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MAJOR ARTICLE

Drug Resistance in HIV-Positive Adults During the Initial Year of Antiretroviral Treatment at Ethiopian Health Centers

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Background. 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 (DRMs) 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-naïve individuals aged ≥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 VLs ≥500 copies/mL obtained on these occasions and on pre-ART samples from those with detectable DRMs 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 a VL ≥500 copies/mL at 6 and/or 12 months. DRMs were detected in 64/98 cases with successful genotyping (65.3%). DRMs 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. drug resistance; Ethiopia; HIV; primary health care; tuberculosis.

INTRODUCTION

Antiretroviral treatment (ART) blocks viral replication, with improved survival and minimized risk of HIV transmission among people with HIV (PWH) [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 onward [4]. Although the global rollout of ART has resulted in reduced AIDS incidence and HIV-related mortality, a successive increase in the prevalence of drug-resistance mutations (DRMs) in treatment-naïve PWH (termed pretreatment 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 DRMs [2, 10].

In low-income countries, most PWH 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 loads (VLs) and low CD4 cell counts [12], factors that may compromise the chances of virologic suppression, with ensuing risk of acquisition of drug resistance [13, 14]. Furthermore, concurrent opportunistic infections are common in PWH 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 VLs than HIV mono-infected individuals [15], and could therefore be at increased risk of acquiring DRMs during ART.

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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 4 years after treatment initiation, 21% had a VL result ≥1000 copies/mL on at least 1 occasion during follow-up. Lack of persistent virological suppression was associated with male sex, pretreatment CD4 count <100 cells/mm^3, 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 pretreatment drug resistance. In addition, we have determined factors associated with acquisition of DRMs, with particular regard to concomitant TB.

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, from 2011 to 2015. Consenting HIV-positive ART-naïve 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/mm^3 and/or World Health Organization [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 was collected with 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 4 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 nonphysician 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, USA; detection limit 40 copies/mL) or the Abbott m2000 RealTime System Automated molecular platforms (Abbott Molecular Inc., Des Plaines, IL, USA; 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 a 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]. Polymerase chain reaction products were directly sequenced using the Sanger method with 6 primers (3 on each strand) on an ABI 3100 or an ABI 3500xl DNA Genetic Analyzer (Applied Biosystems). Sequence assembly and editing were performed using the RECall, version 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 DRMs had evolved during ART (acquired drug resistance [ADR]) or were present before ART initiation (pretreatment 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 DRMs 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 pretreatment 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 the 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 a VL <500 copies/mL in all available samples at 6 and/or 12 months were compared with those with ADR. Individuals with a VL ≥500 copies/mL without detectable ADR, as well as those without genotypic data, were excluded from this analysis. As we specifically aimed to investigate the risk of DRM acquisition during ART, those with

DRMs 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 pretreatment CD4 count and VL, and mid-upper arm circumference (MUAC) was included as a marker of malnutrition. Age was divided into 5-year intervals and CD4 counts into intervals of 25 cells/mm^3 for interpretation of odds ratios. All variables included in the univariate analysis were also included in the multivariate analysis.

Statistical analyses were performed using SPSS, version 26 (IBM Corp, Armonk, NY, USA). *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 (interquartile range [IQR]) was 191 (121–274) cell/mm^3, and 110 (17.7%) had concomitant TB. Efavirenz (EFV) was the most common NNRTI used (83.9%). All participants received lamivudine (3TC), with the 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 counts and MUAC; furthermore, the proportion of concomitant TB was higher among excluded patients (25% vs 18%; *P* = .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 logVL in patients with VL ≥500 copies/mL (IQR) was 4.54 (3.73–5.33) and 4.58 (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 (both 6 and 12 months: 29; only 6 months: 29; only 12 months: 40) (Figure 1). All of the specimens were successfully amplified

