Case report

Selection of an M184V mutation in the cerebrospinal fluid of a treatment-naive HIV-infected individual starting darunavir-based therapy

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Here, we describe a newly diagnosed HIV-1-infected patient, in whom shortly after the initiation of a darunavir-based regimen, the HIV-1 virus exclusively mutated in the cerebrospinal fluid (CSF), leading to an increase in CSF HIV-1 RNA load and neurological complaints.

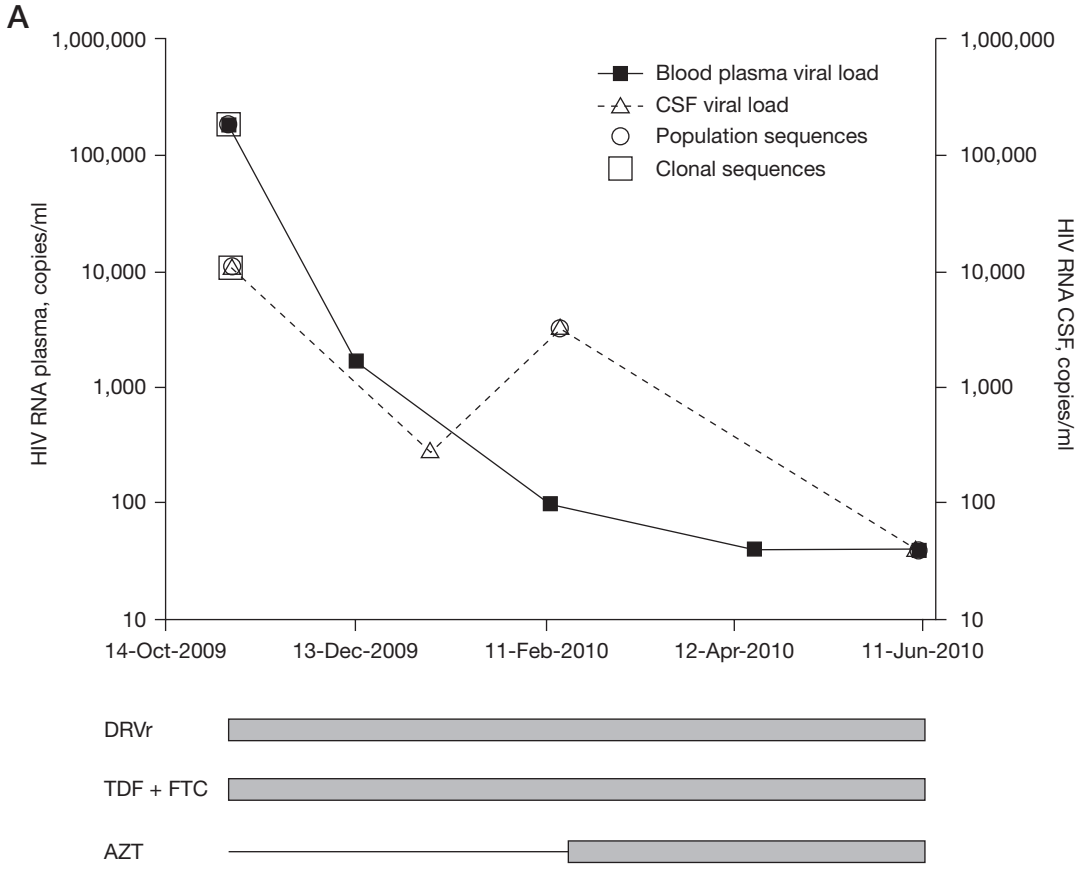
Introduction

The protease inhibitor darunavir has been classified in combination with tenofovir and emtricitabine as a preferred regimen for treatment-naive HIV-1-infected individuals in the latest DHHS and IAS treatment guidelines [1,2]. We describe a severely immunocompromised patient newly diagnosed with HIV, in whom shortly after the initiation of tenofovir/emtricitabine and ritonavir-boosted darunavir the HIV-1 virus exclusively mutated in the cerebrospinal fluid (CSF), leading to a documented rise in CSF HIV-1 RNA load and the development of neurological complaints.

