Low Levels of Antiretroviral-Resistant HIV Infection in a Routine Clinic in Cameroon that Uses the World Health Organization (WHO) Public Health Approach to Monitor Antiretroviral Treatment and Adequacy with the WHO Recommendation for Second-Line Treatment

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A cross-sectional study, performed at a routine human im-munodeficiency virus (HIV)/AIDS clinic in Cameroon that uses the World Health Organization public health approach, showed low rates of virological failure and drug resistance at 12 and 24 months after initiation of antiretroviral therapy. Importantly, the cross-sectional study also showed that the World Health Organization recommendation for second-line treatment would be effective in almost all patients with HIV drug resistance mutations.

To allow a rapid roll-out of antiretroviral therapy (ART), many countries are using the World Health Organization (WHO) public health approach, which proposes standard first-line ther-apy and gives guidelines for treatment initiation and for changes to the treatment regimen that are based on clinical disease progression and, where possible, on CD4$^+$ cell counts (because

monitoring of viral loads is still not feasible for the majority of patients who receive ART) [1–3]. One major consequence of this strategy could be the emergence of high levels of resis-tance to antiretroviral drugs, because many people will continue to receive virologically failing treatment regimens for longer periods, which could compromise the efficacy of second-line therapy and increase the risk of transmission of drug-resistant strains [4]. There is thus an urgent need to study virological failure and drug resistance mutations in routine-care settings in resource-limited countries to evaluate whether the empirical second-line treatment recommended by the WHO would still be efficient under such conditions.

Here, we describe the virological outcome, as measured by viral plasma load and genotypic drug resistance profiles, in patients who were treated according the WHO public health approach in the HIV/AIDS outpatient clinic of the Central Hospital in Yaoundé, Cameroon. At this clinic, access to ART has been available since 2001. From 2001 through 2007, the cost of ART decreased, and ART became free of charge in May 2007. Similarly, the cost of obtaining a CD4$^+$ cell count also decreased over this period but is only partially covered by the government. The cost of a viral load measurement remains entirely the responsibility of the patient. A cross-sectional study was performed among HIV-infected adults ⩾18 years of age who were consecutively enrolled from November 2006 through October 2007 at their follow-up visit after 12 or 24 months of ART (plus or minus 2 months). After informed consent was obtained, a standardized questionnaire was administered to as-sess demographic, epidemiologic, clinical, treatment, and ad-herence information, and 10 mL of whole blood was collected from each patient in EDTA tubes. After centrifugation, plasma aliquots were frozen at −80° C. To differentiate between in-fection due to HIV-1 groups M, N, and O and infection due to HIV-2, serum samples were tested by an in-house indirect ELISA [5]. HIV-1 RNA levels in plasma were measured with a second-generation real-time RT-PCR (Generic HIV Viral Load Assay; Biocentric) with a lower limit of detection of 300 copies/mL [6]. Genotypic antiviral drug resistance testing for HIV-1 group M was performed on plasma samples with HIV-1 RNA levels ⩾1000 copies/mL using an in-house assay described else-where [7]. HIV-1 group O samples were amplified and se-quenced with an in-house assay with use of group O–specific primers. Amino acid sequences were analyzed for the presence of mutations in protease and reverse-transcriptase genes with the drug resistance interpretation algorithm from Agence Na-tionale de Recherche sur le Sida et le Hépatites (version July

2007). The identification of HIV-1 subtypes and circulating recombinant forms (CRFs) was done by phylogenetic tree and recombination analysis, as described elsewhere [8].