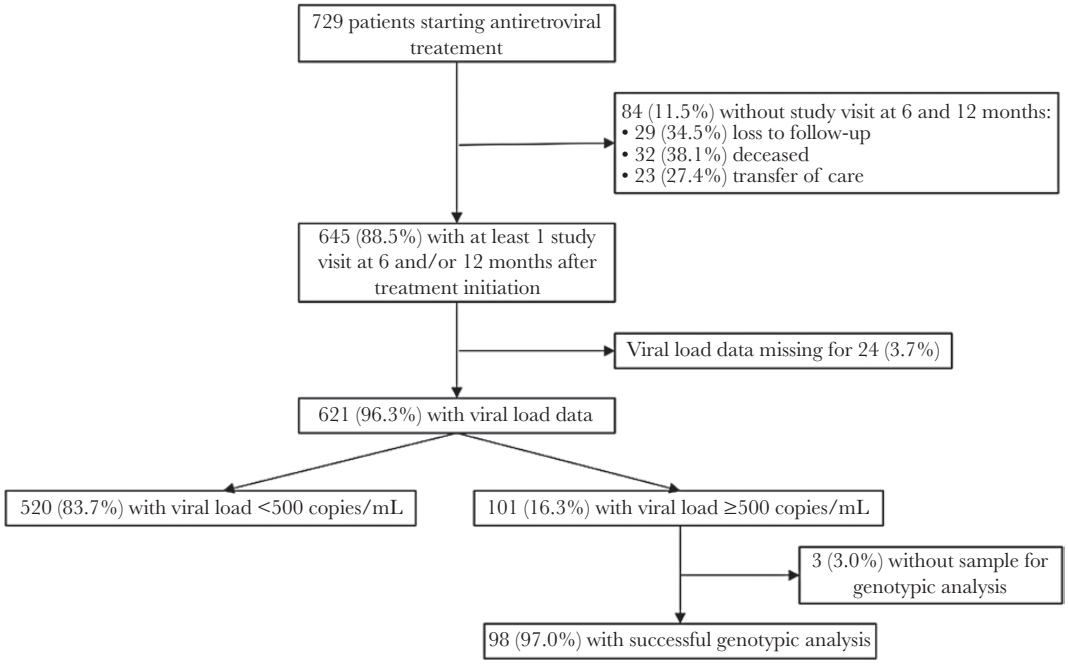

Figure 1. Flowchart of study participants eligible for genotypic analysis. Whereas 493 (79.4%) of the 621 included individuals had viral load (VL) data at both 6 and 12 months, VL data were missing from 27 (4.3%) and 101 (16.3%) participants at 6 and 12 months, respectively. For 7/27 participants with 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)	.287
Female sex		377 (61)	54 (50)	.037
Viral load	log copies/mL	5.11 (4.50–5.55)	5.15 (4.47–5.67)	.595
CD4 count	cells/mm^3	191 (121–274)	154 (101–274)	.042
CD4 strata	<100 cells/mm^3	112 (18)	26 (24)	
	100–200 cells/mm^3	220 (36)	41 (38)	
	>200 cells/mm^3	288 (47)	40 (37)	
MUAC	cm	23.0 (21.0–25.0)	22.0 (20.0–24.0)	<.01
TB co-infection		110 (18)	27 (25)	.074
NNRTI	NVP	100 (16)	17 (14)	.611
	EFV	521 (84)	91 (86)	.611
NRTI	3TC	621 (100)	108 (100)	
	TDF	553 (89)	90 (85)	.217
	AZT	62 (10)	10 (9)	.861
	d4T	6 (1)	6 (6)	<.01

P values were derived using the Mann-Whitney *U* test for continuous variables and the chi-square test for categorical variables. Data presented as No. (%) or median (interquartile range). Viral load data were available for 703/729 (96.4%), and CD4 counts were available for 727/729 (99.7%).

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

and genotyped. All genotyped viruses belonged to subtype C, and DRMs were detected in 64 (65.3%) of these 98 individuals (Table 2; Supplementary Data). NNRTI-associated mutations were present in all individuals with DRMs. Additionally, 35/98 (35.7%) had NRTI-associated DRMs (Table 2). No protease inhibitor (PI)–associated mutations were detected.

