Abstract
In the absence of virological follow-up in people infected with HIV-2, is the ongoing clinical and immunological follow-up in our resource-constrained countries efficient? In this study we document the immuno-virological follow-up of patients infected with HIV-2 in Togo. Thus, a cross-sectional study was carried out at the BIOLIM laboratory of the University of Lome. The sample consisted of HIV-2 infection patients on ART, followed in care centres in Lome, Togo. Confirmation of the HIV type, CD4 + T-cells counting, HIV-2 viral load, HIV-1 viral load and HIV-2 resistance genotyping were carried out. Based on the results of serological retesting, 30 HIV-2 infected patients, including 8 HIV-1 and HIV-2 co-infected, were included. The average age was 52 years IQR [40.7-61.0years]. The median duration on antiretroviral therapy was 7 years IQR [2.00-8.75 years]. At baseline, all patients were on PI-based treatment, 80.0% of them were on lopinavir / ritonavir. The median CD4 + T-cells level was 586 cells per μl IQR [442-732 cells]. The proportion of subjects with detectable HIV-2 viral load (VL> 50 copies / ml) was 13.3% (4/30) with an average VL of 5533 copies / ml. Resistance genotyping of HIV-2 in the RT and Prot regions of the pol gene in virologically challenged subjects revealed the presence of resistance mutations respectively for the IN RT class (M184V, Q151M, K65R, V111I) and PI (I54M, I50V, V47A). Under current conditions, HIV-2-infected patients will face a long-term limit to the choice of treatment due to the onset and accumulation of ARV resistance mutations.

Keywords: HIV-2; Viral load; Drug-resistance mutations; Togo

Introduction
In West Africa, where HIV-2 infects up to 1-2 million people [1], antiretroviral therapy (ART) is becoming increasingly available and ART “scaling-up” programs proliferate. Significant numbers of HIV-2-infected individuals will have access to and will be treated with antiretroviral (ARV) drugs developed against HIV-1 infection [2]. However, HIV-2 is intrinsically resistant to the non-nucleoside reverse transcriptase inhibitors and to enfuvirtide, and reports suggest that HIV-2 may be partially resistant to some protease inhibitors (PIs) and has a low genetic barrier to nucleoside reverse-transcriptase inhibitor (NRTI) resistance [3-6].

In Togo, country located in West Africa, HIV-1 and HIV-2 circulate with a large majority of people infected by HIV-1. In the country, all people living with HIV (PLWHIV) are monitored in care centres accredited by the national AIDS program (PNLS/Togo). Monitoring of PLWHIV is done using a national guide adapted from World Health Organization (WHO) recommendations [7]. However, due to the lack of HIV-2 viral load supply, PLWHIV-2 on ART in our settings, do not have access to virological Follow-up. The aim of this study is to evaluate, based on viral load data, the quality of management of patients infected with HIV-2 in our current conditions in Togo.

Materials and Methods
Data and samples
We conducted a cross-sectional study from January to June 2017 and we included HIV-2 mono-infected patients and those infected with both HIV-2 and HIV-1 followed-up in Lome, the capital city of Togo. All patients were on ART at least for one year. For each patient, 10 ml of venous blood was collected using EDTA tube at the time of enrolment to confirm the HIV status and monitor the immunological and virological status. Using a survey form, we also recorded socio-demographic data, HIV related disease staging according to WHO classification criteria and information on ART. After performing the biological analysis, we completed the form with the number of CD4 + T-cells and the value of HIV-2 RNA viral load. In case of double-infection, we added the result of HIV-1 RNA viral load.

Laboratory methods
The HIV status of patients enrolled in this study was confirmed using INNO LIA HIV I/II Sore (Innogenetics NV Belgium). Serological tests were performed at the national reference center for HIV test and sexual transmitted infections (CNR-VIH/IST) at the university teaching hospital Sylvanus Olympio (CHUSO).

CD4 T-Cells measurement was done at the molecular biology and immunology Laboratory (BIOLIM), located at Health Science...
Faculty of University of Lome with flow cytometry standard on BD FACSCalibur using BD MultiTest reagents and MultiSet software (BD BioSciences).

