ACUTE PRIMARY INFECTION WITH A HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1) GROUP O: A PUZZLING DIAGNOSIS.

Short title: HIV-1 group O primary infection

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Key words: HIV-1 group O, primary infection, genetic diversity

Abbreviations:
HIV-1, human immunodeficiency virus type 1; ELISA, enzyme linked immunosorbent assay.
ABSTRACT

We report a rare case of acute HIV-1 group O infection in a French Caucasian woman. Her sexual partner was secondarily diagnosed with HIV-infection and transmission was confirmed by phylogenetic analysis. Unequal performances of many serologic and molecular assays commercially available lead to delays in diagnosis and affecting patient management.
CASE REPORT

A 58-year-old Caucasian woman was admitted on February 9, 2007, to the internal medicine ward of the hospital of a medium-sized city in central France for a hyperalgic syndrome and fever of 38.5°C evolving of 10 days’duration. Severe myalgia of the lower limbs required analgesic treatment and was associated with non-painful jugular and axillary lymphadenopathies. She had not travelled abroad in the previous few months. The first biological investigation showed the presence of activated lymphocytes and biochemical signs of hepatic cytolysis (ALT 6 N; AST 3.5 N), cholestasis and pancreatitis. Serological investigations for bacterial infections (syphilis, yersinia, Q fever and mycoplasma) were negative. Serological markers for viral hepatitis A (HAV IgM), B (HBs Ag and anti-HBc) and C (anti-HCV) were negative. Serological assays for cytomegalovirus, Epstein-Barr virus and toxoplasmosis revealed the presence of IgG but not IgM antibodies, suggestive of past infections. Screening test for HIV1/2 infection was performed one week later (February 15), when the patient reported having had a sexual relation with an occasional partner at the beginning of January. Two routine fourth generation assays, Axsym HIV 1/2 Combo (Abbott) and Vidas HIV Duo QUICK (bioMérieux), were performed as required by French legislation. They provided positive results. The HIV-1 p24 antigen assay (Vidas HIV Ag, bioMérieux) was negative. HIV-1 RNA was detected (90 copies/mL) with the Versant HIV-1 RNA 3.0 (Bayer) but was undetectable with the Cobas AmpliPrep-Cobas TaqMan HIV-1 test (Roche Diagnostics). Western blot analysis showed the presence of antibody to p24 only (figure 1B). HIV-2 western-blot was negative. Although p24 antigenemia was undetectable, an acute HIV primary infection was suspected and the patient started highly active antiretroviral therapy (HAART) associating KIVEXA™ and KALETRA™ on February 27th, switched for TRUVADA™ and KALETRA™ on March 3, due to hypersensibility reaction to abacavir. HAART was stopped after three weeks on March 23, because of severe adverse intestinal
effects leading to an alteration in clinical status, and because of confusing virological data that
did not provide clear evidence of acute HIV infection. Indeed, at this date, the available
virological results showed that ELISAs were still positive but with a decline in the positivity
index (AxSYM Ag/Ac Combo and Architect HIV Combo, Abbott) (fig. 1A), and no change in
the western-blot reactivity profile except a slight increase in anti-p24 intensity (figure 1B).
Results of additional analyses with other assays performed on sequential sera by i/the local
laboratory of the hospital where the patient was admitted; ii/the laboratory of the regional
teaching hospital; and iii/the National Reference Center for HIV are summarized in figure 1A.
On the basis of these data, RT-PCR assay using group-specific primers (HIV-1 group M and
group O) of a conserved region of the gp41 env gene was performed on a sample taken
February 16, as previously described (1). Unexpectedly, a positive reaction was obtained with
group O primers only. The amplification product (415 bp) was sequenced and submitted to
phylogenetic analysis, which confirmed HIV-1 group O infection (figure 1C).
Close clinical and virological follow-up was established. Fifteen days after
discontinuation of treatment, the patient had persistent nausea and asthenia, and
lymphadenopathies reappeared. On April 17, immunoassays showed an increase in ELISA
index and an evolving western-blot profile, suggestive of seroconversion (figure 1B). Viral
load was quantified at 4.3 log_{10} copies/ml with the RealTime HIV-1 test (Abbott Molecular).
We therefore concluded that she had an acute HIV-1 group O infection. Her clinical status
improved, with regressive but persistent lymphadenopathies, and antiretroviral treatment was
not resumed. Her T CD4^+ cell count was 635/mm^3 in May and 500/mm^3 in July. Subsequent
HIV-1 viral loads in June and August were 5.4 log_{10} copies/mL by RealTime system.
One month after the seroconversion period, the sexual partner of this patient was
identified and diagnosed with HIV-1 group O infection. He was a Caucasian, French resident
and had made regular trips to Cameroon for several years. The phylogenetic analysis of the
gp41 env amplification product from his plasma viral RNA confirmed the common origin of the strains. The two sequenced fragments shared 99.2% homology and cluster with a 100% bootstrap value (figure 1C).

HIV-1 group O infections are rare outside the endemicity region in west central Africa including Cameroon and neighbouring countries (2). The French national surveillance system reported a 0.1% prevalence of HIV-1 group O infections among new HIV diagnoses between 2003 and 2006 (3). In spite of this low prevalence, systematic identification of group O infections, which is made easier by epidemiological data, would be necessary for an accurate assessment of viral replication and adapted HAART, given that most available viral load assays (4) are unable to quantify HIV RNA and that group O HIV-1 strains are naturally resistant to non-nucleoside reverse transcriptase inhibitors.

