Drug resistance in HIV-positive adults during the initial year of antiretroviral treatment at Ethiopian health centers

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Key points:
Among antiretroviral treatment-naïve persons at Ethiopian health centers antiretroviral drug resistance mutations emerged in a majority of those not achieving HIV RNA below 500 copies/ml after 6-12 months treatment. Early recognition of treatment failure is crucial to mitigate resistance accumulation.

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Abstract

Objectives

The increasing prevalence of antiretroviral drug resistance in sub-Saharan Africa threatens the success of HIV programs. We have characterized patterns of drug resistance mutations (DRM) during the initial year of antiretroviral treatment (ART) in HIV-positive adults receiving care at Ethiopian health centers and investigated the impact of tuberculosis on DRM acquisition.

Methods

Participants were identified from a cohort of ART-naive individuals aged \( \geq 18 \) years, all of whom had been investigated for active tuberculosis at inclusion. Individuals with viral load (VL) data at 6 and/or 12 months after ART initiation were selected for this study. Genotypic testing was performed on samples with VL\( \geq 500 \) copies/mL obtained on these occasions, and on pre-ART samples from those with detectable DRM during ART. Logistic regression analysis was used to investigate the association between DRM acquisition and tuberculosis.

Results

Among 621 included individuals (110 [17.5%] with concomitant tuberculosis), 101/621 (16.3%) had VL\( \geq 500 \) copies/mL at 6 and/or 12 months. DRM were detected in 64/98 cases with successful genotyping (65.3%). DRM were detected in 7/56 (12.5%) pre-ART samples from these individuals. High pre-ART VL and low mid-upper arm circumference were associated with increased risk of DRM acquisition, whereas no such association was found for concomitant tuberculosis.
Conclusions

Among adults receiving health-center based ART in Ethiopia, most patients without virological suppression during the first year of ART had detectable DRM. Acquisition of DRM during this period was the dominant cause of antiretroviral drug resistance in this setting. Tuberculosis did not increase the risk of DRM acquisition.

Keywords:

HIV; drug resistance; tuberculosis; Ethiopia; Primary Health Care
Introduction

Antiretroviral treatment (ART) blocks viral replication, with improved survival and minimized risk of HIV transmission among people living with HIV (PLHIV) [1]. In contrast, inadequate virological suppression is associated with worse prognosis, and promotes selection of viruses carrying mutations conferring antiretroviral drug resistance [2,3], which may also be transmitted onwards [4]. Although the global roll-out of ART has resulted in reduced AIDS incidence and HIV-related mortality, a successive increase in the prevalence of drug resistance mutations (DRM) in treatment-naïve PLHIV (termed pre-treatment drug resistance; PDR) has been observed in many world regions [5,6], implying community transmission of drug-resistant viruses [7].

Several factors are involved in the emergence of HIV drug resistance, including irregular drug supply, suboptimal adherence and drug-drug interactions [8]. Importantly, insufficient capacity for virological treatment monitoring leads to delayed recognition of patients with treatment failure [9], which in turn can result in further accumulation of DRM [2,10].

In low-income countries, most PLHIV receive nurse-based care, often decentralized to primary health centers [11]. In these settings, many individuals have advanced disease at ART initiation, with high viral load (VL) and low CD4 cell counts [12], factors which may compromise chances of virologic suppression, with ensuing risk of acquisition of drug resistance [13,14]. Furthermore, concurrent opportunistic infections are common in PLHIV starting ART in resource-limited settings. In this context, tuberculosis (TB) is of special importance. Individuals with TB co-infection at ART initiation have higher VL than HIV mono—infected individuals [15], and could therefore be at increased risk of acquiring DRM during ART.

We have previously reported data on patterns of long-term virological outcomes in a cohort of 630 adults investigated for active TB before starting ART at Ethiopian health centers. Whereas 68% achieved and maintained virological suppression <150 copies/mL for up to four years after
treatment initiation, 21% had a VL result ≥1000 copies/mL on at least one occasion during follow-up. Lack of persistent virological suppression was associated with male sex, pre-treatment CD4 count <100 cells/mm³ and malnutrition, but not with active TB [16].