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

Table 2. Frequency of the 4 Most Common NNRTI and NRTI Drug Resistance Mutations Detected in Individuals With Viral Loads ≥500 Copies/mL at 6 and/or 12 Months After Treatment Initiation

	Total (n = 98)	6 mo (n = 58)	12 mo (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)

Data are presented as No. (%).

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

Pretreatment Drug Resistance

Samples obtained before ART initiation were available for 56/64 (87.5%) of those with DRMs during ART. DRMs were detected in 7/56 (12.5%) of these samples. All 7 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 DRMs comparing pre-ART and ART samples from the same individuals (data not shown).

Among the 61 subjects who were LTFU or died before reaching a 6- or 12-month visit, pretreatment samples were available for 49 (80.3%), with successful genotyping in 43/49 (87.8%). Pretreatment 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 pretreatment VL and MUAC (Table 4).

DISCUSSION

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

Table 3. Frequency of Drug Resistance Mutations Detected in Pretreatment Samples

	Total (n = 125)	DRM at 6 and/or 12 mo (n = 64)	Deceased or LTFU Before Providing 6- or 12-mo 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)

Data are presented as No. (%).

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

Table 4. Factors Associated With Drug Resistance Acquisition During Antiretroviral Treatment

	OR (95% CI)	*P*	aOR (95% CI)	*P*
Tuberculosis	1.09 (0.53–2.24)	.817	0.76 (0.35–1.68)	.503
Age, per 5 y	1.09 (0.96–1.25)	.198	1.00 (0.85–1.17)	.978
Male sex	1.73 (1.00–3.00)	.050	1.50 (0.81–2.78)	.197
CD4 count, per 25 cells/mm^3	0.88 (0.82–0.95)	.001	0.93 (0.87–1.01)	.069
Viral load, log copies/mL	2.57 (1.64–4.03)	<.001	1.96 (1.21–3.16)	.006
MUAC, per cm	0.85 (0.77–0.94)	.002	0.89 (0.80–0.99)	.031

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

Incomplete virological suppression during ART promotes selection of HIV variants carrying DRMs [2]. In particular, NNRTI-associated DRMs have been reported to emerge early in individuals who fail to achieve suppression after starting ART, while NRTI-associated DRMs tend to accumulate after longer periods of persistent replication [10]. In agreement with our findings, 87% of participants in a study conducted in 6 Sub-Saharan African countries had DRM at first occasion of VL ≥1000 copies/mL after at least 6 months of ART (a median of 1 year after first-line ART initiation) [2]. Previous data from Ethiopia also imply high rates of DRMs 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 the proportion of patients with VLs >400 copies/mL with detectable DRMs (40% vs 66%) [25, 26]. In another study, based on data from 7 Ethiopian teaching hospitals, DRMs were detected in 76.6% and 66.7% of patients with VLs >1000 copies/mL after 6 and 12 months of 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 PWH receive ART. To our knowledge, only 1 study in Ethiopia has previously investigated antiretroviral drug resistance at the health center level; among 11 patients with VLs >1000 copies/mL, 9 (81.8%) had DRMs [28]. However, in contrast to our findings, 6/9 (66.7%) of these individuals had detectable DRMs in samples obtained before starting ART. Instead, our results suggest acquisition of DRMs during the first 6–12 months of ART to be the dominant mechanism of drug resistance in this health care 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 as 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 first-line options. In line with this, Kerschberger et al. showed superior ART outcomes when VL was first measured at 3 months compared with 6 months, potentially shortening the time on failing treatment [29]. Although virologic suppression can occur despite the presence of NNRTI-associated DRMs [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 second-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, the VL for those with ADR was high (4.60 log10 copies/mL), indicating the potential of onward 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 >10% in some areas [6]. This situation has prompted recommendations to replace NNRTI with the integrase strand transfer inhibitor dolutegravir in first-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 the NRTI backbone), combined NRTI resistance with K65R and M184V/I was present in 25.5% and 21.7% with VLs ≥500 copies/mL at 6 and 12 months, respectively. In such patients, a 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 DRMs found in this study supports this recommendation if TDF is replaced by AZT, as 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 7 Ethiopian hospitals from 2009 to 2011, PDR was detected in 18/461 (3.9%) randomly selected ART-naïve 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 DRMs detected at 6 or 12 months of ART. Among these, PDR was detected in 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 DRMs in other studies include male sex, higher pretreatment 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 pretreatment 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 PWH [40], as well as unfavorable ART outcomes [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 with next-generation sequencing [43]. It is therefore possible that DRMs occurring at low frequencies were missed and that some of the DRMs detected during ART (and hence categorized as ADR) could also have been detected at inclusion if a sequencing technology with higher resolution were used. Furthermore, pretreatment genotypic data were missing for some of these individuals. Although emergence of DRMs 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 nonsuppressed 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, which 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 of ART. In most of these, DRMs were not detected in samples obtained before 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.