The HIV-2 RNA viral load was performed with an in house Generic HIV-2 BioCentric (Bandol France) test. It is a two main technical steps test based on an automated method of extraction of retroviral RNA followed by Real-Time PCR amplification of extracted RNA. The test targets region located at Long Terminal Repeat (LTRs) and detection threshold is 10copies/ml for a sample portion of 1000μl [8]. In case of dual infection, the HIV-1 viral load measurement was undertook in the same laboratory using Abbott m2000rt after automatically extraction with Abbott m2000sp (Abbott Pack, Illinois, USA). Protease and reverse transcriptase sequencing was performed in samples with a plasma HIV-2 viral load above 50 copies/ml at the virology laboratory Bichat-Claude Bernard in France using in house methods as previously described [9].

Statistical analysis

Data were analysed using descriptive statistics and ++ exact Fisher test, + independence Chi-2 test.

Ethical aspects

The national ethics committee of Togo approved this survey. Patients were informed and had given their written consent before being included.

Table 1: Socio-demographic, clinical and duration on ART of HIV-2 and Dual in HIV-1 and HIV-2 infected patients.

|               | HIV 2 |       | HIV 1&2 |       | p-Value |
|---------------|-------|-------|---------|-------|---------|
|               | n     | %     | n       | %     |         |
| Gender        |       |       |         |       |         |
| Female        | 13    | 59    | 6       | 75    | 0.6722++|
| Male          | 9     | 41    | 2       | 25    |         |
| Age (Years)   |       |       |         |       |         |
| Median [IQR]  | 54    | [40-61]| 49      | [40-56]| 0.5331++|
| ART Duration (Years) | |       |         |       |         |
| ≤5            | 8     | 36.4  | 2       | 25    | 0.6974+ |
| >5            | 14    | 63.6  | 6       | 75    |         |
| WHO Stage     |       |       |         |       | 0.9999++|
| 1             | 19    | 86.4  | 7       | 87.5  |         |
| 2             | 3     | 13.6  | 1       | 12.5  |         |
| ART Observance|       |       |         |       |         |
| Yes           | 17    | 77.3  | 5       | 62.5  |         |
| No            | 5     | 22.7  | 3       | 37.5  |         |

++: Exact Fisher test; +: Independence Chi-2 Test; IQR: Inter-Quartile Range.

Of 30 patients included, 26(86.7%) had undetectable HIV-2 viral load. In the 4(13.3%) patients detected (CV>50 copies/ml), the HIV-2 viral load ranged from 1013 to 9874 copies/ml with a mean of 5533 copies/ml. Dual infected patients (n=8) were all, undetectable in HIV-2 viral load measurement, amongst them, 3 had a detectable HIV-1 viral load but less than 200 copies/ml.

Results

We included 30 PLWHIV-2 and followed-up in 11 care centres in Lome. Among them, 22(73.3%) were HIV-2 infected patients and 8 dual HIV-1/2 infected. Patients’ characteristics are shown in Table 1. The sex ratio M/F was 0.60%. The median age at enrolment was 54 years (Inter-quartile range (IQR): 41-61 years) and 49.5 years (IQR: 40-56 years) respectively for HIV-2 infected patients and dual HIV-1/2 patients. Most of patients (86.7%) were in stage 1 of the WHO classification. Median duration of ART was 7 years (IQR: 2-9 years) in HIV-2 mono-infected patients versus 8 years (IQR: 5-9 years) in dual HIV-1/2 infected patients. Adherence to treatment was found in 73.3% of patients.

Details on treatment and biological characteristics of patients are shown in Table 2. Thus 25(83.3%) patients received a first line treatment based on protease inhibitor (PI) regimen. At enrolment, all patients were on PI regimen comprising in 93.3% of them, Tenofovir (TDF) and Lamivudine (3TC) as nucleoside reverse transcriptase inhibitors (NRTI). The PI used was Lopinavir boosted by ritonavir in 80% of cases. Five patients experienced change in their first line treatment protocol.