In this study, we have characterized antiretroviral drug resistance mutations among participants with inadequate viral suppression during the first year after ART initiation in this cohort and assessed the relative contribution of acquired and pre-treatment drug resistance. In addition, we have determined factors associated with acquisition of DRM, with particular regard to concomitant TB.

Material and Methods

Participants in the study cohort were recruited and followed at public health centers in an uptake area in and around the city Adama, Ethiopia, 2011-2015. Consenting HIV-positive ART-naive adults (≥18 years) who were eligible to start ART according to Ethiopian National ART guidelines at the time of the study (CD4 count <350 cells/mm³ and/or WHO stage 4 disease) were included [17]. The study cohort has been described in detail previously [16,18].

At inclusion, all participants were investigated for active TB, irrespective of symptoms. Sputum samples (and fine-needle aspirates of enlarged lymph nodes, if present) were analyzed with smear microscopy, GeneXpert MTB/Rif, and liquid culture. Sociodemographic and medical information were collected following structured questionnaires. Blood samples were obtained for CD4 count testing, with storage of plasma at -80°C. Medical information was updated along with repeated blood sampling on subsequent follow-up visits scheduled at months 1, 3, 6, and 12, and biannually thereafter for up to four years after ART initiation. If incident TB was suspected at any time during follow-up, bacteriological TB investigations were repeated. Non-nucleoside reverse transcriptase inhibitor (NNRTI)-based ART was initiated according to Ethiopian National guidelines by non-
physician clinicians at the study sites. Participants diagnosed with TB received TB treatment according to Ethiopian National guidelines, provided at the same facilities [17].

VL was performed on stored plasma in batches during the study period using the Abbott Real-Time HIV-1 assay (Abbott Molecular Inc., Des Plaines, IL; detection limit 40 copies/mL) or the Abbott m2000 RealTime System Automated molecular platforms (Abbott Molecular Inc., Des Plaines, IL; detection limit 150 copies/mL). VL results were communicated to the responsible clinicians at the respective health center.

For this study, all individuals with VL data available at 6 and/or 12 months after ART initiation were included. This 6-month time span was used in order to include all patients with lack of virological suppression (defined as ≥1 VL result ≥500 copies/mL) during the first 12 months of ART.

HIV genotype and drug resistance mutation analysis
Genotypic testing was performed on stored samples with VL ≥500 copies/mL obtained at 6 and/or 12 months after starting ART. A 1084-bp fragment of HIV-1 pol (corresponding to the position: 2243–3326 of HXB2, Genbank Accession Number: K03455) comprising amino acids 6–99 of the protease (PR) and 1–251 of the reverse transcriptase (RT) was amplified using an in-house genotyping assay [19,20]. PCR products were directly sequenced using the Sanger method with six primers (three on each strand) on an ABI 3100 or an ABI 3500xl DNA Genetic Analyzer (Applied Biosystems). Sequence assembly and editing were performed using RECall V 2.0 HIV-1 sequencing analysis tool [21]. Sequence quality control was performed to rule out contamination and mislabeling of samples using the online Quality Control program of the Los Alamos HIV sequence database (hiv.lanl.gov). Individuals with contaminated samples were excluded from this study. The presence of DRM was determined using the Stanford HIVdb database algorithm 8.6 (hivdb.stanford.edu) [22].
To determine whether detected DRM had evolved during ART (acquired drug resistance; ADR) or were present before ART initiation (pre-treatment drug resistance; PDR), genotypic analysis was also performed on samples obtained before starting ART for such participants. In order to estimate the prevalence of pre-ART DRM among participants who had died or were lost to follow-up before scheduled sampling at 6 and 12 months (and could hence not be classified with regard to DRM after starting ART), we also genotyped pre-treatment samples from these individuals. PDR mutations were examined according to the Stanford Genotypic Resistance calibrated population resistance (CPR) tool version 6.0 based on the WHO surveillance transmitted drug resistance mutation list of 2009 [23,24].