Supplementary Data

Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Potential conflicts of interest. A.R., D.A.A., T.T.B., H.Y., A.Z., P.M., and P.B. report no conflicts of interest.

Author contributions. A.R., P.B., D.A.A., T.T.B., and P.M. designed the study; A.R., T.T.B., and P.B. collected clinical data; D.A.A., P.M., H.Y., and A.Z. collected biological data and performed experiments; A.R., D.A.A., P.B., and P.M. analyzed results; A.R., P.B., D.A.A., and P.M. wrote the article. All authors reviewed and accepted the final version of the article.

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References

1. World Health Organization (WHO). Guidelines for Managing Advanced HIV Disease and Rapid Initiation of Antiretroviral Therapy. Geneva: World Health Organisation; **2017**.
2. Boender TS, Kityo CM, Boerma RS, et al. Accumulation of HIV-1 drug resistance after continued virological failure on first-line ART in adults and children in Sub-Saharan Africa. J Antimicrob Chemother **2016**; 71:2918–27.
3. Clutter DS, Jordan MR, Bertagnolio S, Shafer RW. HIV-1 drug resistance and resistance testing. Infect Genet Evol **2016**; 46:292–307.
4. Stekler JD, Milne R, Payant R, et al. Transmission of HIV-1 drug resistance mutations within partner-pairs: a cross-sectional study of a primary HIV infection cohort. PLoS Med **2018**; 15:e1002537.
5. Gupta RK, Gregson J, Parkin N, et al. HIV-1 drug resistance before initiation or re-initiation of first-line antiretroviral therapy in low-income and middle-income countries: a systematic review and meta-regression analysis. Lancet Infect Dis **2018**; 18:346–55.
6. World Health Organization (WHO). HIV Drug Resistance Report. Geneva: World Health Organization; **2019**.
7. Hofstra LM, Sauvageot N, Albert J, et al; SPREAD Program. Transmission of HIV drug resistance and the predicted effect on current first-line regimens in Europe. Clin Infect Dis **2016**; 62:655–63.
8. Bertagnolio S, De Luca A, Vitoria M, et al. Determinants of HIV drug resistance and public health implications in low- and middle-income countries. Antivir Ther **2012**; 17:941–53.
9. De Luca A, Marazzi MC, Mancinelli S, et al. Prognostic value of virological and immunological responses after 6 months of antiretroviral treatment in adults with HIV-1 infection in Sub-Saharan Africa. J Acquir Immune Defic Syndr **2012**; 59:236–44.

