Journal of Antimicrobial Chemotherapy

Virological failure after 1 year of first-line ART is not associated with HIV minority drug resistance in rural Cameroon

A. Zoufaly[1,2]*, J. Jochum[1], R. Hammerl[3], N. Nassimi[3], Y. Raymond[4], G. D. Burchard[3], S. Schmiedel[1], J. F. Drexler[5], N. K. Campbell[6], N. Taka[4], C. Awasom[4], K. J. Metzner[6], J. van Lunzen[1] and T. Feldt[3,7]

[1]Infectious Diseases Unit, Department of Medicine 1, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [2]Department of Medicine 4, Kaiser Franz Josef Hospital, Vienna, Austria; [3]Clinical Research Unit, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany; [4]Bamenda Regional Hospital, Bamenda, Cameroon; [5]Institute of Virology, University of Bonn, Bonn, Germany; [6]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, Zurich, Switzerland; [7]Clinic of Gastroenterology, Hepatology and Infectious Diseases, University Hospital Düsseldorf, Düsseldorf, Germany

*Corresponding author. Department of Medicine 4, Kaiser Franz Josef Hospital, Vienna, Austria. Tel: +43-1-60191-2407; Fax: +43-1-60191-2409; E-mail: alex_zoufaly@yahoo.com

Objectives: The aim of this study was to describe clinical and virological outcomes in therapy-naive HIV-1-positive patients treated in a routine ART programme in rural Cameroon.

Methods: In a prospective cohort, 300 consecutive patients starting first-line ART were enrolled and followed for 12 months. Among 238 patients with available viral load data at Month 12, logistic regression was used to analyse risk factors for virological failure ($\geq$1000 HIV RNA copies/mL) including clinical, immunological and virological parameters, as well as data on drug adherence. Population sequencing was performed to detect the presence of drug-resistance mutations in patients with virological failure at Month 12; minority drug-resistance mutations at baseline were analysed using next-generation sequencing in these patients and matched controls.

Results: At Month 12, 38/238 (16%) patients experienced virological failure ($\geq$1000 HIV RNA copies/mL). Patients with virological failure were younger, had lower CD4 cell counts and were more often WHO stage 3 or 4 at baseline. Sixty-three percent of patients with virological failure developed at least one drug-resistance mutation. The M184V ($n=18$) and K103N ($n=10$) mutations were most common. At baseline, 6/30 patients (20%) experiencing virological failure and 6/35 (17%) matched controls had evidence of minority drug-resistance mutations using next-generation sequencing ($P=0.77$). Lower CD4 count at baseline (OR per 100 cells/mm^3 lower 1.41, 95% CI 1.02–1.96, $P=0.04$) and poorer adherence (OR per 1% lower 1.05, 95% CI 1.02–1.08, $P<0.001$) were associated with a higher risk of virological failure. Unavailability of ART at the treatment centre was the single most common cause for incomplete adherence.

Conclusions: Virological failure after 1 year of ART was not associated with minority drug resistance at baseline but with incomplete adherence. Strategies to assure adherence and uninterrupted drug supplies are pivotal factors for therapy success.

Keywords: HIV drug resistance, adherence, Africa

Introduction

The scaling up of antiretroviral treatment programmes in sub-Saharan Africa has led to favourable results.[1,2] However, poor retention in care,[3] inadequate adherence to treatment and lack of stock of antiretroviral drugs among others have been identified as factors contributing to adverse outcomes of ART and the development of drug resistance.[3,4] Furthermore, it has been shown that up to 7.4% of antiretroviral-naive patients harbour a primary drug-resistant virus, although data from central Africa are scarce.[5] In the present prospective cohort study, we aimed to assess the prevalence and determinants of virological failure, including primary drug resistance, in HIV-infected patients in Cameroon.

Methods

In the outpatient HIV clinic of the Bamenda Regional Hospital, patients were assessed for ART eligibility according to national guidelines and WHO recommendations.[6] At the time of the study, two doctors together

with pharmacists and social workers were attending to a total of 4500 HIV patients and more than 30 new patients every month. Ethical approval for the performance of the study was granted by the National Ethics Committee in Yaounde, Cameroon. Written informed consent was obtained from all of the individuals.

From January 2010 to October 2010, 300 consecutive patients being initiated on first-line ART were included in the study. The sample size was determined assuming a prevalence of antiretroviral resistance of 20%, a precision of 5% and a drop-out rate of 20%. Patients were monitored every 3 months by a study physician. Every 6 months, an analysis of blood count, liver function tests and CD4+ T cell count was performed and plasma was cryopreserved. The returned pills were counted and the

adherence ratio was calculated as the number of days a patient was taking the correct number of prescribed antiretroviral drugs divided by the number of days since the start of therapy.