The median CD4+ T-cells count for HIV-2 infected patients and dual HIV-1/2 patients was 595 cells per μl IQR [474.2-771.5 cells] and 535 cells per μl IQR[387.2 -608.8 cells] p(0.899) respectively, and 33.3 % of subjects had a CD4+ T-cells count of less than 500 cells per μl.
Table 2: Biological and treatment characteristics of HIV-2 and dual HIV-1 and HIV-2 infected patients.

| Patient Code | HIV Status | Years on ART | CD4 Cells /µl | ART at Enrolment | Other ART Received | HIV-2 Viral Load Copy/ml | HIV-1 Viral Load Copy/ml |
|--------------|------------|--------------|----------------|------------------|-------------------|-------------------------|-------------------------|
| BLP01        | HIV-2      | 1            | 707            | TDF-3TC-LPV/r    | -                 | 0                       | -                       |
| BLP02        | HIV-2      | 1            | 1713           | TDF-3TC-LPV/r    | -                 | 0                       | -                       |
| BLP03        | HIV-2      | 1            | 965            | TDF-3TC-LPV/r    | -                 | 0                       | -                       |
| BLP06        | HIV-2      | 6            | 391            | TDF-3TC-LPV/r    | -                 | 3837                    | -                       |
| BLP09        | HIV-2      | 8            | 593            | TDF-3TC-LPV/r    | -                 | 1013                    | -                       |
| BLP10        | HIV-2      | 6            | 576            | TDF-3TC-LPV/r    | -                 | 0                       | -                       |
| BLP11        | HIV-2      | 8            | 845            | TDF-3TC-LPV/r    | ABC-DDI-LPV/r     | 0                       | -                       |
| BLP12        | HIV-2      | 2            | 626            | TDF-3TC-LPV/r    | -                 | 0                       | -                       |
| BLP13        | HIV-2      | 2            | 1042           | TDF-3TC-LPV/r    | -                 | 0                       | -                       |
| BLP16        | HIV-2      | 1            | 781            | TDF-3TC-ATV/r    | -                 | 0                       | -                       |
| BLP18        | HIV-2      | 7            | 470            | TDF-3TC-ATV/r    | AZT-3TC-LPV/r     | 0                       | -                       |
| BLP19        | HIV-2      | 12           | 551            | TDF-3TC-LPV/r    | -                 | 0                       | -                       |
| BLP20        | HIV-2      | 2            | 597            | TDF-3TC-ATV/r    | -                 | 0                       | -                       |
| BLP21        | HIV-2      | 8            | 274            | TDF-3TC-LPV/r    | -                 | 9874                    | -                       |
| BLP24        | HIV-2      | 7            | 469            | TDF-3TC-LPV/r    | -                 | 0                       | -                       |
| BLP25        | HIV-2      | 7            | 536            | TDF-3TC-LPV/r    | -                 | 0                       | -                       |
| BLP27        | HIV-2      | 8            | 743            | TDF-3TC-LPV/r    | ABC-DDI-LPV/r     | 0                       | -                       |
| BLP28        | HIV-2      | 12           | 732            | TDF-3TC-LPV/r    | ABC-DDI-LPV/r     | 0                       | -                       |
| BLP31        | HIV-2      | 9            | 884            | TDF-3TC-ATV/r    | D4T-3TC-NVP       | 0                       | -                       |
| BLP35        | HIV-2      | 2            | 369            | TDF-3TC-LPV/r    | -                 | 0                       | -                       |
| BLP37        | HIV-2      | 9            | 388            | TDF-3TC-ATV/r    | TDF-3TC-LPV/r     | 0                       | -                       |
| BLP36        | HIV-2      | 7            | 487            | ABC-3TC-LPV/r    | AZT-3TC-ABC//D4T-3TC-LPV/r | 7408 | - |
| BLP05        | HIV-1&2    | 9            | 653            | TDF-3TC-LPV/r    | -                 | 0                       | 0                       |
| BLP07        | HIV-1&2    | 1            | 360            | TDF-3TC-LPV/r    | -                 | 0                       | 47                      |
| BLP23        | HIV-1&2    | 9            | 594            | ABC-3TC-LPV/r    | ABC-DDI-LPV/r     | 0                       | 143                     |
| BLP26        | HIV-1&2    | 9            | 726            | TDF-3TC-LPV/r    | -                 | 0                       | 0                       |
| BLP29        | HIV-1&2    | 2            | 373            | TDF-3TC-ATV/r    | -                 | 0                       | 0                       |
| BLP30        | HIV-1&2    | 11           | 551            | TDF-3TC-LPV/r    | D4T-3TC-NVP       | 0                       | 0                       |
| BLP32        | HIV-1&2    | 11           | 392            | TDF-3TC-LPV/r    | D4T-3TC-NVP       | 0                       | 0                       |
| BLP33        | HIV-1&2    | 6            | 519            | TDF-3TC-LPV/r    | D4T-3TC-NVP       | 0                       | 60                      |

