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# HIV RNA Load and Antiretroviral Drug Resistance of HIV-1 Strains in Chad on Dried Blood Spots: A Pilot Study

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## Abstract

We performed viral load (VL) and antiretroviral (ARV) drug resistance genotyping assays using dried blood spot samples (DBS) taken from HIV-1 infected patients in Chad. Among 80 adult patients naïve of any antiretroviral treatment regimen, 67 (84%) had a measured VL upper than 40 copies/ml (median; SD: 36891 copies/ml; [603-2 798 968]) of blood. Of them only 24 (36%) demonstrating a viral load upper was than 3.63 log<sub>10</sub> copies/ml were successfully sequenced for performing a drug resistance genotyping assay. Of the 24 patients whom DBS samples were successfully sequenced, seven (29%) displayed the presence of an HIV-1 strain with known mutations conferring resistance against at least one ARV family. Our pilot study provides major data on the high prevalence levels of primary resistance to available ARV drugs in HIV-1 infected patients in Chad and highlights the need for a wide and rational use of HIV anti-integrase drugs in Sahelian area.

**Keywords:** DBS, HIV viral load, Chad, NNRTI- or NRTI- primary resistant HIV strains

In low-income Sub-Saharan Africa countries, peripheral blood HIV RNA levels monitoring twice a year in antiretroviral (ARV) drugs treated patient is a major WHO recommendation since 2013 [1]. In Chad, routine biological assays for testing HIV RNA load in the peripheral blood are not continuously available for antiretroviral (ARV) treated patients even though it is a pivotal viral marker to detect a potential virological failure [1]. In resource-limited settings that organized a free but potentially discontinuous access to the available first line ARV treatment regimens composed of nucleotide reverse transcriptase inhibitor (NRTI) drugs and non-nucleotide reverse transcriptase inhibitor (NNRTI) drugs with a low viral genetic barrier, surveillance of HIV drug resistance by HIV-1 RNA genotyping assays is crucial to ensuring the continued success of ARV therapy programs [1]. To optimize an ARV therapeutic switch to efficacious 2<sup>nd</sup> line or 3<sup>rd</sup> line ARV regimens in HIV-1 infected patients a genotyping assay on peripheral blood plasma is a major biological tool in daily practice [1,2]. Moreover, heterosexual or mother-to-child

transmission of NNRTI- or NRTI- primary resistant HIV strains could represent a major public health issue and have a significant impact on national recommendations regarding the strategic choice of treatment regimens for the ARV treatment of newly discovered HIV-1 infected patients [1]. In case of ARV therapeutic failure, genotyping assays allowing the identification of HIV strains harboring resistance mutations to ARV drugs are not available in Chad using classical EDTA peripheral blood samples or DBS.

In the present time, the use of dried blood spot samples (DBS) is widely documented in published reports and several studies have previously shown that DBS was a good alternative to classic peripheral EDTA-blood samples for measuring peripheral EDTA-blood RNA viral load and identifying HIV-1 ARV resistance using classical genotyping assays [2-5]. In the present report we retrospectively assessed, for the first time, the viral load levels and the prevalence of primary resistance to ARV

drugs in Chad using DBS samples that were collected in ARV-naïve HIV-1 infected adult patients.

From March to December 2012, 80 successive newly HIV-1 seropositive adult patients who were naïve of any ARV treatment (46 F/34 M, median age 35 years [18-56]) and who were attending to the chronic diseases center of the hospital “le Bon Samaritain” (N’Djamena, Chad) were enrolled in the present investigation. The World Health Organization HIV disease clinical stage has been defined elsewhere [6]. Median lymphocyte T-CD4 cell count was performed on FACS® count system. Peripheral blood HIV-1 RNA load was measured on DBS using the Abbott Real Time HIV-1 Amplification Reagent Kit following the manufacturer’s instructions (Abbott, Molecular, Rungis France) [1]. Each DBS sample was then stored at room temperature in Chad during 6 months and then shipped and stored at -80°C until performing HIV-RNA viral load and genotyping assays in France according to previously described protocols [7,8]. Briefly, one DBS spot (70 µl of EDTA whole blood) was cut out per specimen and placed in the sample preparation system RNA for DBS processing (Abbott m200ort Instrument) according to the manufacturer’s instructions (Abbott, Molecular, Rungis France). HIV-1 viral load was automated measured using the Abbott Real Time HIV-1 Amplification Reagent Kit following the manufacturer’s instructions (Abbott, Molecular, Rungis France) and the result was expressed as HIV-1 RNA copies number per ml of peripheral blood [1]. For the specimens giving invalid result on the first test, a repeat test was performed, if an invalid result was produced again, no further attempt was made. Protease and reverse transcriptase gene regions were fully covered by our sequencing strategy and obtained HIV-RNA nucleotide sequences were compared against referenced subtype B, HBX-2 strain (GenBank accession number: K03455.1) [1]. All of our HIV-1 sequences were submitted to the Genbank and obtained an original accession number (MW250353-MW350377). Concerning drug resistance mutations, interpretation of sequencing results was performed according to the French National Agency for AIDS Research resistance algorithm [9].

An oral informed consent for medical research investigations was obtained from each study patient and if needed from their relative family members. This study was approved by the local ethics committee (CHU-BS, Ndjamen, Chad, 2012).