Statistical analysis

Comparison of characteristics of cohort participants who were included and excluded from this study was performed using Mann-Whitney U test for continuous variables and the chi-square test for categorical variables.

We used logistic regression analysis to investigate the association between TB and DRM acquisition. For this analysis, individuals with VL<500 copies/mL in all available samples at 6 and/or 12 months were compared with those with ADR. Individuals with VL≥500 copies/mL without detectable ADR, as well as those without genotypic data, were excluded from this analysis. Since we specifically aimed to investigate risk of DRM acquisition during ART, those with DRM detected before ART initiation were also excluded from this analysis. In addition to TB (defined as bacteriologically or clinically diagnosed TB) at ART initiation, age and gender were included in the regression analysis, as well as pre-treatment CD4 count and VL, and mid-upper arm circumference (MUAC), as a marker of malnutrition. Age was divided into 5-year intervals and CD4 counts in intervals of 25 cells/mm³ for interpretation of
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odds ratios. All variables included in univariate analysis were also included in the multivariate analysis.

Statistical analyses were performed using SPSS, version 26 (IBM Corp, Armonk, NY). P values <.05 were considered statistically significant.

Patient consent statement

Ethical approval was obtained from the national Research Ethics Review Committee at the Ministry of Technology and Innovation of Ethiopia and the Regional Ethical Review Board of Lund University, Sweden. All study participants provided written informed consent.

Results

Participant characteristics

A total of 729/812 (89.8%) individuals enrolled in the original cohort started ART. Among these, 621 (85.2%) had VL data at 6 and/or 12 months after treatment initiation and were included in this study (Figure 1).

Among the 108 excluded individuals, 84 (77.8%) were lost to follow-up, died or transferred out before the 6- and/or 12-month visit, and 24 (21.3%) did not have follow-up VL results (Figure 1). Among the 621 included individuals, 377 (60.7%) were women, the median CD4 count at ART initiation was 191 cell/mm$^3$ (IQR 121-274), and 110 (17.7%) had concomitant TB. Efavirenz (EFV) was the most common NNRTI used (83.9%). All participants received lamivudine (3TC), with third nucleoside reverse transcriptase inhibitor (NRTI) being tenofovir disoproxil fumarate (TDF) in 89.0%, zidovudine (AZT) in 10.0% and stavudine (d4T) in 1.0% (Table 1). Patients who were excluded were more likely to be male, and had lower CD4 count and MUAC; furthermore, the proportion of concomitant TB was higher among excluded patients (25% vs. 18%; p=0.07; Table 1).
Among those with available VL data, 60/534 (10.1%) and 72/520 (13.8%) had VL≥500 copies/mL at 6 and 12 months, respectively. The median log VL in patients with VL≥500 copies/mL was 4.54 (IQR 3.73-5.33) and 4.58 (IQR 4.02-5.15) at 6 and 12 months, respectively. Among participants with VL≥500 copies/mL at these time points, 55/60 (91.7%) and 66/72 (91.7%) had ≥1000 copies/mL at 6 and 12 months, respectively.

Drug resistance during the first year after starting ART

In total, 98 individuals with ≥1 VL ≥500 copies/mL at 6 and/or 12 months had samples available for genotyping (Figure 1; both 6 and 12 months: 29, only 6 months: 29, only 12 months: 40). All of the specimens were successfully amplified and genotyped. All genotyped viruses belonged to subtype C and DRM were detected in 64 (65.3%) of these 98 individuals (Table 2, Supplemental data file). NNRTI-associated mutations were present in all individuals with DRM. Additionally, 35/98 (35.7%) had NRTI-associated DRM (Table 2). No protease inhibitor (PI)-associated mutations were detected.

The median log VL at the time of VL≥500 copies/mL was 4.60 (IQR 3.98-5.11) for patients with DRM, compared to 4.45 (IQR 3.55-5.19) for those without detectable DRM.