TDF: Tenofovir; ABC: Abacavir; D4T: Stavudine; DDI: Didanosine; AZT: Zidovudine; LPV/r: Lopinavir/Ritonavir; NVP: Nevirapine; 3TC: Lamivudine; ART: Antiretroviral Therapy.

In the 4 patients with detectable viremia, protease and reverse transcriptase sequencing were successful for 2 (50%) samples. HIV-2 resistance genotyping in the reverse transcriptase and protease region of the pol gene revealed the presence of resistance mutations respectively for the NRTI class (M184V, Q151M, K65R, V111I, Y115F) and PI (I54M, I50V, V47A) (Table 3).
We aimed to carry out the measurement of the HIV-2 load in order to use the results to assess the quality of immunological and clinical follow-up available in our current conditions in Togo. We found more than 80% HIV-2 infected patients in virological success. Compared with HIV-1 infection, HIV-2 infection is characterized by a much longer asymptomatic stage, lower plasma viral loads, slower decrease in CD4+ T-cells count, decreased mortality rate associated with AIDS, and lower rates of mother-to-child transmission, and sexual transmission [10]. Our findings are in line with these characteristics. However, long-term ART could increase the CD4+ T-cells level and avoid progression to AIDS and related deaths.

The age of the patients confirmed the low transmission of HIV-2 from mother to child even we found a case of 12-year-olds vertically infected. We noted that the age ranges of the patients with HIV-2 and dual HIV-1/2 infections are different from that of HIV-1 infected patients reported in Togo [11-13] and many other African countries [14-15]. While the highest rate of HIV infected adults was from 35 years old in Togo [16], people older than 40 years were persons infected with HIV-2 and dual HIV-1/2 in our study population. As reported in Nigeria [17], there was no significant difference between the HIV-2 and HIV-1/2 dually infected patients.

The slightest virulence of HIV-2 [18] and the use of ART explain easily the fact that patients are found in stage 1 of the WHO classification. Thus, a previous study reported in 2013, in both ARV-naive and starting ART and followed-up in clinical centers in West Africa, that overall, 16.7% of HIV-2 patients on ART had an advanced clinical stage (WHO IV or CDC-C) [19]. Unfortunately we had no information on the clinical stage of the patients at ART initiation. In the same study, the median CD4 count at the ART initiation was 166 cells/mm³, IQR (83-247) among HIV-2 infected patients and 146 cells/mm³, IQR (55-249) among dually seropositive [19]. Certainly because of long-term triple therapy, median CD4+ T-cells levels was above 500 cells/μl, contrary to those reported [9].

We found that in long-term ART, 86.7% of HIV-2 infected patient included in this study were virally suppressed at enrolment. Compare to HIV-1 infected patient in the country, with the same duration on ART, less than 50% of them are found undetectable [20]. It has been shown in West Africa cohort study that 46.5% of HIV-2 infected patients were well controlling infection [21]. This seems due to a better immune response including better protection and less immunopathology [22].

