

## Clinical and virological response to antiretroviral drugs among HIV patients on first-line treatment in Dar-es-Salaam, Tanzania

Fausta Mosha<sup>1,2</sup>, Johanna Ledwaba<sup>3</sup>, Faustine Ndugulile<sup>1</sup>, Zipora Ng'ang'a<sup>4</sup>, Peter Nsubuga<sup>5</sup>, Lynn Morris<sup>3</sup>, Mabula Kasubi<sup>6</sup>, Andrew Swai<sup>6</sup>, Jurgen Vercauteren<sup>2</sup>, Anne-Mieke Vandamme<sup>2,7</sup>

<sup>1</sup> Ministry of Health and Social Welfare, Tanzania

<sup>2</sup> Clinical and Epidemiological Virology, Rega Institute for Medical Research, Department of Microbiology and Immunology, University of Leuven, Leuven, Belgium

<sup>3</sup> National Institute for Communicable Diseases (NICD), South Africa

<sup>4</sup> Jomo Kenyatta University of Agriculture and Technology, Kenya

<sup>5</sup> Global Public Health Solutions, Atlanta, Georgia, United States

<sup>6</sup> Muhimbili National Hospital, Tanzania

<sup>7</sup> Centro de Malária e outras Doenças Tropicais and Unidade de Microbiologia, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisboa, Portugal

### Abstract

**Introduction:** In Tanzania, the follow-up on antiretroviral therapy (ART) response is based on clinical outcomes. We investigated virological response and ARV resistance mutations in relation to clinical response in ARV-treated patients.

**Methodology:** A cross-sectional study of a cohort of 150 patients taking first-line ART in Dar-es-Salaam was conducted. Data were collected using standardized questionnaires and patients' blood samples. HIV viral load testing and genotyping was performed on all viremic samples. Statistical analyses compared clinical responders and non-responders.

**Results:** The median time on ART was 20 months; 71 (47%) patients were ART clinical responders. Clinical non-responders were more likely to have started ART with advanced disease with significantly lower median percentage weight gain (6% versus 20%) with respect to pre-treatment levels. Sixty-one (86%) and 64 (81%) of clinical responders and non-responders, respectively, had undetectable viral loads. Genotyping was successful in 24 (96%) virologically failing patients, among whom 83% had resistance mutations; 67% had dual nucleoside reverse transcriptase inhibitor (NRTI)/non-NRTI (NNRTI) resistance mutations. Seventeen (71%) and 19 (79%) patients had NRTI and NNRTI resistance mutations, respectively, which were related to the ART in use, with no difference between clinical responders and non-responders. The most prevalent subtypes were A and C, found in 9 (38%) and 7 (29%) patients, respectively.

**Conclusions:** The observed virological response was high and did not correlate with clinical response. The prevalence of ARV resistance mutations was high in viraemic patients and was related to the ARV prescribed. We recommend use of viral load monitoring during ART in Tanzania.

**Key words:** antiretroviral drug resistance; Muhimbili hospital; Tanzania.

*J Infect Dev Ctries* 2014; 8(7):845-852. doi:10.3855/jidc.3879

(Received 15 June 2013 – Accepted 08 September 2013)

Copyright © 2014 Mosha *et al.* This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

### Introduction

Tanzania, which in 2012 was estimated to have a population of 45 million, is among the countries highly affected by HIV with a mature, generalized epidemic [1]. The estimated HIV prevalence in 2012 was 5%, and is slightly higher among females (6%) than males (4%) [2]. Since 2003, there has been rapid scale-up of antiretroviral treatment (ART) for HIV infection in resource-limited countries, which has been identified as an international health care priority [3]. ART aims at restoring host immune responses, thus decreasing the risk of morbidity and mortality.

The public health approach to scaling up ART in resource-limited settings involves the use of standardized and simplified treatment regimens that are consistent with international standards and appropriate to local circumstances [4]. The emergence of some HIV drug resistance (HIVDR) is inevitable in populations taking ART, even if appropriate ART regimens are provided and optimal adherence to therapy is supported [3].