Case

On 4 November 2009, a newly HIV-1 diagnosed, 46-year-old Caucasian man was referred to our intensive care unit (Academic Medical Center, Amsterdam, the Netherlands) because of respiratory insufficiency due to pneumosepsis, for which he was already treated with broad-spectrum antibiotics for several days. His $CD4^+$ T-cell count was $<10\times10^6$ cells/ml and the plasma HIV-1 viral load was 170,000 copies/ml (Figure 1A). Bacterial and fungal cultures of the performed bronchoalveolar lavage fluid were negative. Because of confusion and his low $CD4^+$ T-cell count, a CT and MRI of his brain were performed, which showed no abnormalities. Lumbar puncture was negative for bacteria (including syphilis, typical and atypical Mycobacteria), for fungi and for common viral infections. The HIV-1 viral load in his CSF at baseline was 10,000 copies/ml. In 3 days, his clinical and neurocognitive condition improved and he was referred to our department (Infectious Disease ward, Academic Medical Center). He was diagnosed with an HIV-related viral myocarditis with severe impairment of the left ventricular function (ejection fraction 10%) and a disseminated Mycobacterium avium infection, for which he received treatment with clarithromycin 500 mg twice daily, rifabutin 150 mg once daily and ethambutol 1,200 mg once daily. Serology showed evidence of a cleared hepatitis B infection reflected by a positive hepatitis B core antibody only. Hepatitis C and syphilis serology both were negative at baseline. On 19 November 2009 (14 days after the HIV diagnosis), antiretroviral therapy was initiated. A genotypic analysis at baseline of the nucleoside reverse transcriptase (RT) showed an unusual T215A mutation

