Prediction of HIV Drug Resistance Based on Virologic, Immunologic, Clinical, and/or Adherence Criteria in the Stratall ANRS 12110/ESTHER Trial in Cameroon

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Our study in Cameroonian rural district hospitals showed that the immunologic and clinical failure criteria had poor performance to identify human immunodeficiency virus drug resistance in a timely manner. Switching to second-line antiretroviral therapy after 2 consecutive viral loads ≥5000 copies/mL, as recommended by the World Health Organization, appeared to be the most appropriate strategy.

Keywords. HIV; Africa; resistance; switch; failure.

The identification of the optimum time for switching to second-line antiretroviral therapy (ART) remains challenging in resource-constrained settings because of laboratory limitations [1]. Undetected human immunodeficiency virus (HIV) resistance on first-line therapy increases the risk of morbidity, mortality, HIV transmission (including drug-resistant viruses), and accumulation of resistance mutations [2]. The latter subsequently may compromise the efficacy of second-line regimens. Conversely, premature or unnecessary switching to second-line ART may be harmful for both the patients and the health system because these regimens are less accessible, more complex, and more expensive [3]. The issue is especially critical as the usual first-line regimens include 2 drugs with a weak genetic barrier (ie, lamivudine or emtricitabine, and nevirapine or efavirenz).

In routine healthcare in Africa, switching to second-line ART is often decided on the basis of clinical or immunologic failure criteria, and rarely on virologic failure criteria. Moreover, resistance testing is very uncommon, and the World Health Organization (WHO) recognizes that the current recommendation on when to switch ART is based on low quality of evidence. Whereas previous studies compared clinical and/or immunologic failure vs virologic failure [4–8], we assessed the performance of virologic, immunologic, clinical, and adherence criteria alone or combined to identify HIV drug resistance in rural district hospitals in Cameroon.

PATIENTS AND METHODS

A substudy of the Stratall trial—designed to compare monitoring strategies—was performed between May 2006 and April 2010 in 9 district hospitals in Cameroon among ART-naive patients followed up for 24 months after ART initiation [9]. In brief, patients were eligible if they were aged ≥18 years, had HIV type 1 group M infection, and WHO stage 3 or 4 disease or stage 2 disease with a total lymphocyte count ≤1200 cells/μL. The National Ethics Committee of Cameroon and the Institutional Ethics Committee of the French Institut de Recherche pour le Développement approved the protocol.

Clinical visits were scheduled at weeks 0 and 2, months 1 and 3, and every 3 months thereafter. The first-line antiretroviral regimen included 2 nucleoside reverse transcriptase inhibitors (NRTIs) plus 1 nonnucleoside reverse transcriptase inhibitor (NNRTI). Clinical staging of HIV disease was based on the 2006 WHO classification [10]. ART adherence was measured through validated face-to-face questionnaires administrated by community health workers at 1, 3, 6, 12, 18, and 24 months. This tool investigates the respect of the dosing schedule in both the previous 4 days and 4 weeks. Data on adherence collected by physicians or nurses during clinical visits were also used to increase the sensitivity to detect nonadherence behaviors [11].

Plasma viral load (RealTime HIV-1 assay, Abbott Molecular, Des Plaines, Illinois) and CD4 cell count (FACSCount device,

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Becton Dickinson, Mountain View, California) were recorded at baseline and every 6 months thereafter. Genotypic mutations associated with antiretroviral drug resistance (Abbott Viroseq assay, Celera Diagnostics, Alameda, California) were assessed when the viral load was ≥5000 copies/mL on 2 consecutive samples or when the patient's last available viral load was above this threshold. If resistance was detected in those samples, the corresponding baseline samples were also tested to detect primary resistances. Mutations were classified as minor or major using the French National Agency for Research on AIDS and Viral Hepatitis (ANRS) consensus statements on antiretroviral drug resistance from September 2012 [12]. Viral load, CD4 cell count, and resistance testing were performed in a reference HIV laboratory accredited by the WHO for HIV resistance testing and registered to the Centers for Disease Control and Prevention and Quality Assessment and Standardization for Immunological measures relevant to HIV/AIDS external quality-control programs for viral load and CD4 cell count, respectively.