After approval of the study by the National Ethics Committee of Cameroon, a total of 249 and 178 HIV-positive patients who had received ART for 12 and 24 months, respectively, were enrolled. All patients were described as antiretroviral naive at ART initiation, and none of the women included in the study had a history of receiving drugs for prevention of mother-to-child transmission of HIV before beginning ART. Only 1 female patient at month 24 of ART had HIV infection identified as HIV-1 group O infection by serological testing and confirmed by PCR. All patients received treatment at the time of study recruitment, and their demographic and clinical characteristics are described in table 1. Almost all patients received lamivudine-stavudine, zidovudine-efavirenz, and nevirapine as first-line treatment, including 246 (98.8%) of 249 patients at month 12 and 172 (98.6%) of 178 patients at month 24. Thirty-six (8.4%) of the 427 patients switched therapy for medical reasons (e.g., major adverse effects, pregnancy, or incompatibility with tuberculosis treatment); in most of these cases, stavudine was replaced by zidovudine or nevirapine was replaced by efavirenz. Thirty-four (8.0%) of the patients interrupted treatment for personal reasons (e.g., financial constraints), for travel, in favor of traditional medicine, or because of an irregular drug supply.

Fifty-five (22.1%) of 249 patients (95% CI, 17.1%–27.8%) at month 12 and 45 (25.3%) of 178 patients (95% CI, 19.1%–32.3%) at month 24 had plasma HIV RNA levels >500 copies/mL and met the definition for virologic failure. The 95% CIs of the proportions of patients with detectable HIV-1 viral load were estimated using the binomial exact method. Although the proportion of patients with a viral load >500 copies/mL was almost equal at month 12 and month 24, the median viral load for samples with >500 copies/mL was lower at month 12 than it was at month 24 (table 1). Of the 100 patients infected with HIV-1 group M who had plasma HIV RNA levels >500 copies/mL, 81 had plasma HIV RNA levels >1000 copies/mL and had isolates that underwent attempted sequencing for genotypic drug resistance testing. Of these 81 patients, 72 (88.9%) had successful HIV genotyping, and the following group M subtypes and CRFs were identified: CRF02-AG (44 patients), D (7 patients), A-Cam (a subcluster of subtype A; 5 patients), A (4 patients), F (4 patients), CRF11cpx (3 patients), G (2 patients), CRF13cpx (2 patients) and CRF01-AE (1 patient). Overall, at least 41 (50.6%) of 81 patients with a viral load >1000 copies/mL had HIV with at least 1 major drug resistance mutation, corresponding to a minimal overall rate of HIV resistance of 11 (4.4%) of 249 (95% CI, 2.2%–7.8%) at month 12 and 30 (16.9%) of 178 (95% CI, 11.7%–23.2%) at month 24. Importantly, if 41 (56.9%) of the 72 HIV sequences were drug resistant, this proportion was 11 (32.4%) of 34 at month 12 and

30 (78.9%) of 38 at month 24. The WHO recommends that a viral load threshold of 10,000 copies/mL be used to define virologic failure in resource-limited countries; in our study, 51 patients met this definition. Compared with patients who had viral loads >1000 copies/mL, the patients who had viral loads >10,000 copies/mL had a higher proportion of drug-resistant HIV infection (37 [72.5%] of 51). Table 1 also summarizes the drugs to which the strains where resistant and the different mutations that were observed. Of 11 patients with drug-resistant HIV strains at month 12, 10 had strains that were resistant to lamivudine, 10 harbored strains with nonnucleoside reverse-transcriptase inhibitor (NNRTI) mutations that conferred resistance to nevirapine and efavirenz, and 2 harbored strains that also had thymidine analogue mutations (TAMs) that conferred resistance to zidovudine and stavudine. Overall, at month 12, 7 of 11 patients had HIV that was resistant to 2 of the 3 drugs in their regimen, and 2 of 11 had HIV that was resistant to all of the drugs in their treatment regimen. One patient had a strain that accumulated many nucleoside reverse-transcriptase inhibitor (NRTI) mutations, which implied that the strain might also have developed cross-resistance to abacavir and, potentially, to tenofovir. At month 24, 30 drug-resistant HIV strains were observed, 28 of which were isolated from patients who received lamivudine-zidovudine, stavudine-nevirapine, and efavirenz. Two of the 6 patients who received protease inhibitors had strains that were resistant to both of the NRTIs that they received, and 1 of these patients harbored a strain that had major protease inhibitor resistance mutations (V82A and L90M). Of the remaining 28 patients, all had strains that were resistant to nevirapine-efavirenz, 25 had strains that were resistant to lamivudine-emtricitabine, and 5 harbored strains that also had TAMs. Overall, 25 of 28 patients had strains that were resistant to 2 drugs (20 strains) or 3 drugs (5 strains) that were included in their treatment regimen. In strains isolated from 3 additional patients, we also observed the presence of D67DN, which is associated with resistance only when other TAMs are simultaneously present. In a strain isolated from 1 additional patient, we observed Y115FS, which can be associated, in combination with other mutations, with resistance to abacavir. Similar to our findings at month 12, 1 patient at month 24 had a strain that had also accumulated NRTI mutations that caused cross-resistance to other NRTIs, didanosine, and potentially abacavir and tenofovir. At month 12, all NNRTI-resistant strains had only a single major mutation (table 1). Interestingly, there were more NNRTI mutations among isolates obtained from patients who had been receiving ART for 24 months; only 15 of 28 strains had a single mutation, 11 of 28 had 2 major mutations, and 2 of 28 had 3 major mutations. The single patient with HIV-1 group O infection had a strain that was resistant to lamivudine, emtricitabine (M184V was present), and nevirapine-efavirenz (A98G and Y181C were