The use of ARVs to prolong lives of people living with HIV and AIDS (PLWHA) and to prevent mother-to-child transmission of HIV is now firmly established

in Tanzania. The country adopted a care and treatment program for PLWHA in 2003, with the goal of providing ARVs to 400,000 HIV-infected patients by the end of 2008 [4]; by 2010, nearly half of Tanzanians in need of treatment were receiving it [5]. The national policy is to provide ARV to individuals with CD4 counts < 350 cells/ $\mu$ L. The first-line ARV regimen is a combination of zidovudine, stavudine, lamivudine, nevirapine, and efavirenz. In case of drug intolerance or clinical failure, a second-line regimen is provided, containing a combination of protease inhibitors (PIs) [4].

In order to monitor ART response in Tanzania, the National AIDS Control Programme (NACP) adopted World Health Organization (WHO) guidelines as clinical and immunological criteria for monitoring ARV response, due to limited means and expertise for expensive virological follow-up [4]. The positive impact of ART in Tanzania has been remarkable, yet the patient monitoring system is exclusively dependent on clinical and immunological methods, which may not accurately detect virological failures early enough [5]. This has raised concerns with regard to the associated risk of antiretroviral drug resistance development, especially with the finding of 9% nucleoside reverse transcriptase inhibitor (NRTI) and/or non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance mutations in a population of ARV-naive newly diagnosed youth (between 13 and 25 years of age) in Tanzania [6].

We decided to investigate the factors that predict ART clinical response and associate them with virological responses in patients taking ART. We also determined the prevalence of ARV drug resistance mutations in the same cohort of patients.

## **Methodology**

### *Study population*

Eligibility of patients for ART was as recommended by the Tanzania HIV/AIDS care and treatment guidelines [4]. After ART initiation, patients were seen by the clinician every month for clinical assessment and ARV refill. For immunological monitoring, testing for the level of CD4 cells count was done every six months. There was no routine virological monitoring at the time of data collection. The inclusion criteria for this study was being on first-line treatment for at least six months and at most three years, and being over 18 years of age. All patients who met these inclusion criteria were interviewed for consent between August and October 2007. The Epi Info StatCalc for unmatched case-control study was

used to estimate the sample size of the two studied populations, clinical responders and clinical non-responders, powered at 80%. The prevalence of ARV resistance in clinical responders that was used in this calculation was 57.40% [7]. The sample size obtained included a total of 144 patients (72 clinical responders and 72 non-clinical responders).

### *Study design*

A cross-sectional study in a cohort of patients taking ARV in Dar-es-Salaam was conducted. The clinical stage of HIV disease for each patient was determined according to WHO guidelines [8]; patients were grouped into stage I, II, III, or IV accordingly. Clinical failure was defined by clinicians as either new, recurrent (after being resolved), or persistent stage III or stage IV conditions at six months or later after the start of treatment based on the Tanzanian Ministry of Health and Social Welfare guidelines [4]. Since there were more clinical responders than non-responders, in order to obtain a balanced number from each group, two randomly selected ART clinical non-responders were interviewed every day, and after every selected clinical non-responder, the third-next HIV infected patient who was clinically responding well to ART was also selected. In this way, a maximum of four patients were enrolled daily for three months.

Ethical clearance to conduct this study was obtained from the National Institute for Medical Research in Tanzania. All patients were enrolled after they had provided their informed consent. All patients' information was removed from the obtained data.

### *Data collection*

Tools for data collection included standardized questionnaires, patients' records, and patients' blood samples. The variables collected included social and demographic information at study entry, and clinical progression from HIV diagnosis to the start of treatment. Descriptive analysis was done for the basic demographic and clinical characteristics of patients as well as continuous variables such as age. Patients' records were consulted to determine the percentage of patients who kept all of their appointments in the previous six months.

### *Defining ART compliance based on consistency of keeping appointments*

Clinic attendance for each patient was reviewed for the previous six months based on the given clinic appointment days for ARV refills. Compliance based

on consistency of keeping appointments was based on 100% for each patient visiting the clinic for their refills on the scheduled appointment dates for the entire six months [9].

#### *Laboratory testing*

Blood for HIV-1 RNA levels testing and genotyping was collected in EDTA tubes from all patients and stored at -20°C before being shipped to South Africa for viral load testing and DNA sequencing under cold chain.

HIV-1 RNA levels in plasma were detected by nucleic acid amplification polymerase chain reaction (PCR) technology using the Amplicor HIV-1 Monitor Test (Roche Diagnostics, Mannheim Germany) [10] with a detection limit of 400 copies/mL.