Pre-treatment drug resistance

Samples obtained before ART initiation were available for 56/64 (87.5%) of those with DRM during ART. DRM were detected in 7/56 (12.5%) of these samples. All seven had NNRTI resistance (K103N=5, K181V=1, K103N and G190S combined=1). The sample with dual NNRTI resistance also had multiple thymidine analogue mutations (TAMs): D67N, T215C, and K219E (Table 3). Only minor changes were observed in patterns of DRM comparing pre-ART and ART samples from the same individuals (data not shown).
Among the 61 subjects that were LTFU or died before reaching a 6- or 12-month visit, pre-treatment samples were available for 49 (80.3%), with successful genotyping in 43/49 (87.8%). Pre-treatment DRM was detected in 2 of these (4.7%; both NNRTI-associated) (Table 3).

Factors associated with drug resistance acquisition during antiretroviral treatment

In this analysis, 57 cases with ADR were compared with 520 individuals with VL<500 copies/mL at 6 and/or 12 months. Concomitant TB was not significantly associated with ADR. In univariate analysis, CD4 count, VL, and MUAC were associated with ADR (Table 4). In multivariate analysis, the statistically significant association remained for pre-treatment VL and for MUAC (Table 4).

Discussion

In this cohort of PLHIV receiving care at Ethiopian health centers, we detected DRM in a majority of patients with VL ≥500 copies/mL at 6-12 months after ART initiation. In most of these treatment-naïve individuals (87.5%), DRM were not detected in samples obtained before starting ART, implying acquired drug resistance as the major mechanism for drug resistance in this setting.

Incomplete virological suppression during ART promotes selection of HIV variants carrying DRM [2]. In particular, NNRTI-associated DRM have been reported to emerge early in individuals who fail to achieve suppression after starting ART, while NRTI-associated DRM tend to accumulate after longer periods of persistent replication [10]. In agreement with our findings, 87% of participants in a study conducted in six Sub-Saharan African countries had DRM at first occasion of VL ≥1000 copies/mL after at least 6 months ART (in median 1 year after first-line ART initiation) [2]. Previous data from Ethiopia also imply high rates of DRM in patients with virological failure. Two repeated surveys performed in a hospital clinic in Northwestern Ethiopia in 2011 and 2015, respectively, showed an
increase in proportion of patients with VL >400 copies/mL with detectable DRM (40% vs. 66%) [25,26]. In another study, based on data from seven Ethiopian teaching hospitals, DRM were detected in 76.6% and 66.7% of patients with VL >1000 copies/mL after 6 and 12 months ART, respectively [27].

In contrast to most other studies from sub-Saharan Africa, our cohort was recruited and followed at health centers, where the majority of PLHIV receive ART. To our knowledge, only one study in Ethiopia has previously investigated antiretroviral drug resistance at health center level; among 11 patients with VL >1000 copies/mL, 9 (81.8%) had DRM [28]. However, in contrast to our findings, 6/9 (66.7%) of these individuals had detectable DRM in samples obtained before starting ART. Instead, our results suggest acquisition of DRM during the first 6-12 months of ART to be the dominant mechanism of drug resistance in this healthcare setting. In turn, this emphasizes the importance of adherence, and implies that adherence support needs to be strengthened in Ethiopian ART programs in order to secure effective treatment options.

We could not determine the exact time point after ART initiation that DRM mutations emerged, but since the prevalence of NNRTI mutations was similar at 6 and 12 months, it is likely that these mutations occur during the first months of ART. VL testing and early identification of those with failing treatment during the initial 6 months of ART could therefore be effective for saving 1st line options. In line with this, Kerschberger et al. showed superior ART outcome when first VL was measured at 3 months compared to 6 months, potentially shortening the time on failing treatment [29]. Although virologic suppression can occur despite presence of NNRTI-associated DRM [30,31], the recommendation of enhanced adherence counseling followed by repeat VL testing is unlikely to be successful in most patients with incomplete viral suppression due to drug resistance, constituting two thirds in our population. In these cases, change to 2nd line ART regimens is indicated, whereas persons without DRM will not benefit from treatment modification. This dichotomy illustrates the
need for access to methods to determine the presence of major drug resistance in patients with virologic failure, in order to provide effective interventions to optimize treatment outcomes.