Table 1. Demographic and clinical characteristics of the HIV-infected study population, genotypic drug resistance after 12 months and 24 months of antiretroviral therapy (ART) in patients with viral loads >1000 copies/mL, and patient characteristics associated with drug-resistant infection.

Variable	Month 12 (*n* = 249)	Month 24 (*n* = 178)
Sex		
Female	176	130
Male	73	48
Age, median years (IQR)		
Female	35 (27–49)	37 (31–44)
Male	40 (34–46)	41 (36–49)
First-line ART		
3TC + D4T + NVP	118	104
3TC + AZT + NVP	9	1
3TC + D4T + EFV	58	40
3TC + AZT + EFV	61	27
3TC + D4T + IDV	…	3
3TC + AZT + IDV	1	2
3TC + DDI + EFV	2	1
Treatment switch[a]	20/249	16/178
Treatment interruption[b]	10/249	24/178
Viral load test during ART	0	14/178
CD4$^+$ cell count within 6 months	136/249	78/178
Viral load >500 copies/mL	55/249 (22.1)	45/178 (25.3)
Viral load[c], median log$_{10}$ copies/mL	3.13 (2.97–4.39)	4.39 (3.4–5.22)
Viral load >1000 copies/mL[d]	41 (16.4)	40 (22.5)
PCR amplification of samples with viral load >1000 copies/mL	34/41	38/40
Presence of ≥1 major drug resistance mutation in amplified samples	11/34	30/38
Genotypic resistance		
3TC-FTC only	1	0
NVP-EFA only	1	3
3TC-FTC + NVP-EFV	7	20
3TC-FTC + AZT-D4T + NVP-EFV	1	4
3TC-FTC + AZT-D4T + NVP-EFV + ABC + TDF	1	0
3TC-FTC + AZT-D4T + NVP-EFV + DDI + ABC-TDF	0	1
3TC-FTC + AZT-D4T	0	1
3TC-FTC + AZT-D4T + IDV	0	1
Mutations associated with NNRTI resistance[e]		
K103N	6	8
Y181C	3	3
Y188L	1	1
G190A	…	1
K101EK	…	1
V106A	…	1
K101E, G190S/A	…	2
K103N, G190A	…	1
K103N , A98S	…	2
K103N, P225H	…	3
K103N, Y181C	…	1
K103N, M230L	…	1
K103N, K101KP	…	1
K101EK, Y181CY, G190A	…	1
K103N, Y188L, A98S	…	1

Table 1. *(Continued.)*

Variable	Month 12 (n = 249)	Month 24 (n = 178)
Mutations associated with NRTI resistance[f]		
T215Y	1	1
M41L, D67N, L210W, T215Y	1	…
M41L, T215FY	…	2
M41L L74V, T215Y	…	1
D67N, K70R, K219Q	…	1
D67N, K70R, K219E	…	1
D67N, K70R, T215Y, K219Q	…	1
M184V	10	27
Resistance after treatment switch[a]	2/20 (10.0)	3/16 (18.8)
Resistance after treatment interruption[b]	0/10	14/24 (58.3)

NOTE. Data are no. (%) of patients, unless otherwise indicated. ABC, abacavir; AZT, zidovudine; DDI, didanosine; D4T, stavudine; EFV, efavirenz; FTC, emtricitabine; IDV, indinavir; IQR, interquartile range; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; NVP, nevirapine; TDF, tenofovir; 3TC, lamivudine.