Figure 1. HIV plasma and cerebrospinal fluid viral load

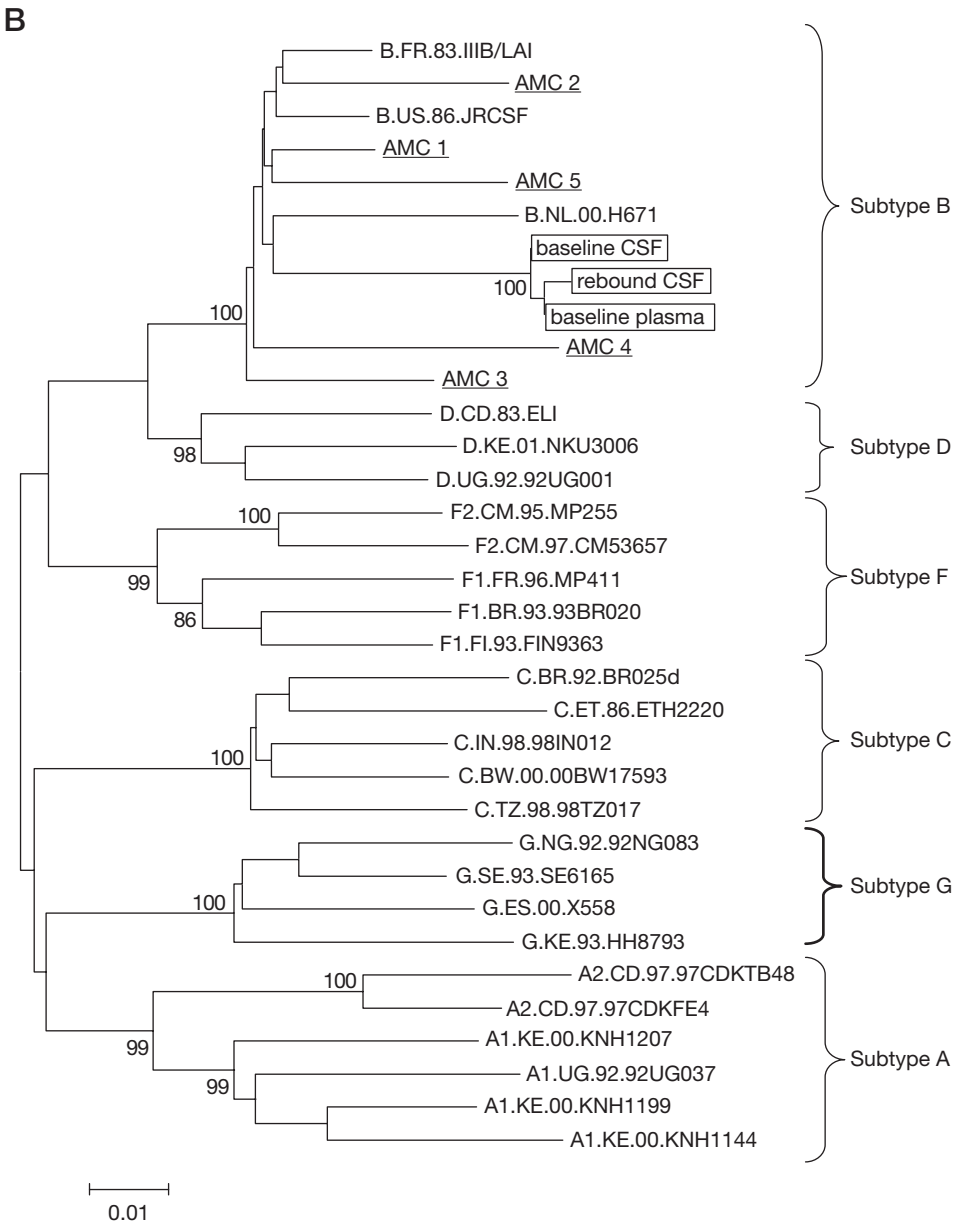


(A) HIV-1 plasma and HIV cerebrospinal fluid (CSF) viral load in copies/ml. **(B)** Neighbour-joining phylogenetic analysis based on population sequences. Baseline HIV pol sequences (plasma or CSF), obtained through routine drug resistance testing and the HIV pol sequence of the rebound CSF were used to construct a phylogenetic tree. The three sequences obtained from the patient (presented in boxes) were subtype B and clustered highly together (bootstrap 100). Local controls, named AMC, were randomly selected from the database. These samples were collected around the same time and tested identical as the described patient samples. Reference sequences were downloaded from HIV sequence database of the Los Alamos National Laboratory. The phylogenetic analysis was performed with MEGA version 4.0 software package [12]. Distances were estimated with the Kimura 2 parameter and the tree was generated with the neighbour-joining method and bootstrap resampling with 1,000 replicates. AZT, zidovudine 300 mg twice daily; DRVr, ritonavir 100 mg once daily and darunavir 800 mg once daily; FTC, emtricitabine 300 mg once daily; plasma, plasma HIV-1 viral load; TDF, tenofovir 245 mg once daily.

both in plasma and CSF. No other major mutations in RT and/or protease were observed [3]. We started with tenofovir/emtricitabine and once-daily ritonavir-boosted darunavir (100/800 mg), which resulted in an improvement of his left ventricular function and a rapid decay of the plasma HIV-1 viral load to 1,670 copies/ml after 4 weeks.

From January 2010 onwards he developed cognitive impairment, initially characterized by memory loss only. An MRI scan of his brain and a lumbar puncture were repeated, which both showed no abnormalities. Because his neurological condition deteriorated the next month with severe behavioural abnormalities, including loss of decorum, as seen in HIV dementia, an MRI scan was repeated. It now showed increased signal intensity in the white matter, compatible with HIV encephalopathy. Analysis of his CSF showed a significant increase in the HIV-1 viral load, from 270 to 3,255 copies/ml. CSF white cell count was 25 cells/mm^3 and total protein 1.52 g/l. Apart from a borderline positive PCR for Epstein–Barr virus that could not be quantified, CSF was negative for bacteria, common neurotropic viruses and mycobacteria. Furthermore, immunophenotyping showed no evidence for a haematological malignancy. The plasma HIV-1 viral load 3 days prior to the lumbar puncture was 98 copies/ml. In both samples, the darunavir concentration was determined. The plasma darunavir concentration was 3.7 mg/l 12 h after dosing and was judged to be adequate. The concentration in the CSF 7 h after dosing

Figure 1. Continued



was 0.050 mg/l, which is comparable to CSF levels for darunavir recently described [4]. We did not calculate the CSF–plasma ratio because samples were not drawn on the same day nor at the same time after intake.

Given the 1 log increase in CSF HIV-1 viral load, we performed a genotypic resistance analysis of the HIV from the CSF, which showed an M184V mutation in addition to the unusual T215A. The chromatogram of the sequence analysis did not appear as a mixture. The M184 was apparently entirely converted to 184V. This mutation was not found at baseline both in blood plasma and CSF. To exclude contamination, a phylogenetic analysis was performed using the baseline HIV pol sequences together with the HIV pol sequence, obtained from the rebound CSF (Figure 1B). The high bootstrap value (100) of the monophyletic group showed the high relationship of the three viruses. However, genotyping analysis was done by population sequencing, which can miss sequences constituting <20% of the major population. To search for the existence of an M184V mutant