Despite the low-level of HIV-2 viral load in patients enrolled in our study we found (n=4) (13.3%) subjects with detectable viremia. Similar results have been reported in others studies about patient on long term ART [9,23]. This viral replication may be due to emergence of virus with drug-resistance mutations. Other mechanisms that contribute to HIV persistence during ART, including HIV latency, immune dysfunction and persistent low-level spread of the virus to uninfected cells could lead to detectable viremia [24]. Otherwise limited drug penetration within tissues and the presence of immune sanctuaries have been argued as potential mechanism allowing HIV to spread during ART [24-26]. Amongst 4 patients with detectable viremia, 2 harbored virus with drug-resistance mutations which compromising triple therapy in progress. In one patient, after 7 years on ART, appearance of drug resistance mutations such as M184V, V111I, Q151M and I54M indicates difficulties in the future therapeutic choice. The same drug-mutations are reported in HIV-2-treated patients in Abidjan (Ivory Coast) [9]. As reported by Jallow et al. [23], these findings justify the need to offer the viral load for patient follow-up in order to detect virological escapes early [23]. Thus, it seems that keeping the viral load to undetectable or very low level will be a good marker to predict the good evolution of the infection, which remain stable for many years. However our number of study patients is very low to draw a solid conclusion regarding virological issue.

We found that in long-term ART, 86.7% of HIV-2 infected patient included in this study were virally suppressed at enrolment.
Acknowledgement

The PLHIV care centres which kindly accepted to participate to this study: CHU SO, EVT, ACS, AMC-Lome, CAPS JPII, AST-Baguida, AIDER, ATBEF, CHR Lome-Commune, le JADE, GCCST. Cephas Sehonou and Rene E. Amonde for their critical roles in sample and data collections. Dieudonne Sewu for the data analysis.

Conflict of Interest

The authors declare no conflicts of interest concerning this article.