Viral load testing was retrospectively performed using the Abbott real-time HIV-1 amplification reagent kit (University of Bonn). Drug-resistance testing in patients virologically failing at Month 12 by population sequencing was performed using the Viroseq version 2 (Abbott) (University of Zurich). The retrospective drug-resistance testing of baseline samples by next-generation sequencing was performed using 454 pyrosequencing technology as previously described[7] (University of Zurich; see the Supplementary data available at JAC Online for details) on patients with virological failure at Month 12 and an equal number of patients

Table 1. Baseline characteristics of patients with available viral load (VL) data at Month 12 ($n=238$)

	Virological success (VL <1000 HIV RNA copies/mL, $n=200$)	Virological failure (VL ≥1000 HIV RNA copies/mL, $n=38$)	P
Gender, n (%)			0.07[a]
male	60 (30)	9 (24)	
female	140 (70)	29 (76)	
Education, n (%)			0.29[a]
none	6 (3)	1 (3)	
primary	111 (56)	22 (58)	
secondary	74 (37)	13 (34)	
university/higher	9 (5)	2 (5)	
History of TB, n (%)			0.10[a]
no	179 (90)	31 (82)	
yes	21 (11)	6 (16)	
Current TB, n (%)			0.12[a]
no	191 (96)	35 (92)	
yes	9 (5)	2 (5)	
WHO stage, n (%)			0.01[a]
1	94 (47)	12 (32)	
2	50 (25)	10 (26)	
3	40 (20)	9 (24)	
4	15 (8)	5 (13)	
NRTI backbone, n (%)			0.35[a]
ZDV/3TC	165 (83)	28 (74)	
d4T/3TC	2 (1)	0 (0)	
TDF/3TC	13 (7)	6 (16)	
TDF/FTC	20 (10)	4 (11)	
Third drug, n (%)			0.92[a]
ABC	1 (1)	0 (0)	
EFV	49 (25)	10 (26)	
LPV/r	9 (5)	1 (3)	
NVP	141 (71)	27 (71)	
Age (years), median (range)	37.8 (19.5–78.1)	34.9 (22.3–79.5)	0.03[b]
CD4+ T cells/mm^3, median (range)	184 (1–889)	116 (3–349)	0.05[b]
VL (log$_{10}$ HIV RNA copies/mL), median (range)	5.4 (2.3–6.7)	5.5 (3.3–6.4)	0.99[b]
Haemoglobin (mg/dL), median (range)	11 (6.3–20.3)	10.7 (5.6–17)	0.54[b]

ZDV, zidovudine; 3TC, lamivudine; d4T, stavudine; TDF, tenofovir; FTC, emtricitabine; ABC, abacavir; EFV, efavirenz; LPV/r, lopinavir boosted with ritonavir; NVP, nevirapine.
[a]χ^2 test.
[b]Wilcoxon rank sum test.

Table 2. Univariable and multivariable logistic regression analysing risk factors for virological failure

	Univariable logistic regression			Multivariable logistic regression		
	OR	95% CI	*P*>z	OR	95% CI	*P*>z
Female sex (versus male)	1.33	0.59–3	0.49			
Age (per 10 years older)	0.71	0.49–1.02	0.07	0.79	0.54–1.15	0.21
CD4 baseline (per 100 cells/mm^3 lower)	1.41	1.02–1.96	0.04	1.47	1.02–2.08	0.04
WHO Stage 3 or 4 (yes versus no)	1.96	0.95–4.02	0.07	1.38	0.61–3.11	0.43
Viral load at baseline (per 1 log$_{10}$ HIV RNA copies/mL higher)	1.06	0.64–1.77	0.81			
Efavirenz at baseline (yes versus no)	1.24	0.56–2.77	0.59			
Nevirapine at baseline (yes versus no)	0.96	0.44–2.07	0.91			
Current TB (yes versus no)	1.25	0.26–6.03	0.78			
Haemoglobin at baseline (per 1 mg/dL higher)	0.91	0.76–1.09	0.33			
Adherence ratio (per 1% lower)[a]	1.05	1.02–1.08	<0.001	1.04	1.02–1.07	<0.001

[a]From baseline to last clinical follow-up.

with a viral load <1000 HIV RNA copies/mL at Month 12 who were matched according to gender, age, CD4+ T cell count and viral load at baseline.