The emergence of HIV resistance during follow-up was compared to (1) a concomitant viral load ≥5000 copies/mL; (2) a concomitant fall of CD4 count to baseline, 50% fall from on-treatment peak CD4 value, or persistent CD4 count of <100 cells/μL; (3) a concomitant, new, or recurrent WHO stage 4 condition, pulmonary tuberculosis, or severe bacterial infection; and (4) an adherence <100% from month 1 to the visit preceding the point of failure by each of the above definitions. This threshold was chosen to increase the sensitivity to detect nonadherence behaviors. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value and 95% confidence intervals (CIs) of criteria for resistance development were estimated using bootstrap procedures. Patients (n = 12) who switched to second-line regimens during follow-up were excluded from the analysis thereafter.

RESULTS

Of 459 Stratall participants, 2 with primary resistances and 1 infected with a group O virus were excluded from this analysis. The remaining 456 patients were mostly women (70.8%) and had a median age at inclusion of 37 years (interquartile range [IQR], 30–45 years). Of them, 73.5% and 26.3% were at WHO clinical stage 3 and stage 4, respectively. Median CD4 cell count was 181 cells/μL (IQR, 87–336 cells/μL) and viral load was 5.6 $\log_{10}$ copies/mL (IQR, 5.2–6.1 $\log_{10}$ copies/mL). The first-line antiretroviral regimen was stavudine, lamivudine, and nevirapine (n = 301 [66.0%]); stavudine, lamivudine, and efavirenz (n = 82 [18.0%]); zidovudine, lamivudine, and efavirenz (n = 39 [8.6%]); or zidovudine, lamivudine, and nevirapine (n = 34 [7.5%]). Thirty-eight patients (8.3%) were lost to follow-up at month 24. The total and median follow-up durations equaled 712 person-years and 24 months (IQR, 18–24 months), respectively.

Seventy-four patients had at least 1 follow-up viral load ≥5000 copies/mL. Of them, 15 had isolated viral loads ≥5000 copies/mL followed by lower viral loads thanks to adherence intervention, 35 had 2 consecutive viral loads ≥5000 copies/mL, and 24 had isolated viral loads ≥5000 copies/mL at their last measurement. The latter 2 groups were tested for resistance. Forty-five patients (34 with 2 consecutive viral loads ≥5000 copies/mL and 11 with 1 only) had resistance after a median time of 12.3 months (IQR, 6.5–18.1 months) from ART initiation. All 45 patients had resistance to NNRTIs—specifically to nevirapine (n = 45) and efavirenz (n = 42)—and 31 (68.9%) also had resistance to NRTIs, specifically to lamivudine/emtricitabine (n = 31). Thirty-eight patients had resistance from the first or isolated viral load ≥5000 copies/mL. The PPV to identify resistance was 50.8% (95% CI, 39.3–63.0) for the first or isolated viral load ≥5000 copies/mL and 97.3% (95% CI, 90.3%–100.0%) for 2 consecutive viral loads ≥5000 copies/mL.

Table 1 shows that the performance was low for all other criteria. Immunologic failure alone had a sensitivity of 42.2% and a PPV of 15.0%, and occurred a median of 5.1 months (IQR, 0.0–7.2 months; range, 0.0–18.9 months) after the emergence of resistance. When a viral load ≥5000 copies/mL confirmed the immunologic failure, the sensitivity remained similar but the PPV increased to 54.1%. The highest sensitivity (76.7%) was achieved with the combined virologic and adherence criterion; the PPV was then 50.8%. The median delay between resistance emergence and this combined criterion was 0.0 month (IQR, 0.0–4.8 months; range 0.0–17.7 months). The clinical-based criteria had the lowest sensitivity (4.3%–6.5%). The specificity was <95% for most criteria. Finally, the negative predictive value ranged between 90% and 98% for all criteria.

DISCUSSION

This study in rural district hospitals in Cameroon showed that switching to second-line ART after 2 consecutive viral loads ≥5000 copies/mL as recommended by the WHO appeared appropriate in almost all cases [1]. However, the second viral load should be measured shortly after adherence intervention following the first elevated viral load (ideally 1 month) because most patients have resistance from this measurement. By contrast, switching after a single viral load ≥5000 copies/mL would result in numerous patients being prescribed prematurely or unnecessarily second-line ART. Indeed, half of our patients with a single or the first of 2 viral loads ≥5000 copies/mL had no resistance. This figure is comparable to a previous finding in routine healthcare in Cameroon [13] but higher than reported elsewhere [14].