References

1. Gottlieb GS, Badiane MN, Hawes SE, Fortes L, Toure M, et al. (2009) Emergence of multiclass drug-resistance in HIV-2 in antiretroviral-treated individuals in Senegal: implications for HIV-2 treatment in resource-limited West Africa. Clin Infect Dis 49(4): 476-483.
2. Menéndez-Arias I, Alvarez M (2014) Antiviral therapy and drug resistance in human immunodeficiency virus type 2 infection. Antiviral Res 102: 70-86.
3. Desbois D, Roquebert B, Peytavin G, Damond F, Collin G, et al. (2008) In vitro phenotypic susceptibility of human immunodeficiency virus type 2 clinical isolates to protease inhibitors. Antimicrob Agents Chemother 52(4): 1545-1548.
4. Poveda E, Rodes B, Torro C, Soriano V (2004) Are fusion inhibithors active against all HIV variants? AIDS Res Hum Retroviruses 20(3): 347-348.
5. Smith RA, Gottlieb GS, Anderson DJ, Pyrak CL, Preston BD (2008) Humman immunodeficiency virus type 1 and 2 exhibit comparable sensitivities to zidovudine and other nucleoside analog inhibitors in vitro. Antimicrob Agents Chemother 52(1): 329-332.
6. Witvrouw M, Pannecouque C, Switzer WM, Folks TM, De Clercq E, et al. (2004) Susceptibility of HIV-2, SIV and SHIV to various anti-HIV-1 compounds: implications for treatment and postexposure prophylaxis. Antivir Ther 9(1): 57-65.
7. Witvrouw M, Pannecouque C, Switzer WM, De Clercq E, et al. (2004) Genotypic resistance profiles of HIV-2-treated patients in West Africa. AIDS 28(8): 1161-1169.
8. Markovitz D, Bock P, Markovitz D (2001) Infection with HIV-2. AIDS 15 (Suppl 5): S35-S45.
9. Charpentier C, Eholié S, Anglaret X, Bertine M, Rouzioux C, et al. (2014) Extraordinary heterogeneity of virological outcomes in patients receiving highly antiretroviral therapy and monitored with the World Health Organization public health approach in Sub-Saharan Africa and Southeast Asia. Clin Infect Dis 58(1): 99-109.
10. Gottlieb GS, Badiane MN, Hawes SE, Fortes L, Toure M, et al. (2009) Emergence of multiclass drug-resistance in HIV-2 in antiretroviral-treated individuals in Senegal: implications for HIV-2 treatment in resource-limited West Africa. Clin Infect Dis 49(4): 476-483.
11. d'Almeida JF, Vidal N, Mensah A, Patassi A, Aho K, et al. (2011) High prevalence of HIV-1 drug resistance among patients on first-line antiretroviral treatment in Lomé, Togo. J Int AIDS Soc 14: 30.
12. Dagnra AY, Vidal N, Mensah A, Patassi A, Aho K, et al. (2011) High prevalence of HIV-1 drug resistance among patients on first-line antiretroviral treatment in Lomé, Togo. J Int AIDS Soc 14: 30.
13. Konou A, Salou M, Vidal N, Kodah P, Kombate D, et al. (2015) Virological outcome among HIV-1 infected patients on first-line antiretroviral treatment in semi-rural HIV clinics in Togo. AIDS Res Ther 12: 38.
14. da Silva ZJ, Oliveira I, Andersen A, Dias F, Rodrigues A, et al. (2008) Changes in prevalence and incidence of HIV-1, HIV-2 and dual infections in urban areas of Bissau, Guinea-Bissau: is HIV-2 disappearing? AIDS 22(10): 1195-1202.
15. Prince PD, Master A, van Tienen C, Whittle HC, Schim van de Loef MF (2014) Mortality rates in people dually infected with HIV-1/2 and those infected with either HIV-1 or HIV-2: a systematic review and meta-analysis. AIDS Lond Engl 28(4): 549-558.
16. PNLS-IST-Togo Ministère de la Santé (2016) Rapport annuel 2016 des activités du PNLS-IST, pp. 11-98.
17. Odaibo GN, Olakere DO (2013) Laboratory profile of HIV-2 and dual HIV-1/HIV-2 associated acquired immunodeficiency syndrome in Nigeria. World J AIDS 3(3): 192-196.
18. Marlink R, Kanki P, Thorl I, Travers K, Eisen G, et al. (1994) Reduced rate of disease development after HIV-2 infection as compared to HIV-1. Science 265(5178): 1587-1590.
19. Elouevi DK, Balestre E, Coffie PA, Minta D, Messou E, et al. (2013) Characteristics of HIV-2 and HIV-1/HIV-2 dually seropositive adults in West Africa presenting for care and antiretroviral therapy. The IeDEA-West Africa HIV-2 cohort study. PloS One 8(6): e66135.
20. Konou A, Dagnra AY, Vidal N, Salou M, Adam Z, et al. (2015) Alarming rates of virological failure and drug resistance in patients on long-term antiretroviral treatment in routine HIV clinics in Togo. AIDS 29(18): 2527-2530.
21. Elouevi DK, Avettand-Fénot V, Tchounga BK, Coffie PA, Sawadogo A, et al. (2015) Plasma HIV-2 RNA according to CD4 count strata among HIV-2-infected adults in the IeDEA-West Africa collaboration. PloS One 10(6): e0129886.
22. De Silva TL, Cotton M, Rowland-Jones SL (2008) HIV-2: the forgotten virus. Trends Microbiol 16(12): 588-595.
23. Jallow S, Alabi A, Sarge-Njie R, Peterson K, Whittle H, et al. (2009) Virological response to highly active antiretroviral therapy in patients infected with human immunodeficiency virus type 2 (HIV-2) and in patients dually infected with HIV-1 and HIV-2 in the Gambia and emergence of drug-resistant variants. J Clin Microbiol, 47(9): 2200-2208.
24. Martinez-Picado J, SG Deeks (2016) Persistent HIV-1 replication during antiretroviral therapy. Curr Opin HIV AIDS 11(4): 417-423.
25. Honecutt JB, Thayer WO, Baker CE, Ribeiro RM, Lada M, et al. (2017) HIV persistence in tissue macrophages of humanized myeloid-only mice during antiretroviral therapy. Antimicrob Agents Chemother 61(4): 2200-2208.
26. Wong JK, Yukl SA (2016) Tissue reservoirs of HIV. Curr Opin HIV AIDS 11(4): 362-370.