For physicians regularly involved in the management of patients suffering from HIV infection, the clinical presentation of this patient was retrospectively strongly suggestive of acute HIV infection. However, the discordant results of various virological assays confused the interpretation. Discordant results of HIV antibody assays, p24-antigen assays and nucleic acid-based assays might be suggestive of either false-positive or false-negative reactivities. They strongly complicated the early diagnosis of this acute HIV-1 group O infection and were largely instrumental in the decision to stop antiretroviral treatment soon after. One fourth-generation ELISA, Genscreen HIV Ultra (BioRad), was negative during the 2 months following sexual exposure and was only weakly reactive more than 3 months later. Failure to detect HIV antibodies because of HIV genetic diversity has been regularly reported, even when using these highly sensitive assays. Single variant sequences were generally responsible for failure to detect HIV-1 groups M or O infections (5, 6). In the present case, the sequence of the gp41 immunodominant epitope (IDE) was similar to that of the group O consensus
IDE, and it could not therefore explain the failure to detect antibodies. The sensitivity of p24 antigen detection of newly developed fourth-generation assays varies, and failures have been described, particularly for group O strains at p24 concentrations below 25 pg/mL (7). Western blot profile showed only a reactive p24 band on early sequential samples although with a slight increase in intensity. A significant drop in the positivity index of ELISAs was observed under therapy. This could be explained by early initiation of HAART. Of note, one third-generation assay, Ortho HIV1/2 Antibody Capture (Ortho Diagnostics), although positive on earlier samples, became negative (fig. 1A). Such a seroreversion was previously described in acutely infected individuals treated with early HAART (8).

Direct detection using p24 antigen assays or viral load assays is particularly useful in cases of indeterminate antibody response. However the assays all provided discordant results in our case. P24 antigenemia was negative or slightly positive, and became rapidly undetectable. HIV-1 RNA quantification was negative with Cobas AmpliPrep – Cobas TaqMan HIV-1 assay, and was particularly under-estimated with the branched-DNA assay, since it was retrospectively determined at 4.9 log_{10} copies/mL with the Real Time HIV-1 assay (fig. 1A). At present, only the Abbott RealTime system, among commercially available assays, can quantify HIV-1 group O RNA, because it uses primers and probes designed in the highly conserved region of integrase in the pol gene (4). Finally, we had no epidemiological information concerning our patient’s sexual partner during the first weeks of diagnosis, it was the slight increase in reactivity to p24 by western blot, associated with the various discordant results of the different assays, that prompted us to explore a possible group O infection using specific tools available at the National Reference Center.

This case of acute primary infection with an HIV-1 group O variant underlines the impact of the genetic diversity of HIV on virological diagnosis and its consequences for patient management. HIV divergent strains must no longer be considered as limited to
developing countries, and the development of diagnostic tools better suited to the genetic diversity of HIV must be encouraged to improve the diagnosis and management of HIV-infected patients.
ACKNOWLEDGEMENTS

We thank Dr Thomas Bourlet for his help in performing the HIV-1 viral load with Real Time HIV-1 assay, Dr Catherine Gaudy-Graffin for her help in the phylogenetic analysis and Jeffrey Watts for revision of the English manuscript.
REFERENCES


Legends to figure.

Serological and molecular findings on sequential samples from the reported case.

(A) Results of the different immunoassays and viral load assays.

Bold indicates reactivity. Results of serological tests are expressed as signal to cut-off (S/CO). S/CO values greater than or equal to 1.00 were considered reactive, except for VIDAS HIV Duo Quick (F): positive>0.25. Shaded area: period under HAART.

* Commercial tests: VIDAS Agp24 II (bioMérieux); Modular HIV Antigen (Roche Diagnostics); Ortho HIV1/HIV2 Antibody Capture (Ortho Diagnostics); Anti-HIV TETRA ELISA (Biotest); Axsym Ag/Ac Combo (Abbott); VIDAS HIV Duo QUICK (bioMérieux); Architect HIV Combo (Abbott); Genscreen HIV Ultra (BioRad); Versant HIV-1 3.0 (Bayer); Cobas AmpliPrep-CobasTaqMan HIV-1 (Roche Diagnostics); RealTime HIV-1 (Abbott Molecular).

HIV-1 p24 antigen is expressed in pg/mL; HIV-1 viral load is expressed in log_{10} copies/mL.

* Asterisks indicate values obtained on samples that were analyzed retrospectively.

(B) Western blot analysis of sequential serum samples from February to April 2007 (HIV-1/2 Blot, Genelabs). From left to right, the strips are shown in chronological order (month/day). N: negative control; WP: weakly positive control; P: positive control.

(C) Phylogenetic tree. The env sequences (415 bp) from the index case (cnr208507) and her partner (cnr2171262) were analyzed using the neighbour-joining method. The amplified sequences from the two patients were amplified and sequenced separately, and were compared with the 22 closest group O sequences (indicated by an asterisk) selected in the Los Alamos database (http://hiv-web.lanl.gov) and 3 group O reference sequences and 17 group M reference sequences representative of various clades (one strain per pure subtypes and major circulating recombinant forms). Distances were calculated using the Kimura two-parameter method (ratio T/t = 2.0) and the MEGA analysis program (http://www.megasoftware.net). Bootstrap analysis was used to test the reliability of the branching order. Bootstrap values above 50 are indicated. The tree was rooted using a SIVCPZ sequence (CPZ.CM.98.AJ271369) as outgroup.
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