For genotyping, extraction of viral ribonucleic acid (RNA) was performed using a Total Nucleic Acid Isolation Kit (Roche Diagnostics, Mannheim Germany) on automated Magna Pure LC. RNA was reverse-transcribed using the ThermoScript RT-PCR system (Invitrogen, Carlsbad, USA) in the presence of a gene-specific primer. A nested PCR was performed with Long Template PCR Kit (Roche Diagnostics, Mannheim Germany) to generate a 1.7 Kb fragment spanning the pol gene. PCR products were sequenced using Big Dye Terminator (Applied Biosystems, Foster City, USA) on a 3100 ABI genetic analyzer (Applied Biosystems, Foster City, USA) [11].

#### *Sequence analysis*

Sequences were manually edited using Sequencher version 4.5 (Gencode, Los Angeles, USA) and further aligned using Bioedit (Biosciences, Carlsbad, USA). Subtyping was done using REGA subtyping tool version 2 [12].

#### *Resistance interpretation and genotypic susceptibility scores (GSSs)*

Resistance interpretation was done according to the HIVdb V5.1.2 [13] or REGA HIV-1 automated subtyping tool 8.01 criteria. For HIVdb V5.1.2, the drug-specific GSS for each ARV was calculated; the assigned drug-specific GSS was 1.0 for ARV (to which HIV was predicted to be fully susceptible) and 0 for ARV (to which HIV was predicted to be fully resistant), and 0.5 for intermediate resistance. For the Rega algorithm, GSS was scored as indicated by the algorithm; the main difference with HIVdb was that a fully active boosted PI was given a higher weight (GSS = 1.5) than a fully active unboosted PI (GSS = 1.0).

#### *Statistical analysis*

Epi Info version 3.3.2 and Microsoft Excel were used for general data analysis. Clinical progression was assessed by comparing clinical characteristics at hospital registration, baseline, and at the time of the study, taking into account the change in clinical characteristics over time. Median percentage weight gain was adjusted for amount of time on treatment. ART clinical non-responders were compared to ART clinical responders, and the association was checked with virological failure. The Yates-corrected Chi-squared test analysis and the Wilcoxon two-sample test were used to compare responders with non-responders for categorical and continuous variables, respectively, at 95% CI and  $p < 0.05$ .

## **Results**

### *Demographics, clinical and virological characteristics of patients*

Among the 150 recruited HIV-infected patients, 71 patients (47%) clinically responded after a median of 16.5 months (range, 12.5-34.0) of first-line treatment. Seventy-nine patients (53%) did not clinically respond after a median of 21.0 months (range, 14.5-34.5) of first-line treatment. There was no statistical difference in the average time receiving treatment for both groups ( $p = 0.3$ ). Sixty-one of the 71 clinical responders and 64 of the 79 clinical non-responders had an undetectable viral load. The majority were females (65%), most were single (54%), and 88 (59%) had attained primary school education only (first seven years of schooling).

There were no significant demographic differences between the responders and non-responders with respect to appointment adherence, gender (overall, 64% were women), marital status (overall, 46% were married), education level (overall, 20% had attained post-primary education), and age (40 years was the overall average age). Sixty-three patients had 100% appointment compliance, with no significant differences between clinical non-responders and responders (Table 1).

Clinically, there was no difference between the responders and non-responders with regard to type of ARV used, treatment used against opportunistic infections (mainly fluconazole and cotrimoxazole in Tanzania), plasma viral load among those detectable (3.0 Log<sub>10</sub> plasma HIV-1 RNA on average), mean BMI after one year of treatment (24.8 on average), median CD4 at hospital registration and during ART initiation, period of illness from diagnosis to ART initiation, and period of ART (Table 1).