As expected, clinical failure criteria and, to a lesser extent, immunologic failure criteria had poor performance to identify HIV resistance in a timely manner. The use of a single viral

Table 1. Performance of Virologic, Immunologic, Clinical, and/or Adherence Criteria to Identify HIV Drug Resistance Among 456 Patients Receiving Antiretroviral Therapy in 9 District Hospitals in Cameroon, 2006–2010, Stratall ANRS 12110/ESTHER Trial

	Sensitivity			Specificity			Positive Predictive Value			Negative Predictive Value		
Criteria	no./No.	%	95% CI	no./No.	%	95% CI	no./No.	%	95% CI	no./No.	%	95% CI
Virologic (unconfirmed)[a]	NA	NA	NA	NA	NA	NA	38/74	50.8	39.3–63.0	NA	NA	NA
Virologic (confirmed)[b]	NA	NA	NA	NA	NA	NA	34/35	97.3	90.3–100.0	NA	NA	NA
Immunologic	17/40	42.2	27.9–58.1	321/416	77.2	73.1–81.1	17/112	15.0	8.8–21.8	321/344	93.3	90.5–96.1
Clinical	3/44	6.5	.0–15.4	379/412	92.0	89.1–94.5	3/36	8.1	.0–18.8	379/420	90.2	87.4–93.1
Virologic and immunologic	18/42	42.5	28.0–58.3	399/414	96.4	94.5–98.1	18/33	54.1	37.1–72.0	399/423	94.3	92.0–96.6
Virologic and clinical	3/44	6.5	.0–15.4	410/412	99.5	98.8–100.0	3/5	60.0	.0–100.0	410/451	90.9	88.2–93.6
Virologic and adherence	30/39	76.7	63.0–88.9	388/417	93.0	90.5–95.3	30/59	50.8	37.5–63.0	389/397	97.7	96.1–99.0
Immunologic and clinical	2/44	4.3	.0–11.5	404/412	98.1	96.6–99.3	2/10	18.8	.0–50.0	404/446	90.6	87.9–93.3
Immunologic and adherence	15/42	35.9	20.5–51.4	356/414	86.0	82.4–89.0	15/73	20.3	11.7–29.7	356/383	92.9	90.3–95.6
Clinical and adherence	2/45	4.3	.0–11.1	386/411	93.9	91.5–96.1	2/27	6.9	.0–18.8	386/429	90.0	87.1–92.8

Virologic criterion: viral load ≥5000 copies/mL. Immunologic criteria: fall of CD4 count to baseline, 50% fall from on-treatment peak CD4 value, or persistent CD4 count of <100 cells/μL. Clinical criteria: new or recurrent, World Health Organization stage 4 disease, pulmonary tuberculosis, or severe bacterial infection. Adherence criterion: adherence <100%. The number of data available at months 6, 12, 18, and 24 was 370, 352, 340, and 318 for viral load; 369, 353, 340, and 318 for CD4 cell count; 384, 363, 348, and 330 for clinical assessment; and 389, 378, 364, and 343 for adherence assessment, respectively. Adherence <100% was achieved in 184, 217, 245, and 263 patients at months 6, 12, 18, and 24, respectively.

Abbreviations: CI confidence interval; NA, not applicable.

[a] A single viral load ≥5000 copies/mL.

[b] Two consecutive viral loads ≥5000 copies/mL.

load measurement of ≥5000 copies/mL to confirm treatment failure reduced the number of false-positive results. However, half the patients classified as having a treatment failure would have only required adherence intervention. On the other hand, the use of immunologic failure criteria to confirm clinical failure (also recommended by the WHO) only slightly improved the performance compared to clinical failure alone and was less sensitive than immunologic failure alone. ART adherence criterion was only helpful when a single viral load ≥5000 copies/mL was used.

Our study has several limitations. First, resistance testing was only performed in samples with viral load ≥5000 copies/mL corresponding to the threshold recommended by the WHO to define virologic failure in resource-constrained settings. We cannot rule out a few cases of resistance despite viral loads <5000 copies/mL. Patients who resuppressed following a single viral load ≥5000 copies/mL were not genotyped because one can reasonably assume that they had not developed resistance. Second, viral load and CD4 cell count were monitored every 6 months. The performance of criteria could have been different with a longer schedule [15], which is more likely to be encountered in routine clinical care. Third, the number of events for clinical-based combined criteria was small, limiting the precision of our estimates. These estimates should therefore be seen as indicative only.

