

Virological failure predicts mortality in  
HIV-infected patients who receive antiretroviral  
treatment in rural Tanzania



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# **Virological failure predicts mortality in HIV-infected patients who receive antiretroviral treatment in rural Tanzania**

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## **I. ABSTRACT**

### **Background**

Virological monitoring of HIV-infected patients on antiretroviral treatment (ART) is normally not available in resource-limited settings and many patients experience unrecognized virological failure. We studied the clinical, immunological and virological outcome of virological failure in rural Tanzania.

### **Method**

Previously, virological efficacy was measured in a cohort of HIV-infected patients treated with ART at Haydom Lutheran Hospital. In the present study, patients with virological failure (VF; HIV-1 RNA > 400 copies/ml) were followed-up and compared to those with virological response (VR; HIV-1 RNA < 400 copies/ml) with regard to mortality, CD4 change and subsequent virological outcome. For each patient with VF, one patient with VR was randomly selected. Mortality and virological failure rates were analyzed using Chi-squared test, while comparison of CD4 T-cell counts was performed with Mann Whitney U test.

### **Results**

Fifty-six patients with VF had a median CD4 T-cell count of 358 cells/ul (interquartile range (IQR) 223–635) and a median HIV-RNA of 13,573 copies/ml (IQR 2326-129,736). Median CD4 T-cell count for those with VR was 499 cells/ul (IQR 290-636). During a median follow-up time of 39 months (IQR 18-42), 8 of 56 patients (14.3%) with VF died, compared to 1 of 63 patients (1.6%) with VR ( $p=0.009$ ). All registered deaths were HIV-related. Of 55 patients with subsequent HIV-RNA measurements, only 12 of 30 (40%) patients with VF achieved virological suppression, compared to 80% of those with VR ( $p=0.003$ ).

## **Conclusion**

Virological failure predicts death and subsequent virological failure in patients on ART in a resource-limited setting. Access to viral load testing and better follow-up of patients with virological failure is warranted.

## **Keywords:**

HIV, ART, resource-limited settings, long-term outcome, viral load, mortality, CD4.

## II. INTRODUCTION

The human immunodeficiency virus (HIV) epidemic is a major global challenge. By the end of 2010, an estimated 34 million people were living with HIV/AIDS globally [1], of whom 69% reside in Sub-Saharan Africa [2]. The introduction of antiretroviral therapy (ART) and the commitments made of the United Nations and the World Health Organization (WHO) to achieve universal access to HIV prevention, treatment, care and support for all in need, has radically changed the face of the HIV epidemic. The scaling up of ART in resource-limited settings has led to a more than 100-fold increase in access to HIV treatment in less than a decade, and by 2011, an estimated 6.2 million people were receiving treatment [2]. New infections and AIDS-related deaths are declining, also in high-burden countries [2]. Today, the estimated life expectancy of HIV-positive individuals approaches that of the general population, as long as treatment is initiated in an early stage and CD4 T-cell count is restored to a normal level [3]. Unfortunately, the necessary conditions to achieve proper treatment are still beyond reach for many HIV-infected individuals in resource-limited settings.

The key to long-term benefit of ART is sustained suppression of viral replication [4]. In western industrialized countries HIV-positive patients on ART are monitored with HIV-RNA quantification and genotypic resistance testing. Because of high costs and the need for sophisticated laboratory equipment, these tests have not been recommended by WHO in rural Africa. WHO's guidelines for resource-limited settings are to initiate and monitor treatment, as well as base the decision on when to switch to second-line ART, on clinical criteria and CD4 T-cell counts [5]. However, several studies have shown that these criteria are insufficient to correctly identify virological failure [6, 7]. Hence, the recognition of virological failure might be delayed for months and years. The clinical consequences of this are not well described.

In order to elucidate the long-term consequences of virological failure in resource-limited settings, we carried out a study of children and adults on ART in rural Tanzania. Clinical, immunological and virological outcomes in patients with previous virological failure were compared to a control group with previous virological suppression.

### **III. METHOD**

#### **Study setting**

Tanzania is a large developing country on Africa's east coast. It has a population of nearly 47 million. The adult prevalence (15-49 years) of HIV was estimated in 2008 to be 5.7% [8].

With efforts from multiple international donors, access to ART has increased over the past 10 years. By December 2010, 384 816 people were receiving ART [8].

Haydom Lutheran Hospital (HLH) is located in northern Tanzania. ART has been offered since 2003 in accordance with WHO's guidelines [5, 9]. In the study period the first-line treatment available was stavudine or zidovudine, together with lamivudine, plus either nevirapine or efavirenz. From December 2006 second-line treatment has also become available; lopinavir/ritonavir, didanosine and abacavir, and later also tenofovir. Patients are monitored with regular CD4 T-cell counts, but HIV-RNA measurements are not available at a regular basis. Thus, the patient group reflects the conditions in similar rural, resource-limited settings.