The characteristics of patients with and without virological failure were compared using the Wilcoxon rank sum test for continuous data and χ^2 tests for categorical data.

Risk factors were assessed by logistic regression models. All the tests were two-sided and *P*<0.05 was considered significant. STATA v12 was used for all analyses. One patient with HIV group O virus, which is naturally resistant to NNRTI drugs, was excluded from the risk factor analysis.

Results

A total of 300 HIV-positive patients who started ART were included from January 2010 to October 2010. Viral load testing at Month 12 was successfully performed for 238 patients (Figure S1) and these patients were included in the analyses. Of these patients, 200 (84%) had a viral load of <1000 HIV RNA copies/mL, 160 (67%) patients had a viral load of <50 HIV RNA copies/mL and 38 (16%) patients experienced virological failure (Table 1).

At least one HIV-1 drug-resistance mutation was detected in 27/38 (71%) patients with virological failure at Month 12 using population sequencing. In 24 patients (63%), mutations associated with high-level resistance to any of the prescribed drugs were found. The M184V mutation was the most frequently detected NRTI mutation (*n*=18), the most commonly observed NNRTI resistance mutations being K103N (*n*=10), Y181C (*n*=7) and G190A (*n*=6) (Table S1).

Drug-resistance mutations at baseline were detected in 6/30 patients (20%) experiencing virological failure and 6/35 (17%) matched controls using next-generation sequencing (*P*=0.77). The most common mutations were V90I (*n*=4) and V108I (*n*=3). The M184V mutation was detected in one patient at a frequency of 1.3% and the K103N mutation was detected in another patient at a frequency of 1.7%. Both patients had virological failure at Month 12, when neither the M184V nor the K103N mutation was detected (Table S1).

In the multivariable logistic regression, a lower baseline CD4 cell count (OR per 100 cells/mm^3 lower 1.47, 95% CI 1.02–2.08, *P*=0.04) and a lower adherence ratio (OR per 1% lower 1.04, 95% CI 1.02–1.07, *P*<0.001) were associated with a higher risk of virological failure (Table 2). A higher age was associated with a higher adherence ratio (*P*=0.01) but not with lower virological failure rates (*P*=0.21). The most common reasons for incomplete drug intake (adherence ratio <95%) included 'drugs were out of stock' (37%), 'patient was out of town and could not collect drugs in time' (8%) and 'discontinued drugs because of side effects' (8%).

Discussion

In this study in rural Cameroon, 67% of patients achieved a viral load of <50 HIV RNA copies/mL after 1 year of treatment. HIV-1 drug-resistance mutations were detected in 71% of patients with virological failure. A previous study from the capital of Cameroon reported an undetectable viral load in 49% of patients after 2 years of ART, with major drug mutations being detected in only 10%.[8] Being treated in a rural centre has previously been shown to be a risk factor for virological failure in a study from Gabon.[9]

Poorer adherence was clearly associated with a higher risk of virological failure. Interestingly, younger patients (<36 years) showed poorer adherence but similar rates of virological failure and resistance compared with older patients. This supports previous findings that good adherence does not protect from the development of drug resistance if the drugs are continued in the absence of complete viral suppression.[10]

The predominant reasons for non-adherence were organizational; 41% of patients stated that their refill date was scheduled on a public holiday or that the ART drugs were out of stock. This suggests that repeated interruptions of drug supply and subsequent intake of antiretroviral drugs may be a specific problem in rural Africa. Our results are in line with those from studies from South Africa and Malawi suggesting that incomplete adherence is associated with a higher risk of virological failure[11] and that patients with previous treatment interruptions have higher rates of drug resistance.[12]

Using next-generation sequencing with a detection limit of 1%, we found primary drug-resistance mutations prior to ART initiation in 20% of patients with later virological failure, and at a

similar frequency in matched controls without virological failure. None of the mutations occurred at a frequency high enough to be picked up by conventional population-based sequencing. A previous pooled analysis shows an association between the existence of minority drug-resistance mutations and virological failure,[13] but the clinical use of detecting minorities in longitudinal studies seems, so far, to be limited.[14–16] We found that acquired drug-resistance mutations became detectable within the first year of antiretroviral therapy in a substantial number of patients. Because of cross-class resistance, future therapeutic options are limited to drugs from other classes such as protease or integrase inhibitors, which are not yet widely available in Africa.[17]

Our study had the limitation that follow-up was limited to 1 year and the number of patients with virological failure was limited. Minority variants were only assessed in a subset of patients with virological failure and matched controls. However, our study is among the very few to provide information on the existence of minority variants in an African setting.