[a] Medical reasons included major adverse effects, pregnancy, and incompatibility with tuberculosis treatment.
[b] Personal reasons included financial constraints related to medical visits, travel, preference for traditional medicine, or irregular drug supply.
[c] Median viral load was calculated for samples with a viral load >500 copies/mL.
[d] Viral load >1000 copies/mL corresponds to the detection limit of genotypic drug resistance tests.
[e] Only A98S and K101KP are associated with possible resistance to NNRTIs, all other NNRTI mutations shown in the table are associated with NNRTI resistance.
[f] NRTI mutations include the thymidine analogue mutations M41L, D67N, K70R, T215Y/F, and K219Q/E.

present); however, the possibility that the NNRTI mutations were natural polymorphisms characteristic for HIV-1 group O cannot be excluded.

Overall, relatively low rates of genotypic drug resistance were observed among these patients, who were monitored according to the WHO public health approach. However, because the first-line regimen included 2 drugs with low genetic barriers to resistance, the majority of patients with HIV strains that had drug resistance mutations had strains that were resistant to at least 2 of the 3 drugs in their treatment regimen (36 [87.8%] of 41 patients). It is probable that we underestimated the rate of drug-resistant infection among treated patients, because we used a cross-sectional approach, and only patients who were still receiving treatment were observed. Therefore, our findings are useful for clinicians managing patients and are also an indicator of the efficacy of the ART program among patients who are still receiving treatment, but our study does not provide any information on how many patients withdrew from care or died. However, patient retention in ART programs is an important issue [9], and studies are ongoing to measure this parameter for the evaluation of the overall efficacy of the national ART program in Cameroon. Similar to other studies [10, 11], we showed that >90% of patients with HIV-resistant strains had strains that were resistant to nevirapine-efavirenz and lamivudine-emtricitabine. An important finding of our study is that, with the exception of 2 cases, the second-line regimen recommended by the WHO (emtricitabine-lamivudine plus tenofovir or didanosine-abacavir with a boosted protease inhib-

itor) would still be effective. This contradicts the findings of a recent report from Malawi, but diagnosis of treatment failure was made on the basis of clinical criteria only, which possibly delayed detection, and HIV subtype C, which preferentially selects the K65R mutation, predominated in this study population [11].

The absence of genotypic drug resistance in strains isolated from patients with virological failure raises the problem of the interpretation of a single viral load test. We showed that only one-half of patients with a viral load >1000 copies/mL harbored drug-resistant strains, compared with 70% of patients with viral loads >10,000 copies/mL, which thus reinforces the WHO recommendation to switch to second-line therapy at a viral load of 10,000 copies/mL [2]. However, in both scenarios, the use of viral load data without adherence intervention and without drug resistance testing after subsequent repeated detectable viral load measurements (if available) will lead to unnecessary therapeutic switching in a substantial proportion of nonadherent patients, with the risk of wasting the financial resources of the program on more-expensive second-line therapy. On the other hand, for one-half of the patients with viral loads >1000 copies/mL, an adequate use of viral load data would avoid the accumulation of drug-resistant mutations, which can jeopardize long-term prognosis and some second-line options. The results of our study show the importance of viral load data for the detection of nonadherent patients; however, alternative, less sophisticated, and less expensive methods of adherence monitoring have to be evaluated in parallel, especially in the context

of implementing ART programs in nonurban areas [12]. Importantly, the WHO recommendations for second-line treatment would still be effective for almost all patients in this setting in Cameroon.

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