**Table 1.** Demographic, clinical and virological characteristics of ART clinical non-responders and ART clinical responders

Characteristics	ART on-responders (N = 79)	ART responders (N = 71)	P value
Any reported missed appointment	32 (41%)	23 (32%)	0.4
At least one ARV change	28 (35%)	17 (24%)	0.2
Above primary school level	13 (16%)	17 (24%)	1.0
Female	50 (63%)	46 (65%)	0.8
Married	35 (44%)	34 (48%)	0.8
Prophylaxis against opportunistic infections	26 (33%)	26 (37%)	0.8
Viral Load detected (> 400 copies/mL)	15 (19%)	10 (14%)	0.3
Stage IV at ART initiation	14 (18%)	5 (7%)	<b>0.08</b>
Stage III at ART initiation	22 (28%)	36 (51%)	<b>0.07</b>
Stage II at ART initiation	42 (53%)	29 (41%)	0.2
Stage IV after one year on ART	2 (3%)	2 (3%)	1.0
Stage III after one year on ART	10 (13%)	9 (13%)	0.99
Stage II after one year on ART	58 (73%)	46 (65%)	0.9
<b>Continuous variables</b>	<b>Median (IQR)</b>	<b>Median (IQR)</b>	<b>P value</b>
Age	40 (36.5-47) years	39 (30-46) years	0.15
CD4 at ART initiation	153 (65-252) cells/microliter	103 (38-178) cells/microliter	0.2
CD4 six months on treatment	193 (111-315) cells/microliter	247 (175-339) cells/microliter	<b>0.05</b>
CD4 one year on treatment	376 (250-558) cells/microliter	392 (169-393) cells/microliter	<b>0.03</b>
Period of illness before ART	5.5 (3.5-8.5) months	6.5 (4.5-9.5) months	0.3
Period on ART (months)	21.0 (14.5-34.5)	16.5 (12.5-34.0)	0.3
Percentage weight gain (kgs)	6 (3-9)	20 (14-28)	<b>&lt; 0.01</b>
<b>Continuous variables</b>	<b>Mean (SD)</b>	<b>Mean (SD)</b>	<b>P value</b>
BMI at baseline	23.5 (4.1)	22.8 (4.5)	0.3
BMI one year on treatment	24.3 (4.2)	25.3 (4.6)	0.3
Log10 plasma HIV-1 RNA level (copies/mL)	3.0 (0.9)	2.9 (0.7)	0.4

**Table 2.** Summary of ARV resistance mutations detected, MNH 2007

Mutations	N = 24	Non-responders (N = 15)	Responders (N = 9)	P value
Any RT or major PI resistance mutation	20 (83%)	11	9	0.13
Dual NRTI and NNRTI mutations	16 (67%)	10	6	0.70
Any NRTI mutations	17 (71%)	10	7	0.46
M184V/I	18 (75%)	12 (80%)	6 (67%)	
K70R/E	7 (29%)	6 (40%)	1 (11%)	
K219Q/E	7 (29%)	5 (33%)	2 (22%)	
D67N	8 (33%)	6 (40%)	2 (22%)	
T215I/Y/F	4 (17%)	4 (27%)	0 (0%)	
M41L	2 (8%)	2 (13%)	0 (0%)	
L210W	1 (4%)	1 (7%)	0 (0%)	
L74I	2 (8%)	1 (7%)	1 (11%)	
T69D	4 (17%)	2 (13%)	2 (22%)	
A62V	2 (8%)	1 (7%)	1 (11%)	
<b>Any NNRTI mutations</b>	19 (79%)	11	8	0.36
G190A/S	9 (38%)	7 (47%)	2 (22%)	