at diagnosis, we studied the viral variants in CSF and blood plasma by performing an RT-PCR encompassing codon 184 of the HIV-1 pol gene. Subsequently, the amplified products were cloned and a random number of clones were selected for sequencing. For this purpose, a specific nested RT-PCR primer set around the M184 position in RT–pol was designed. RNA was isolated from blood plasma and CSF with a method using silica and guanidium thiocyanate [5]. Reverse transcription was done with primer 3′R10-M184V4 (5′-CTTCTGTATGTCATTGACAGTCCA-3′), followed by a PCR with this primer and with primer 5′R10-M184V1 (5′-TACTGCATTTACCATACCTAGTGT A-3′). One tenth volume of the first PCR was used in the second nested PCR with primers 5′R10-M184V2 (5′-GGATGGAAAGGATCACCATCAAT-3′)and3′R10-M184V3 (5′-GGCAGCGTTATGGGCTGTACTG-3′) [6]. In total, two independent RNA isolations were performed, and 70 and 71 clonal sequences from blood plasma and CSF, respectively, were analysed. In none of the 141 clonal sequences the M184V mutation was observed. To estimate genetic diversity an adequate sample size is required. Using probability consideration with 70 sequences there is a 5% probability of not sampling sequences present at a frequency of <4% [7]. This suggests that the M184V mutation in the CSF population of February 2010 can be the result of active viral replication in the central nervous system (CNS). Pending the results, we intensified treatment with zidovudine 300 mg twice daily. After intensification of his antiretroviral therapy, his cognitive function slowly improved and he was discharged to a nursing home for further revalidation. Three months later, plasma and CSF HIV-1 viral load were <40 copies/ml.

Discussion

This case, to our knowledge, is the first to demonstrate that in a previously treatment-naive HIV-1-infected individual starting tenofovir, emtricitabine and ritonavir-boosted darunavir, HIV can mutate selectively in the CSF, leading to a significant rise in CSF HIV-1 viral load. The development of the M184V mutation suggests that antiretroviral concentrations were not sufficient to prevent ongoing viral replication. Canestri *et al*. [8] recently described 11 patients with adequately suppressed plasma HIV-1 viral load in whom neurological symptoms developed due to ongoing viral replication in the CSF. Interestingly, two of the described patients were using a darunavir-based regimen. Plasma HIV-1 viral load were undetectable in both patients for >5 years. One of these patients was treated with darunavir monotherapy. In this patient, darunavir concentration was <0.005 mg/l in the CSF, whereas plasma darunavir concentration was considered adequate. The second patient was, in addition to darunavir, treated with lamivudine/abacavir/tenofovir and had adequate CSF and plasma darunavir concentrations. van Lelyveld *et al*. [9] report a similar case in which enfuvirtide resistance selectively developed in the CSF of a treatment-experienced patient, in whom this eventually led to therapy failure due to the emergence of the resistant strain in plasma. Although our patient fortunately did not show virological failure in plasma, the latter case confirms that mutated virus indeed can migrate from CSF to plasma and can potentially lead to virological failure.

Genotypic analyses in the baseline CSF and plasma as well as at the time of virological failure in CSF surprisingly showed a T215A mutation. A T215A is a very unusual mutation as shown in the Stanford database; in untreated patients, 0.1% of all records show this mutation (24/23,587 records). The T215A mutation is not reported to be associated with decreased susceptibility to nucleoside reverse transcriptase inhibitors, in contrast to the T215S/C/D/E/I/V mutations. The latter are transition mutations between susceptible and the high potentially resistant T215Y/F mutations.

We could not exclude residual undetected M184V in this patient since we tested only a fraction of the patients' total amount of plasma. However, we made a considerable effort to convincingly show that there were not detectable levels of M184V in this patient before therapy. In all 141 clonal sequences, we did not detect a single GTG valine amino acid substitution, which is suggestive for a *de novo* development of the mutation in the CSF.

Darunavir, like other protease inhibitors (PIs), is bound to plasma proteins, with only a small proportion of unbound PI available to enter the CSF. Yilmaz *et al*. [4] measured darunavir concentrations in the CSF of eight selected individuals and found these to be comparable to the 50% inhibitory concentrations previously determined in infected macrophages. Based on these findings, darunavir was scored as a drug with a good CNS penetration effectiveness score (CPE) in a recent update from the CHARTER cohort [10]. The CPE score is based on available data concerning CSF penetration of the different agents used and was previously validated [11]. In this large cross-sectional cohort study, a clear association has been demonstrated between a lower CPE score and ongoing viral replication in the CSF. Interestingly, all but one of the patients described by Yilmaz *et al*. [4] were simultaneously treated with zidovudine, which is known to have an excellent penetration in the CSF. Therefore, in our opinion, it remains uncertain whether darunavir penetration is sufficient to significantly contribute to suppression of viral replication in the brain/CSF. Our case should raise awareness to perform a CSF analysis

in patients who recently start antiretroviral therapy if new neurological complaints occur.

Disclosure statement

The authors declare no competing interests.

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