As expected, and in agreement with other studies, mutations conferring NNRTI resistance were the most commonly observed type of DRM. Importantly, VL for those with ADR was high (4.60 log10 copies/mL) indicating the potential of onwards transmission of viruses harboring DRM. Several studies, performed in different parts of sub-Saharan Africa (as well as other low- and middle-income settings) show increasing rates of pre-ART resistance, paralleling scale-up of ART programs [6]. In particular, rates of NNRTI mutations are high, with levels above 10% in some areas [6]. This situation has prompted recommendations to replace NNRTI with the integrase strand transfer inhibitor dolutegravir in 1st line regimens [32]. Although the genetic barrier to resistance of dolutegravir is higher than for NNRTIs, dolutegravir monotherapy promotes selection of resistant variants [33]. Functionally, this situation could arise if NNRTIs are replaced with dolutegravir in patients with combined NRTI mutations. In our cohort (in which nearly 90% had TDF as NRTI backbone), combined NRTI resistance with K65R and M184V/I was present in 25.5% and 21.7% with VL ≥500 copies/mL at 6 and 12 months, respectively. In such patients, regimen switch from NNRTI to dolutegravir could lead to functional dolutegravir monotherapy, with a risk of emergence of dolutegravir resistance [34].

Dolutegravir is also recommended as a second-line alternative for patients failing NNRTI-based ART [32]. The pattern of NRTI DRM found in this study support this recommendation if TDF is replaced by AZT, since mutations conferring AZT resistance were rare in our population.

The proportion of PDR among individuals starting ART in Ethiopia is not well known. In a study conducted at seven Ethiopian hospitals 2009-2011, PDR was detected in 18/461 (3.9%) randomly selected ART-naive individuals [35]. We did not aim to assess PDR in this study. Nonetheless, in order to differentiate between PDR and ADR in our participants, we genotyped samples obtained before
ART initiation for those with DRM detected at 6 or 12 months of ART. Among these, PDR was detected in a 7/56 (12.5%).

In this cohort, concomitant TB was not associated with increased risk of acquired drug resistance in patients receiving NNRTI-based ART. This is in line with previously reported findings from this cohort of similar short- and long-term ART outcomes with regard to TB co-infection [16,36]. Factors that have been associated with acquisition of DRM in other studies include male sex, higher pre-treatment VL and lower CD4 counts [37–39]. Interestingly, although participants with TB were more likely to have these characteristics [18], they were not at increased risk of DRM acquisition. This could suggest an indirect protective effect of concomitant TB related to closer contact with health care.

The only variables independently associated with ADR in this cohort were high pre-treatment VL and low MUAC. Both of these factors indicate more advanced HIV disease. We have previously shown that low MUAC is associated with concomitant TB in ART-naïve PLHIV [40], as well as unfavorable ART outcome [16,36]. Low MUAC could also reflect unrecognized opportunistic infections, as well as poverty and food insecurity [41,42].

This study was based on a well-characterized cohort in which all participants had been subjected to intensified TB case-finding. These patients received nurse-based care at health centers, which we consider to be a representative setting for Ethiopia, as well as for other countries in Sub-Saharan Africa. Nonetheless, this study has several limitations. Genotyping was performed with Sanger sequencing, which has a lower sensitivity compared to next generation sequencing [43]. It is therefore possible that DRM occurring at low frequencies were missed, and that some of the DRM detected during ART (and hence categorized as ADR) could also have been detected at inclusion if a sequencing technology with higher resolution was used. Furthermore, pre-treatment genotypic data was missing for some of these individuals. Although emergence of DRM is most common in the setting of high viral replication, this can occur also during low-level viremia [44]. For this reason, we
chose 500 copies/mL to select cases for genotypic testing, in contrast to most prior studies on antiretroviral drug resistance in sub-Saharan Africa (which have used a threshold of 1000 copies/mL [2,27]). Therefore, direct comparisons with our findings require consideration of this circumstance. However, 83.3% of non-suppressed individuals had VL>1000 copies/mL. Finally, this study was not specifically powered to test the hypothesis that concomitant TB increases the risk of ADR. However, the 95% confidence intervals of both the unadjusted and adjusted odds ratio do indicate that a clinically relevant association was not missed. The prevalence of concomitant TB tended to be higher among the 108 individuals excluded due to lack of follow-up viral load and genotypic data, which could imply selection bias that may have had an impact on these results.