10. De Luca A, Sidumo ZJ, Zanelli G, et al. Accumulation of HIV-1 drug resistance in patients on a standard thymidine analogue-based first line antiretroviral therapy after virological failure: implications for the activity of next-line regimens from a longitudinal study in Mozambique. BMC Infect Dis **2017**; 17:605..
11. Joint United Nations Programme on HIV/AIDS (UNAIDS). Ending AIDS: Progress Towards the 90–90–90 Targets. Geneva: UN Joint Programme on HIV/AIDS (UNAIDS); **2017**..
12. Ford N, Mills EJ, Egger M. Immunodeficiency at start of antiretroviral therapy: the persistent problem of late presentation to care. Clin Infect Dis **2014**; 60:1128–30..
13. The PLATO II Project Team and COHERE group. Triple-class virologic failure in hiv-infected patients undergoing antiretroviral therapy for up to 10 years. Arch Intern Med **2010**; 170:410–9..
14. Raffi F, Hanf M, Ferry T, et al; Dat'AIDS Study Group. Impact of baseline plasma HIV-1 RNA and time to virological suppression on virological rebound according to first-line antiretroviral regimen. J Antimicrob Chemother **2017**; 72:3425–34..
15. Olsson O, Björkman P, Jansson M, et al. Plasma profiles of inflammatory markers associated with active tuberculosis in antiretroviral therapy-naive human immunodeficiency virus-positive individuals. Open Forum Infect Dis **2019**; 6:XXX–XX..
16. Reepalu A, Balcha TT, Sturegård E, Medstrand P, Björkman P. Long-term outcome of antiretroviral treatment in patients with and without concomitant tuberculosis receiving health center–based care—results from a prospective cohort study. Open Forum Infect Dis **2017**; 4:1–8..
17. Ethiopian Federal Ministry of Health. Guidelines for Clinical and Programmatic Management of TB, Leprosy and TB/HIV in Ethiopia. Addis Ababa: Ethiopian Federal Ministry of Health; **2012**. Available at https://etharc.org/index.php/resources/download/finish/33/709. Accessed 16 February 2021..
18. Balcha TT, Sturegård E, Winqvist N, et al. Intensified tuberculosis case-finding in HIV-positive adults managed at Ethiopian health centers: diagnostic yield of Xpert MTB/RIF compared with smear microscopy and liquid culture. PLoS One **2014**; 9:e85478..
19. Zhou Z, Wagar N, DeVos JR, et al. Optimization of a low cost and broadly sensitive genotyping assay for HIV-1 drug resistance surveillance and monitoring in resource-limited settings. PLoS One **2011**; 6:e28184..
20. Arimide DA, Abebe A, Kebede Y, et al. HIV-genetic diversity and drug resistance transmission clusters in Gondar, Northern Ethiopia, 2003–2013. PLoS One **2018**; 13:2003–13..
21. Woods CK, Brumme CJ, Liu TF, et al. Automating HIV drug resistance genotyping with RECall, a freely accessible sequence analysis tool. J Clin Microbiol **2012**; 50:1936–42..
22. Rhee SY, Kantor R, Katzenstein DA, et al; International Non Subtype B HIV-1 Working Group. HIV-1 pol mutation frequency by subtype and treatment experience: extension of the HIVseq program to seven non-B subtypes. AIDS **2006**; 20:643–51..
23. Bennett DE, Camacho RJ, Otelea D, et al. Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update. PLoS One **2009**; 4:e4724..
24. Gifford RJ, Liu TF, Rhee SY, et al. The calibrated population resistance tool: standardized genotypic estimation of transmitted HIV-1 drug resistance. Bioinformatics **2009**; 25:1197–8..
25. Mulu A, Maier M, Liebert UG. Low incidence of HIV-1C acquired drug resistance 10 years after roll-out of antiretroviral therapy in Ethiopia: a prospective cohort study. PLoS One **2015**; 10:e0141318..
26. Mulu A, Maier M, Liebert UG. Upward trends of acquired drug resistances in Ethiopian HIV-1C isolates: a decade longitudinal study. PLoS One **2017**; 12:e0186619..