In conclusion, this study showed that only a virologic failure confirmed on 2 consecutive viral loads allows adequate switches to second-line ART. The use of other criteria may result in many patients with resistance remaining on failing first-line ART on the one hand and many other patients switched unnecessarily or prematurely to expensive second-line ART on the other hand. These findings emphasize the need for viral load monitoring (eg, through point-of-care testing) and support the WHO-recommended strategy of switching to second-line ART after a confirmed virologic failure despite adherence intervention.

Notes

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References

1. World Health Organization. Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public health approach. 2010 revision. Available at: http://whqlibdoc.who.int/publications/2010/9789241599764_eng.pdf. Accessed 19 May 2013.
2. Hamers RL, Sigaloff KCE, Kityo C, Mugyenyi P, de Wit TFR. Emerging HIV-1 drug resistance after roll-out of antiretroviral therapy in sub-Saharan Africa. Curr Opin HIV AIDS **2013**; 8:19–26.
3. Médecins Sans Frontières (MSF). Untangling the web of antiretroviral price reductions. 15th ed. **2012**; 1–120. Available at: http://aids2012.msf.org/wp-content/uploads/2012/07/MSF_Access_UTW_15th_Edition_2012_webres.pdf. Accessed 19 May 2013.
4. Rawizza HE, Chaplin B, Meloni ST, et al. Immunologic criteria are poor predictors of virologic outcome: implications for HIV treatment monitoring in resource-limited settings. Clin Infect Dis **2011**; 53: 1283–90.
5. Sigaloff KC, Hamers RL, Wallis CL, et al. Unnecessary antiretroviral treatment switches and accumulation of HIV resistance mutations; two arguments for viral load monitoring in Africa. J Acquir Immune Defic Syndr **2011**; 58:23–31.
6. Reynolds SJ, Nakigozi G, Newell K, et al. Failure of immunologic criteria to appropriately identify antiretroviral treatment failure in Uganda. AIDS **2009**; 23:697–700.
7. Kantor R, Diero L, Delong A, et al. Misclassification of first-line antiretroviral treatment failure based on immunological monitoring of HIV infection in resource-limited settings. Clin Infect Dis **2009**; 49: 454–62.
8. Mee P, Fielding KL, Charalambous S, Churchyard GJ, Grant AD. Evaluation of the WHO criteria for antiretroviral treatment failure among adults in South Africa. AIDS **2008**; 22:1971–7.
9. Laurent C, Kouanfack C, Laborde-Balen G, et al. Monitoring of HIV viral loads, CD4 cell counts, and clinical assessments versus clinical monitoring alone for antiretroviral therapy in rural district hospitals in Cameroon (Stratall ANRS 12110/ESTHER): a randomised non-inferiority trial. Lancet Infect Dis **2011**; 11:825–33.
10. World Health Organization. Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public health approach. **2006 revision**. Available at: http://www.who.int/hiv/pub/guidelines/artadultguidelines.pdf. Accessed 18 April 2013.
11. Meresse M, Carrieri MP, Laurent C, et al. Time patterns of adherence and long-term virological response to non-nucleoside reverse transcriptase inhibitor regimens in the Stratall ANRS 12110/ESTHER trial in Cameroon. Antivir Ther **2013**; 18:29–37.
12. French National Agency for Research on AIDS and Viral Hepatitis. HIV-1 genotypic drug resistance interpretation's algorithms, **2012**. Available at: http://www.hivfrenchresistance.org/2012/tab1.html. Accessed 19 May 2013.
13. Kouanfack C, Montavon C, Laurent C, et al. 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. Clin Infect Dis **2009**; 48:1318–22.
14. Marconi VC, Sunpath H, Lu Z, et al. Prevalence of HIV-1 drug resistance after failure of a first highly active antiretroviral therapy regimen in KwaZulu Natal, South Africa. Clin Infect Dis **2008**; 46:1589–97.
15. Gupta RK, Hill A, Sawyer AW, et al. Virological monitoring and resistance to first-line highly active antiretroviral therapy in adults infected with HIV-1 treated under WHO guidelines: a systematic review and meta-analysis. Lancet Infect Dis **2009**; 9:409–17.