In conclusion, antiretroviral drug resistance was observed in a majority of individuals not achieving virological suppression after 6-12 months ART. In most of these, DRM were not detected in samples obtained prior to starting ART, implying DRM acquisition during the initial year of ART as the dominant cause of drug resistance in this population. This demonstrates the importance of earlier identification of patients without virological suppression, so that interventions can be implemented before drug resistance acquisition has occurred.
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Author’s contribution: AR, PB, DAA, TTB, and PM designed the study; AR, TTB and PB collected clinical data; DAA, PM, HY, and AZ collected biological data and performed experiments; AR, DAA, PB, and PM analyzed results; AR, PB, DAA, and PM wrote the article. All authors reviewed and accepted the final version of the article.
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Figure 1. Flow chart of study participants eligible for genotypic analysis.

Whereas 493 (79.4%) of the 621 included individuals had VL data at both 6 and 12 months, VL data were missing from 27 (4.3%) and 101 (16.3%) participants at 6 and 12 months, respectively. For 7/27 participants with missing VL data at 6 months, this represented a missed study visit; the respective proportion at 12 months was 33/74; 27 individuals had not reached the 12-month visit at study closure. For the remaining cases, study visits were registered but blood samples for VL testing were not available.
Table 1. Characteristics of cohort participants at antiretroviral treatment initiation with comparison of individuals included and excluded in the current study.

|                      | Included N=621 | Excluded N=108 | P-value |
|----------------------|---------------|---------------|---------|
| **Age** years        | 32 (28-40)    | 31 (28-39)    | 0.287   |
| **Female sex**       | 377 (61)      | 54 (50)       | 0.037   |
| **Viral load** log copies/mL | 5.11 (4.50-5.55) | 5.15 (4.47-5.67) | 0.595   |
| **CD4 count** cells/mm$^3$ | 191 (121-274)  | 154 (101-274) | 0.042   |
| **CD4 strata** cells/mm$^3$ |             |               |         |
| <100                 | 112 (18)      | 26 (24)       |         |
| 100 - 200 cells/mm$^3$ | 220 (36)      | 41 (38)       |         |
| >200                 | 288 (47)      | 40 (37)       |         |
| **MUAC** cm          | 23.0 (21.0-25.0) | 22.0 (20.0-24.0) | <0.01   |
| **TB co-infection**  | 110 (18)      | 27 (25)       | 0.074   |
| **NNRTI** NVP        | 100 (16)      | 17 (14)       | 0.611   |
|                      | 521 (84)      | 91 (86)       | 0.611   |
| **NRTI** 3TC         | 621 (100)     | 108 (100)     |         |
|                      | 553 (89)      | 90 (85)       | 0.217   |
| **AZT**              | 62 (10)       | 10 (9)        | 0.861   |
|                      | 6 (1)         | 6 (6)         | <0.01   |
| **d4T**              |               |               |         |

Abbreviations: MUAC, mid-upper arm circumference; TB, tuberculosis; NNRTI, non-nucleoside reverse transcriptase inhibitor; NVP, nevirapine; EFV, efavirenz; NRTI, nucleoside reverse transcriptase inhibitor; 3TC, lamivudine; TDF, tenofovir disoproxil fumarate; AZT, zidovudine; d4T, stavudine.

P value derived using Mann-Whitney U test for continuous variables and Chi-square test for categorical variables.

Data presented as n (%) or median (interquartile range).