Among the 80 study patients, the median CD4 cell count was 348 [13-1665] cells/ml prior to initiation of HAART. Fifty percent of patients had a CD4<sup>+</sup>-T lymphocyte count <350 cells per mm<sup>3</sup>, 16% had a count between 350 and 500 cells per mm<sup>3</sup> and 34% had a count >500 cells per mm<sup>3</sup>. Concerning the quantification of HIV-1 RNA, 84% (67 out of 80) patients had an HIV RNA viral load upper than 40

copies/ml of peripheral blood recovered on DBS. Among these 67 patients, the median HIV-1 RNA load value was 36,891 (SD values: [603-2 798 968]) copies/ml on DBS. In the remaining 13 patients, six of them had a viral load of less than 40 copies/ml and seven were undetectable. Regarding our resistance genotyping test results, only 24 (36%) of 67 detectable viral RNA extracted from DBS were successfully sequenced. We observed that HIV sequencing on RNA extracted from DBS was only successful when the viral load value was upper than 3.63 log<sub>10</sub> copies/ml [7]. Of the 24 patients whom HIV RT and Protease genes were successfully sequenced, seven (29%) displayed a viral strain with known mutations conferring resistance against at least one ARV drug family. The most frequently detected mutation was V106I (21%). The K103N mutation was observed in 2 subjects (8%). K65E and V179D were also detected in two separate individuals. The major resistance mutation to the protease inhibitor L90M was observed in one patient (4%). Other polymorphism mutations (L10I, G16E, K20I, M36I, I62V, V77I, L89M) were present in 21 HIV RNA sequences (88%). HIV protease sequences in 3 patients (12.5%) identified one case of resistance to saquinavir and one case of a combined resistance to nelfinavir, indinavir and atazanavir. At the 3-month follow-up, 9 patients were lost to follow-up, 6 died and 5 moved to another hospital center. A total of 60 patients were followed-up whom 50 of them were initially treated by classical first line HAART regimen.

Our retrospective monocentric investigation using DBS taken from HIV-1-infected patients who were naïve of any ARV treatment regimens allowed to assess a prevalence of primary resistance to antiretrovirals which amounted to 29% of 67 subjects with a detectable HIV RNA load on DBS. The low adherence to drug regimens and the use of a first therapeutic line with one NNRTI drug with a low genetic barrier may explain these high levels of ARV resistance [10]. These resistance rates are compatible with those observed in 2014 in Malia with 17.5% of primary drug resistance among sequenced HIV strains [7]. In the present work, the most frequently detected mutation was V106I described as a natural polymorphism in non-B subtypes of HIV and conferring a major resistance to etravirine when associated with at least one other mutation [1]. Other identified mutations conferred major resistance to efavirenz and nevirapine or resistance to tenofovir and to rilpivirine. Interestingly, a high frequency of natural polymorphisms was observed in the HIV protease gene. The HIV protease sequences showed only one resistance to saquinavir and one conferring a combined resistance to nelfinavir, indinavir and atazanavir [11]. The main limitation of our published study was the small number of samples and the absence of standard HIV-RNA load results obtained on classical blood plasma samples. We believed that poor preservation (room temperature storage

during 6 months in Chad and frozen storage during 2 years in France until viral RNA extraction processing was performed in a virological laboratory) having destroyed or fragmented the viral genomic RNA could explain the negative results obtained in viral load and genotyping assays. The low sampling volume (two peripheral EDTA-blood spots of 70µl) could be an additional explanation for a genotyping failure. However, the new optimized Abbott DBS protocol that we used, appeared to meet the WHO performance criteria for the use of DBS for viral load monitoring with a strong correlation between peripheral blood EDTA-plasma and DBS as demonstrated previously [3]. In the present retrospective study, 50% of study patients demonstrated CD4<sup>+</sup>-T lymphocytes levels lower than 350 cells/mm<sup>3</sup> at the time of HIV serological diagnosis clearly indicating that Chadian adult patients were lately diagnosed during the natural course of HIV disease with only 37% of patients screened at WHO stage 1. This virological and immunological stage of HIV infection obviously has an impact at the individual level with an increased risk of morbidity and mortality (8.4% of deaths in our present retrospective study) but also more seriously at the collective level with a major risk of horizontal transmission to one or more partners before a documented HIV-1 infection diagnosis. In this context of immunosuppression, it is important to note that it has also been suggested that African ethnicity was independently associated with a lower T-CD4<sup>+</sup> lymphocyte reconstitution during HAART than in Caucasian ethnicity adult patient cohorts [12]. Improving adherence to the health care system also involves simplifying screening modalities and tests: performing self-sampling and using POC-type systems with DBS-type support systems. The use of DBS offers the advantage of a stable sample that is easy to collect by fingertip puncture, to transport without cold chain and with minimal risk of infection and with a biological preparation that does not require a laboratory centrifugation. DBS can be used on the main platforms presently available in laboratories located in central Africa [13]. We propose that DBS can be largely used to strengthen HIV RNA load levels survey in order to achieve the last target 90 of the UNAIDS goals (90-90-90) and finally support fighting against AIDS in Africa.

In conclusion, our pilot study provides preliminary data on the prevalence levels of primary resistance to ARV drugs in Chad and supports the concept of a widespread routine use of HIV RNA load and antiretroviral drug resistance of HIV-1 strains on DBS in Sahelian area. Moreover, our observed high levels of HIV-1 primary resistance to available ARV drugs (NNRTI, NRTI, PI) highlights the need for a wide accessibility and a rational use of new antiretroviral regimens including anti-integrase HIV drugs, as well the development of an ARV resistance surveillance network program in African resource-limited countries.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

## Ethics Approval

This study was approved by the local ethics committee (CHU-BS, Ndjamen, Walia, Chad, 2012) (non-interventional study).

## Consent to Participate

All patients were informed of the study and gave oral consent for the analysis of their samples abroad.

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