27. Telele NF, Kalu AW, Gebre-Selassie S, et al. A viral genome wide association study and genotypic resistance testing in patients failing first line antiretroviral therapy in the first large countrywide Ethiopian HIV cohort. BMC Infect Dis **2019**; 19:569..
28. Abdissa A, Yilma D, Fonager J, et al. Drug resistance in HIV patients with virological failure or slow virological response to antiretroviral therapy in Ethiopia. BMC Infect Dis **2014**; 14: 181..
29. Kerschberger B, Boulle AM, Kranzer K, et al. Superior virologic and treatment outcomes when viral load is measured at 3 months compared to 6 months on antiretroviral therapy. J Int AIDS Soc **2015**; 18:20092..
30. Hoffmann CJ, Charalambous S, Sim J, et al. Viremia, resuppression, and time to resistance in human immunodeficiency virus (HIV) subtype C during first-line antiretroviral therapy in South Africa. Clin Infect Dis **2009**; 49:1928–35..
31. Derache A, Iwuji CC, Baisley K, et al. Impact of next-generation sequencing defined human immunodeficiency virus pretreatment drug resistance on virological outcomes in the ANRS 12249 treatment-as-prevention trial. Clin Infect Dis **2019**; 69:207–14..
32. World Health Organization (WHO). Update of First and Second Line Antiretroviral Regimens. Geneva: World Health Organization; **2019**..
33. Wijting I, Rokx C, Boucher C, et al. Dolutegravir as maintenance monotherapy for HIV (DOMONO): a phase 2, randomised non-inferiority trial. Lancet HIV **2017**; 4:e547–54..
34. Rhee SY, Grant PM, Tzou PL, et al. A systematic review of the genetic mechanisms of dolutegravir resistance. J Antimicrob Chemother **2019**; 74:3135–49..
35. Telele NF, Kalu AW, Gebre-Selassie S, et al. Pretreatment drug resistance in a large countrywide Ethiopian HIV-1C cohort: a comparison of Sanger and high-throughput sequencing. Sci Rep **2018**; 8:7556..
36. Reepalu A, Balcha TT, Skogmar S, et al. High rates of virological suppression in a cohort of human immunodeficiency virus-positive adults receiving antiretroviral therapy in Ethiopian health centers irrespective of concomitant tuberculosis. Open Forum Infect Dis **2014**; 1:XXX–XX..
37. Palumbo PJ, Fogel JM, Hudelson SE, et al. HIV drug resistance in adults receiving early vs delayed antiretroviral therapy. J Acquir Immune Defic Syndr **2018**; 77:484–91..
38. Fogel JM, Hudelson SE, Ou S-S, et al. HIV drug resistance in adults failing early antiretroviral treatment: results from the HIV Prevention Trials Network 052 trial. J Acquir Immune Defic Syndr **2016**; 72:304–9..
39. Xing H, Wang X, Liao L, et al. Incidence and associated factors of HIV drug resistance in Chinese HIV-infected patients receiving antiretroviral treatment. PLoS One **2013**; 8:4–9..
40. Skogmar S, Balcha TT, Jemal ZH, et al. Development of a clinical scoring system for assessment of immunosuppression in patients with tuberculosis and HIV infection without access to CD4 cell testing—results from a cross-sectional study in Ethiopia. Glob Health Action **2014**; 7:23105..
41. Koethe JR, Heimburger DC. Nutritional aspects of HIV-associated wasting in Sub-Saharan Africa. Am J Clin Nutr **2010**; 91:1138–42S..
42. Weiser SD, Tuller DM, Frongillo EA, et al. Food insecurity as a barrier to sustained antiretroviral therapy adherence in Uganda. PLoS One **2010**; 5:e10340..
43. Inzaule SC, Hamers RL, Noguera-Julian M, et al; PanAfrican Studies to Evaluate Resistance. Clinically relevant thresholds for ultrasensitive HIV drug resistance testing: a multi-country nested case-control study. Lancet HIV **2018**; 5:e638–46..
44. Delaugerre C, Gallien S, Flandre P, et al. Impact of low-level-viremia on HIV-1 drug-resistance evolution among antiretroviral treated-patients. PLoS One **2012**; 7:e36673..