In conclusion, minority drug-resistance mutations were detected by next-generation sequencing at baseline in a substantial proportion of patients but were not associated with virological failure after 1 year of first-line ART. An important reason for incomplete adherence to ART, as a major determinant of the substantial virological failure rates in our study, was the unavailability of drugs at the treatment centre. Strategies for an uninterrupted supply chain to rural parts in Africa seem pivotal in order to achieve sustainable treatment success.

Transparency declarations

None to declare.

Supplementary data

Supplementary data, including Figure S1 and Table S1, are available at *JAC* Online (http://jac.oxfordjournals.org/).

References

1 Coetzee D, Hildebrand K, Boulle A *et al*. Outcomes after two years of providing antiretroviral treatment in Khayelitsha, South Africa. *AIDS* 2004; **18**: 887–95.

2 Stringer JS, Zulu I, Levy J *et al*. Rapid scale-up of antiretroviral therapy at primary care sites in Zambia: feasibility and early outcomes. *JAMA* 2006; **296**: 782–93.

3 Rosen S, Fox MP, Gill CJ. Patient retention in antiretroviral therapy programs in sub-Saharan Africa: a systematic review. *PLoS Med* 2007; **4**: e298.

4 Sigaloff KC, Hamers RL, Menke J *et al*. Early warning indicators for population-based monitoring of HIV drug resistance in 6 African countries. *Clin Infect Dis* 2012; **54** Suppl 4: S294–9.

5 Gupta RK, Jordan MR, Sultan BJ *et al*. Global trends in antiretroviral resistance in treatment-naive individuals with HIV after rollout of antiretroviral treatment in resource-limited settings: a global collaborative study and meta-regression analysis. *Lancet* 2012; **380**: 1250–8.

6 WHO. *Antiretroviral Therapy for HIV Infection in Adults and Adolescents: Recommendations for a Public Health Approach*. 2010. http://whqlibdoc. who.int/publications/2010/9789241599764_eng.pdf.

7 Di Giallonardo F, Zagordi O, Duport Y *et al*. Next-generation sequencing of HIV-1 RNA genomes: determination of error rates and minimizing artificial recombination. *PLoS One* 2013; **8**: e74249.

8 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.

9 Liegeois F, Vella C, Eymard-Duvernay S *et al*. Virological failure rates and HIV-1 drug resistance patterns in patients on first-line antiretroviral treatment in semirural and rural Gabon. *J Int AIDS Soc* 2012; **15**: 17985.

10 Bangsberg DR, Charlebois ED, Grant RM *et al*. High levels of adherence do not prevent accumulation of HIV drug resistance mutations. *AIDS* 2003; **17**: 1925–32.

11 El-Khatib Z, Katzenstein D, Marrone G *et al*. Adherence to drug-refill is a useful early warning indicator of virologic and immunologic failure among HIV patients on first-line ART in South Africa. *PLoS One* 2011; **6**: e17518.

12 Luebbert J, Tweya H, Phiri S *et al*. Virological failure and drug resistance in patients on antiretroviral therapy after treatment interruption in Lilongwe, Malawi. *Clin Infect Dis* 2012; **55**: 441–8.

13 Li JZ, Paredes R, Ribaudo HJ *et al*. Low-frequency HIV-1 drug resistance mutations and risk of NNRTI-based antiretroviral treatment failure: a systematic review and pooled analysis. *JAMA* 2011; **305**: 1327–35.

14 Balduin M, Oette M, Daumer MP *et al*. Prevalence of minor variants of HIV strains at reverse transcriptase position 103 in therapy-naive patients and their impact on the virological failure. *J Clin Virol* 2009; **45**: 34–8.

15 Boltz VF, Bao Y, Lockman S *et al*. Low-frequency nevirapine (NVP)-resistant HIV-1 variants are not associated with failure of antiretroviral therapy in women without prior exposure to single-dose NVP. *J Infect Dis* 2014; **209**: 703–10.

16 Metzner KJ, Scherrer AU, Von Wyl V *et al*. Limited clinical benefit of minority K103N and Y181C-variant detection in addition to routine genotypic resistance testing in antiretroviral therapy-naive patients. *AIDS* 2014; **28**: 2231–9.

17 De Luca A, Hamers RL, Schapiro JM. Antiretroviral treatment sequencing strategies to overcome HIV type 1 drug resistance in adolescents and adults in low-middle-income countries. *J Infect Dis* 2013; **207** Suppl 2: S63–9.