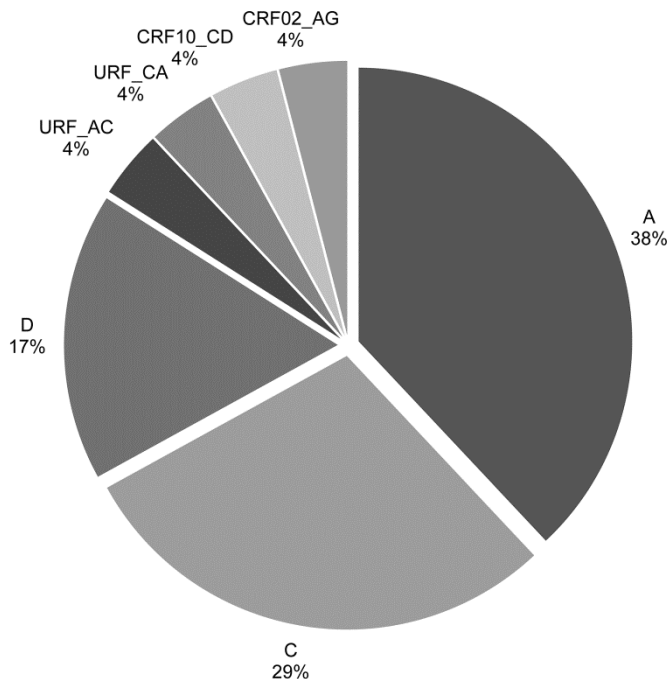
Initiation of therapy at advanced disease stage (WHO clinical stage IV) was not significantly associated with clinical failure (OR = 2.8 [2.1-8.4];  $p = 0.08$ ). ART clinical responders had a significantly higher weight gain (6% in non-responders versus 20% in responders) and higher CD4 count gain with respect to pre-treatment levels (median CD4 count of 376 cells/ $\mu$ L for the non-responders versus 392 cells/ $\mu$ L for the responders) (Table 1). However, the percentage with undetectable viral load was high in both groups (83% on average) and was not significantly different between non-responders and responders. Patients with detectable viral load received treatment for an average 25 months ( $\pm 9.3$  months), while those with undetectable viral load received treatment for 21 months ( $\pm 9.6$  months).

*HIV-1 drug resistance mutations and subtypes*

Among the 25 patients who were viremic (having viral load > 400 copies/mL), genotyping was successful on 24, of whom 9 were clinical responders and 15 were clinical non-responders. One of the samples from an ART clinical responder could not be successfully sequenced.

None of the drug resistance parameters were significantly different in clinical non-responders as compared to clinical responders (Table 2). Eighty-three percent of the genotyped samples had resistance mutations (excluding minor PI resistance mutations) and 67% had dual NRTI/NNRTI resistance mutations. Only four clinical non-responders had no resistance mutations. There were no major PI mutations, though eight (33%) samples had minor PI mutations (L10I or L10V), consistent with the distribution of these polymorphisms in the wild type subtypes found and thus consistent with the absence of PI drug selective pressure. There were 17 (71%) patients with NRTI-associated mutations and 19 (79%) had NNRTI-associated mutations, both with no significant difference between ART clinical non-responders and responders. With respect to individual mutations, the difference among ART clinical non-responders and responders could not be assessed statistically due to the low number of sequenced samples. M184V/I was the most common NRTI mutation, found in 18 (75%) samples, while 9 (38%) samples had thymidine analogue mutations (TAMs). The most common TAMs were of the TAM-2 pathway (67N/70R/215F/219EQ), and one (11%) patient had TAM-1 pathway mutations (41L/210W/215Y) (Table 2). G190A/S, K103N, and Y181C were the predominant mutations observed, found in nine (38%),

**Figure 1.** Percentage of observed Subtypes and Recombinants, Dar-es Salaam, Tanzania



eight (25%), and five (21%) samples, respectively, among NNRTIs. Mutations detected were related to the ARV therapy in use; more were observed for lamivudine (M184V/I), stavudine (M41L, D67N, K60R, L210W, T215F/Y, K219Q/E), and nevirapine (K103N/S/T, Y181C, G190A, V106A). The genotypic susceptibility score was not significantly different between clinical responders and non-responders (Table 2).

The most prevalent subtypes were A (n = 9; 38%) and C (n = 7; 29%), followed by subtype D (n = 4; 17%), URF\_AC (n = 1; 4%), URF\_CA (n = 1; 4%), CRF10\_CD (n = 1; 4%), and CRF02\_AG (n = 1; 4%) (Figure 1). L10I and L10V, known as minor PI drug resistance mutations, were found among subtypes A, D, and CRF02\_AG. However, due to the low number of viremic patients per subtype, subtype-specific differences on specific ARV resistance mutations could not be assessed.

**Discussion**

We conducted this study to investigate the association between a number of variables with clinical response to first-line ART because clinical response – not virological response – is the evaluation criterion used in low-resource countries such as Tanzania. Our patients were selected from those who were on first-line ART for at least six months and at

most three years, up to a study size of 150. The mean percentage weight gain, median CD4 count after starting treatment, and median CD4 cell increase were lower among our ART clinical non-responders compared to responders, a result to be expected since weight gain is related to the clinical definition of failing treatment, and declining CD4 cell count causes disease progression. It is thus obvious that patients failing ART clinically have poorer weight gain and slower CD4 increase, as has already been reported [14].