Viral load data available for 703/729 (96.4%) and CD4 counts available for 727/729 (99.7%).
Table 2. Frequency of the four most common NNRTI and NRTI drug resistance mutations detected in individuals with viral load ≥500 copies/mL at 6 and/or 12 months after treatment initiation.

|                        | Total (n=98) | 6 months (n=58) | 12 months (n=69) |
|------------------------|--------------|-----------------|------------------|
| Any NNRTI and/or NRTI  | 64 (65.3)    | 41 (70.7)       | 46 (66.7)        |
| NNRTI                  | 64 (65.3)    | 41 (70.7)       | 46 (66.7)        |
| K103N                  | 39 (39.8)    | 23 (39.7)       | 29 (42.0)        |
| V106A/M                | 16 (16.3)    | 9 (15.5)        | 11 (15.9)        |
| Y181C/I                | 15 (15.3)    | 12 (20.7)       | 12 (17.4)        |
| G190A/C/E/Q/S          | 12 (12.2)    | 12 (20.7)       | 4 (5.8)          |
| NRTI                   | 35 (35.7)    | 26 (44.8)       | 25 (36.2)        |
| M184V/I                | 30 (30.6)    | 20 (34.5)       | 23 (33.3)        |
| K65R                   | 24 (24.5)    | 20 (34.5)       | 17 (24.6)        |
| A62V                   | 9 (9.2)      | 7 (12.1)        | 7 (10.1)         |
| Y115F                  | 7 (7.1)      | 5 (8.6)         | 7 (10.1)         |

Abbreviations: NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor.

Data presented as n (%).
Table 3. Frequency of drug resistance mutations detected in pre-treatment samples.

|                          | Total (n=125) | DRM at 6 and/or 12 months (n=64) | Deceased or LTFU before providing 6- or 12-month samples (n=61) |
|--------------------------|---------------|----------------------------------|---------------------------------------------------------------|
| Genotype missing         | 26 (20.8)     | 8 (12.5)                         | 18 (29.5)                                                    |
| Genotype available       | 99 (79.2)     | 56 (87.5)                        | 43 (70.5)                                                    |
| Any sDRM detected        | 9 (9.1)       | 7 (12.5)                         | 2 (4.7)                                                      |
| NNRTI sDRM               | 9 (9.1)       | 7 (10.9)                         | 2 (4.7)                                                      |
| NRTI sDRM                | 1 (1.0)       | 1 (1.8)                          | 0 (0)                                                        |

Abbreviations: DRM, drug resistance mutation; LTFU, lost to follow-up; sDRM, surveillance drug resistance mutation included in the WHO 2009 sDRM list; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor.

Data presented as n (%).
Table 4. Factors associated with drug resistance acquisition during antiretroviral treatment.

| Factor                          | OR (95% CI)     | P    | aOR (95% CI) | P    |
|---------------------------------|-----------------|------|--------------|------|
| Tuberculosis                    | 1.09 (0.53-2.24)| 0.817| 0.76 (0.35-1.68) | 0.503|
| Age, per 5 years                | 1.09 (0.96-1.25)| 0.198| 1.00 (0.85-1.17) | 0.978|
| Male sex                        | 1.73 (1.00-3.00)| 0.050| 1.50 (0.81-2.78) | 0.197|
| CD4 count, per 25 cells/mm³     | 0.88 (0.82-0.95)| 0.001| 0.93 (0.87-1.01) | 0.069|
| Viral load, log copies/mL       | 2.57 (1.64-4.03)| <0.001| 1.96 (1.21-3.16) | 0.006|
| MUAC, per cm                    | 0.85 (0.77-0.94)| 0.002| 0.89 (0.80-0.99) | 0.031|

Abbreviations: OR, odds ratio; aOR, adjusted odds ratio; MUAC, mid-upper arm circumference.
Figure 1

729 patients starting antiretroviral treatment

84 (11.5%) without study visit at 6 and 12 months:
  • 29 (34.5%) loss to follow-up
  • 32 (38.1%) deceased
  • 23 (27.4%) transfer of care

645 (88.5%) with at least one study visit at 6 and/or 12 months after treatment initiation

Viral load data missing for 24 (3.7%)

621 (96.3%) with viral load data

520 (83.7%) with viral load <500 copies/mL

101 (16.3%) with viral load ≥500 copies/mL

3 (3.0%) without sample for genotypic analysis

98 (97.0%) with successful genotypic analysis