as A1 stage, following CDC classification). A few days later, the HIV-1 genotypic resistance test, obtained using Trugene HIV-1 Genotyping kit (Siemens Health Care Diagnostics), revealed no resistance mutations in the *transcriptase* gene, but some mutations in the *protease* gene including L10I, K20I, M36I, and M46I (Table 1). Moreover, the analysis performed with REGA HIV-1 Subtyping Tool - Version 3.0 attributed the HIV-1 sequence to the CRF02_AG recombinant form. At this point all laboratory tests were confirming the clinical impression of a primary HIV-1 infection, thus requiring immediate initiation of cART. Because of his low viral load the patient was put on a single tablet regimen with tenofovir, emtricitabine and rilpivirine (EACS, 2014). However, when the virus was sequenced and identified as a CRF02_AG recombinant form, a second real-time PCR assay was performed on an addi-

tional aliquot of the same sample tested with Nuclisens EasyQ®, using the Versant® HIV-1 RNA 1.0 Assay (kPCR) (Siemens HealthCare Diagnostics). This assay revealed a HIV-1 RNA of 230,000 copies/ml. Thus, cART was potentiated by adding an integrase inhibitor. By using kPCR, two weeks later, HIV-1 RNA was 104 copies/ml and after an 18 week course of four-drug cART, the viral load was 38 copies/ml. At the same time points, the EQ assay detected an HIV-1 RNA of 40 copies/ml and undetectable (<10 copies/ml), respectively (Table 2).

DISCUSSION

HIV-1 is characterized by high genetic variability, due to the large number of errors made by the reverse transcriptase enzyme, in a setting of high viral replication and host immunological pressure. The majority of HIV-1 viral forms circulating in Europe belong to the M group, B subtype. However, the number of patients newly infected by non-B subtypes and by HIV-1 CRFs, which result from the recombination of different HIV-1 subtypes, is rising. In particular, according to Hemelaar *et al.* (2011), from 2001 to 2007 the prevalence of the recombinant form CRF02_AG increased from 5.4 to 7.7% globally, with a major growth in African countries. However, a major increase was also observed in Western and Central Europe, where CRF02_AG accounted for 2.9% of HIV-1 infections at the beginning of the study period and for 4.5% at the end of 2007 (Hemelaar *et al.*, 2011). In Italy, the prevalence of non-B strains increased from 2.6% in 1980-1992 to 18.9% in 1993-2008 (Lai *et al.*, 2010), and the most prevalent non-B subtype are currently C, F and CRF02_AG (Santoro *et al.*, 2012). This phenomenon is most likely due to the increasing number of migrants and travellers from areas of high HIV-1 prevalence where non-B subtypes are the predominant variant. Knowing and investigating the spread of non-B strains is not only essential for epidemiological and clinical reasons but also for laboratory implications. Indeed, the wide genetic variability of HIV-1 subtypes may affect the capability of the commonly used assays to de-

Table 1 - Patient's HIV-1 genotypic resistance test.

<i>Protease gene</i>	
Resistance mutations	10I 13V 20I 36I 46I
Other mutations	14R 15V 16E 39S 41K 43R 63Q 64L 69K 70R 89M
<i>Polymerase gene</i>	
Resistance mutations	None
Other mutations	37F 39A 45R 60I 123E 135V 162A 173A 174K 177E 200A 207E 245Q
<i>Integrase gene</i>	
Resistance mutations	None
Other mutations	101I 111Q 112V 124A 125A 133T 134N 135V 136T

Table 2 - HIV-1 RNA (copies/ml) using different assays at baseline evaluation and after 2 and 18 weeks of combined antiretroviral therapy (cART).

	Nuclisens EasyQ® HIV-1 2.0	Versant® HIV-1 RNA 1.0 Assay (kPCR)
Baseline	6,700	230,000
Week 2	40	104
Week 18	<3	38

tect and accurately quantify HIV-1 RNA in non-B subtypes and CRFs, as previously reported in African experiences (Peeters *et al.*, 2010; Luft *et al.*, 2011; Bruzzone *et al.*, 2014), as well as in European series (Holguin *et al.*, 2008). Despite primers and probes used in the commercial HIV-1 RNA test are designed to annealing the most conserved region of HIV-1 virus, it is impossible for them to recognize all HIV-1 strains with the same efficiency. To investigate the concordance among the newly and commonly used assays [EQ, Abbott RealTime HIV-1 (m2000sp/rt), COBAS AmpliPrep/COBAS Taqman® HIV-1 test v2.0 (CTM) and kPCR] several studies were carried out. On the whole, all assays show a good correlation and concordance with each other.

Only small differences were observed. Briefly, EQ assay in comparison with the others shows an under-quantification of HIV-1 RNA, mainly for G subtypes and CRF02_AG (Gomes *et al.*, 2013; Ndiaye *et al.*, 2015); the quantitative values obtained using kPCR assay were usually lower than those obtained by CTM assay (Tropan KT *et al.*, 2009) whilst the quantitative values obtained using CTM, mainly for B subtypes, (Bourlet T *et al.*, 2011) were usually higher than those obtained by EQ and m2000sp/rt assays (Ndiaye *et al.*, 2015).

The discrepancy in the detection and/or quantification of some subtypes and CRFs between the used tests may be due to differences in technology platforms but, above all, to the target region of viral genome that each assay uses. The gag gene targeted by the EQ assay is less conserved than others such as the pol-int gene (m2000sp/rt, CTM and kPCR) and LTR region (CTM). The under-quantification by EQ may be related to primer and probe mismatches in the gag target region whilst the over-quantification by CTM may be due to the amplification of double target: gag and LTR (Ndiaye *et al.*, 2015).

In such scenario, the method that measures the highest value of viremia in the same sample should be considered the most reliable. A non-B subtypes or CRF HIV-1 strain infection should be suspected when lower than expected viral load values are found. In the above-described case, several factors were in accordance with a diagnosis of HIV-1 acute infection (a history of recent unprotected sexual intercourse, the mononucleosis-like syndrome and the typical indeterminate Western Blot test report): therefore, high levels of HIV-1 RNA were expected. Surprisingly, the HIV-1 RNA resulted 6,700 copies/ml. Nevertheless, when the genotypic resistance test revealed a CRF02_AG strain, the need of a second, different HIV-1 RNA test on the same samples was clear. The second result (230,000 copies/ml), two logarithms higher than the first one, was certainly more consistent with the clinical picture, and led to the choice of potentiating the ongoing cART with the addition of suitable drug against >100,000 copies/ml viral loads. The subsequent HIV-1 RNA controls, performed with both the virological assays, confirmed the discrepancy between the two tests. Finally, as previously reported, in the *protease* gene the following amino acids substitutions were detected: L10I, K20I, M36I, and M46I. In subtype B, the 10I, 20I and 36I mutations contribute to secondary resistance to protease inhibitors (PIs) whereas the 46I mutation reduces susceptibility to atazanavir, fosamprenavir, indinavir, lopinavir and nelfinavir (Rhee *et al.*, 2010) and increases the PIs catalytic efficiency (Henderson *et al.*, 2012). However, in CRF02_AG the 20I is wild type, the 36I is detected in 99% of CRF02_AG (Taylor *et al.*, 2008) and

the real meaning of the 46I is unknown, although caution in PIs use is advisable.

In conclusion, physicians should be aware of the critical issues they might find when interpreting laboratory results of non-B HIV-1 infection, also because of possible implications in the therapeutic choices. Dialogue with virologists should be sought and can prove crucial whenever clinical and laboratory data seem not to match properly.

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