Despite the fact that only 71 patients (47%) met the definition of good clinical response, only 15 (19%) were viremic (> 400 copies/mL). A similar discordance was also observed in a study in Uganda [15]. Since decisions for change of therapy are based on the clinical progression of the patients, 64 of our patients with undetectable viremia would be eligible for changing therapy, while 10 viremic patients would have been considered to do well on their therapy. The majority of the patients had started therapy at WHO clinical stage II, which could explain the high virological success rate. This indicates effective treatment regimens and early access to care and treatment [14]. These findings are similar to a study in Uganda, which found that more than 70% of the patients remained in care for more than one year of ART [16].

Importantly, our study demonstrated that initiation of therapy in later stages of the disease was associated with more clinical treatment failure, though this was not related to virological failure. It is indeed known that patients who start ART with low CD4 counts have slow recoveries [16], even though in a minority of patients with advanced disease and low CD4 counts when therapy is initiated, clinical improvement may be observed in the absence of CD4 improvement [17]. Making therapy decisions based on clinical response, especially among those starting treatment at advanced disease stages, may result in unnecessary treatment changes. It seems unrealistic to expect no clinical progression in these late-stage patients after only six months of treatment.

Other major goals of this study were to evaluate ARV resistance mutations among the virologically failing patients, to assess the association with the clinical definition of ART failure and with the different first-line treatment regimens available in Tanzania, and to evaluate the prospects of these patients for second-line treatment response. All the viremic samples were sequenced in the pol region with a success rate of 96% (24 of 25). Not counting minor

protease inhibitor mutations, the prevalence of drug resistance was high, as almost all patients with detectable viral load (except four clinical non-responders) had resistance mutations all related to the ART regimen received. The occurrence of multi-NRTI resistance mutations and dual resistance to NRTIs and NNRTIs in 67% of the virologically failing patients is especially worrying. Such a profile may compromise the second-line treatment, which consists of dual NRTIs and a PI [4]. The NRTI mutations found – a high number of TAMs (high level resistance to stavudine and zidovudine) and a high prevalence of M184V (high level resistance to lamivudine) – are typical for the first-line regimen in Tanzania, which consists of mainly stavudine, nevirapine, efavirenz, zidovudine, and lamivudine [18]. Among the NNRTI-associated mutations, G190A was the most prevalent, consistent with the use of nevirapine in first-line treatment. This calls for a more careful consideration of the use of nevirapine as a single dose for prevention of mother-to-child transmission.

Consistent with our previous report, the most prevalent subtypes were A and C, followed by D and recombinants [6]. We could not thoroughly assess the association between therapy response and resistance pattern because of our small sample size and the different HIV-1 subtypes, but we found no contradiction to current observations that subtype does not influence therapy response [19]. However, our data may contribute to larger studies on this topic.

We estimated therapy adherence based on the consistency of clinic attendance, due to the fact that this is the only time that the patients can get ARV refills. Thus, we assumed that if a patient misses clinic, he or she will run out of medication. In the absence of virological follow-up parameters, it may be of paramount importance to carefully monitor adherence. We anticipate that such information could be more predictive of virological failure, and it might be more feasible to combine adherence parameters with clinical failure in resource-limited settings where virological monitoring remains rare. In Tanzania, Muhimbili Hospital started to provide ART earlier than many other centers. It has been demonstrated that virological failure, both in terms of insufficient suppression of viral load, and in terms of drug resistance, precedes CD4 cell decline, which ultimately leads to clinical progression and death [20]. To follow up patients using clinical parameters, thus, means that any therapy change will be delayed compared to following up using virological parameters. In our study, some of the patients who

responded to therapy using clinical parameters only were already failing virologically. Other reports have suggested similar findings in resource-poor settings [20]. Similar to our study findings, these reports show that clinical criteria are poor predictors for virological outcome. However, to our surprise, there were many patients defined to have clinical failure who were actually not virologically failing.

Observing equally high drug resistance levels in both clinically failing and responding patients is alarming. Thus, virological testing is important and might even be cost effective in resource-limited settings, if one wants to avoid drug failure for the individual patient and ARV resistance virus transmission at the population level. Similar findings were observed in Ethiopia where clinico-immunological assessments were found to have lower performance in diagnosing virological failures [21].

#### *Limitations of the study*

Our study was designed to balance the number of clinical responders and clinical non-responders, and thus we cannot expand on the predictors for virological failure. Our estimates are only valid for patients retained in follow-up, and may therefore not be representative of all patients on first-line ART in Tanzania. Also, our results were based on a limited number of samples and may not be representative of all patients using ART in Tanzania.

#### **Conclusions**

In our study population of 150 patients in ART follow-up for six months to three years in Dar-es-Salaam, Tanzania, we found that the criteria for defining clinical failure of ART did not reflect the actual virological failure. While half of our patients failed clinically, few patients had a detectable viral load, and in them, the prevalence of ARV drug resistance was high and was related to the ARV drugs prescribed. We recommend the use of viral load monitoring to patients on ARVs and the use of ARV resistance testing, preferentially before but especially during treatment for patients experiencing viral load failure. Monitoring the emergence of drug-resistant HIV in populations starting and using ARV therapy would be a useful approach for planning effective treatment programs in Tanzania.

#### **Acknowledgements**

We would like to thank the study participants for consenting to participate in this study, the staff at MNH for supporting data collection, Field Epidemiology and Laboratory Training Programme (FELTP) in Kenya, and all collaborating partners. The study was supported by the Centre for Disease Control and Prevention (CDC) through FELTP. AMV and JV acknowledge the Seventh Framework Programme (FP7/2007-2013) under the project Collaborative HIV and Anti-HIV Drug Resistance Network (CHAIN) - grant agreement 223131; FWO grant G.0611.09, and a grant from the Interuniversity Attraction Poles Programme, Belgian State, Belgian Science Policy (IAP-VI P6/41).

#### **References**

1. National Bureau of Statistics (NBS) Tanzania (26<sup>th</sup> August 2012) population and housing census counts. Available: <http://www.nbs.go.tz/nbs/takwimu>. Accessed 15 March 2013.
2. National Bureau of Statistics. (2013) Tanzania HIV/AIDS and Malaria Indicator Survey 2011-2012. Available: <http://nbs.go.tz/tnada/index.php/catalog/23>. Accessed 22 April 2013.
3. World Health Organization (2010) HIV Drug resistance early warning indicators to monitor HIV drug resistance prevention and antiretroviral treatment sites. Available: <http://new.paho.org/hq/dmdocuments/2010/hivdr-early-warning.pdf>. Accessed 9 June 2012.
4. Ministry of Health and Social Welfare Tanzania (2005) National Guidelines for the Clinical Management of HIV and AIDS (2005/2009) National Aids Control Programme (NACP). Available: <http://www.nacp.go.tz/site/publications/care-and-treatment>. Accessed 21 May 2006 and 16 July 2010.
5. WHO/UNAIDS/UNICEF (2011) Global HIV/AIDS Response: Epidemic update and health sector progress towards Universal Access. Available: [http://www.who.int/hiv/pub/progress\\_report2011/en](http://www.who.int/hiv/pub/progress_report2011/en). Accessed 22 February 2013.
6. Moshia F, Urassa W, Aboud S, Lyamuya E, Sandstrom E, Bredell H, Williamson C (2011) Genetic Diversity and Prevalence of Genotypic Resistance to Antiretroviral Drugs in Treatment Naive HIV-infected Youths in Dar-es-Salaam, Tanzania 2005. *AIDS Res Hum Retroviruses* 27: 377-382.
7. Adje C, Cheingsong R, Roels T, Maurice C, Djomand G, Verbiest W, Hertogs K, Larder B, Monga B, Peeters M, Eholie S, Bissagene E, Coulibaly M, Respass R, Wiktor SZ, Chorba T, Nkengasong J; UNAIDS HIV Drug Access Initiative, Abidjan, Côte d'Ivoire (2001) High Prevalence of Genotypic and Phenotypic HIV-1 Drug-Resistant Strains among patients receiving Antiretroviral therapy in Abidjan, Ivory Coast. *J Acquir Immune Defic Syndr* 26: 501-506.
8. World Health Organization (2006) Disease Staging System for HIV Infection and Disease. Available: <http://www.who.int/hiv/pub/guidelines/HIVstaging150307.pdf>. Accessed 28 June 2007.
9. American Public Health Association (2004) Adherence to HIV treatment Regimens. Recommendations for Best Practices. Available:

- <http://www.apha.org/Bestpracticesnew.pdf>. Accessed 19 May 2006.
10. Barlow KL, Tosswill JH, Clewley JP (1995) Analysis and genotyping of PCR products of the Amplicor HIV-1 kit. *J Virol Methods* 52: 65-74.
  11. Pillay V, Ledwaba J, Hunt G, Rakgotho M, Singh B, Makubalo L, Bennett D, Puren A, Morris L (2008) Antiretroviral drug resistance surveillance among drug-naïve HIV-1-infected individuals in Gauteng Province, South Africa in 2002 and 2004. *Antiviral Therapy* 13: 101-107.
  12. De Oliveira T, Deforche K, Cassol S, Salminen M, Paraskevis D, Seebregts C, Snoeck J, van Rensburg E, Wensing A, van de Vijver D, Boucher C, Camacho R, Vandamme A (2005) An automated genotyping system for analysis of HIV-1 and other microbial sequences. *Bioinformatics* 21: 3797-3800.
  13. Stanford University (1998-2014) HIV Drug Resistance Database. Available: <http://hivdb.stanford.edu>. Accessed 25 August 2009.
  14. Egger M, May M, Chêne G, Phillips A, Ledergerber B, Dabis F, Costagliola D, D'Arminio Monforte A, de Wolf F, Reiss P, Lundgren J, Justice A, Staszewski S, Leport C, Hogg R, Sabin C, Gill M, Salzberger B, Sterne J (2002) Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. *Lancet* 360: 119-129.
  15. Mermin J, Ekwaru J, Were W (2011) Utility of routine viral load, CD4 cell count, and clinical monitoring among adults with HIV receiving antiretroviral therapy in Uganda: randomised trial. *BMJ* 343: d6792.
  16. Ahoua L, Guenther G, Pinoges L, Anguzu P, Chaix M, Tiec C, Balkan S, Olson D, Olaro C, Pujades-Rodríguez M (2009) Risk factors for virological failure and sub-therapeutic antiretroviral drug concentrations in HIV-Positive adults in rural north-western Uganda. *BMC Infectious Diseases* 9: 81.
  17. Richmann D, Grimes J, Lagakos S (1990) Effect of stage of disease and drug dose on zidovudine susceptibility of isolates of human immunodeficiency virus. *J Acquir Immune Defic Syndr* 3: 743-746.
  18. Ross L, Scarsella A, Raffanti S, Henry K, Becker S, Fisher R, Liao Q, Hirani A, Graham N, St Clair M, Hernandez J (2001) Thymidine analog and multinucleoside resistance mutations are associated with decreased phenotypic susceptibility to stavudine in HIV type 1 isolated from zidovudine-naïve patients experiencing viremia on stavudine-containing regimens. *AIDS Res Hum Retrovirus* 17: 1107-1115.
  19. Holguin A, Paxinos E, Hertogs K, Womac C, Soriano V (2004) Impact of frequent natural polymorphisms at the protease gene on the in vitro susceptibility to protease inhibitors in HIV-1 non-B subtypes. *J Clin Virol* 31: 215-220.
  20. Rawizza H, Chaplin B, Meloni S, Eisen G, Rao T, Sankalé J, Dieng-Sarr A, Agbaji O, Onwujekwe D, Gashau W, Nkado R, Ekong E, Okonkwo P, Murphy R, Kanki P; APIN PEPFAR Team (2011) Immunologic criteria are poor predictors of virologic outcome: implications for HIV treatment monitoring in resource-limited settings. *Clin Infect Dis* 53: 1283-1290.
  21. Misgena D (2011) The pattern of immunologic and virologic responses to Highly Active Antiretroviral Treatment (HAART): Does success bring further challenges? *Ethiopia Journal of Health Development* 25: 61-67.

#### Corresponding author

Fausta Moshia.  
 Ministry of Health and Social Welfare  
 Samora Avenue  
 Box 9083,  
 Dar es Salaam, Tanzania  
 Phone: +255-22-2120261  
 Fax: +255-22-2139951  
 Email: [fausta\\_mosha@yahoo.com](mailto:fausta_mosha@yahoo.com)

**Conflict of interests:**No conflict of interests is declared.