#### **Study design**

A cohort study of patients consecutively starting ART was established in 2003. As part of a previous research project, patients treated with ART underwent viral load testing between 2007 and 2010, as described by Johannessen *et.al.* and Bratholm *et.al.* [10, 11] (Figure 1).



Based on these results, a number of patients switched treatment regimen in accordance to WHO's criteria for switching to second-line ART (HIV-RNA > 10,000 copies/ml).

A total of 236 children and adults, of whom all had received ART for at least six months, were enrolled in this study. Fifty-six of these patients demonstrated virological failure (VF) (HIV-RNA > 400 copies/ml) and were thereby included in the present study (Figure 2). For each patient with virological failure, another patient with virological response (VR) was selected by choosing the patient with the closest hospital ID number who had HIV-RNA < 400 copies/ml. In total 119 patients were included in the present study, 56 with VF and 63 with VR. Baseline was defined as the date when VF or VR was first recognized during the previous studies running from 2007 to 2010.

All patients were followed up until August 2011. Blood samples for viral load testing were collected in July and August 2011, when the patients came to their monthly control. For those who died, were transferred out, lost to follow-up or missing, the follow-up time was right-censored at the date of the last known contact with the clinic (Figure 1). Demographic, clinical (death) and immunological (CD4 T-cell counts) data was collected from medical charts at the clinic. Death during the follow-up time was ascertained by home-visitors.

All data were typed and stored anonymously in the study database. National Institute for Medical Research in Tanzania and Regional Committee for Medical Research Ethics in Norway granted ethical approval, and all patients gave written consent to participate in the study.

### **Laboratory methods**

Recorded hematology and CD4 T-cell counts were measured at Haydom Lutheran Hospital using the Sysmex KX-21 Hematology Analyzer (Sysmex Corp., Kobe, Japan) and FACSCount flow cytometer (Becton Dickinson, San Jose, California, USA). The HIV-RNA

analyses from 2007-2010 and 2011 were performed at Muhimbili National Hospital, Dar Es Salaam, Tanzania, using either the Cobas TaqMan 48 Analyzer (Roche Diagnostics, Branchburg, New Jersey, USA), or the Cobas Amplicor HIV-1 Monitor v1.5 (Roche Diagnostics, Branchburg, New Jersey, USA). Due to logistical constraints, a subset of the HIV-RNA analyses from 2011 were performed at Oslo University Hospital, using the Cobas TaqMan 48 Analyzer. Lower limit of detection ranged from 20 to 400 copies/ml depending on the different instruments. Hence, a cut-off at 400 copies/ml was used to define virological failure.

Baseline resistance data were analyzed at Oslo University Hospital, Norway, using ViroSeq HIV-1 Genotyping System (Abbott Molecular, De Plains, Illinois, USA). All specimens with viral load > 1000 copies/ml were tested. Only drug resistance mutations listed in the Spring 2008 update from the International AIDS Society were considered [12]. Resistance profiles to antiretroviral drugs were interpreted according to the Stanford University HIV Drug Resistance Database [13].

Plasma samples for virological analyses performed in 2011 were centrifuged within one hour of venepuncture, and frozen at -20 degrees Celsius. The samples were transported at ambient temperature for less than 20 hours to the reference laboratories.

### **Statistical methods**

Categorical data were analysed using Chi-squared test or Fisher's exact test, as appropriate. Mann-Whitney U test was used for comparison of continuous data throughout, since some of the data were not normally distributed. Continuous data are presented as median values and corresponding interquartile range (IQR 25 and 75 percentile).

The main three endpoints of interest in this study were death, subsequent virological failure and immunological progression, based on median values of recorded CD4 T-cell counts with

intervals of 6 months (+/- 3 months). Difference in clinical and virological outcome was analysed using Chi-squared test or Fisher's exact test as appropriate. Risk of mortality was calculated with logistical regression. Differences in progression of CD4 T-cell count between the groups were analysed with Mann-Whitney U test.

SPSS version 19.0 (SPSS Inc., an IBM Company) was used for all the analyses. All tests were two sided and level of significance was set at  $p < 0.05$ .

## **IV. RESULTS**

### **Population characteristics**

The two study groups, VF (N=56) and VR (N=63), consisted of 36 (64%) and 49 (78%) women and 18 (32%) and 3 (5%) children  $\leq 15$  years, respectively (Table 1). The median CD4 T-cell count at baseline was 358 cells/ul (IQR 223-635) in the VF group and 499 cells/ul (IQR 290-636) in the VR group. Median HIV-RNA at baseline in the VF group was 13,573 copies/ml (IQR 2326-129,736), and undetectable in all patients in the VR group. Median time on ART before baseline was 29 months for the VF group (IQR 22-36) and 25 months for the VR group (IQR 14-34). Median follow-up time from baseline was 28 months for the VF group (IQR 18-42) and 40 months for the VR group (IQR 18-44).

There were no significant differences between the two groups with regard to gender, ART duration at baseline, median CD4 T-cell count at baseline and follow-up time. However, there were significantly more children  $\leq 15$  years in the VF group than in the VR group (mean age at baseline 26 years vs. 40 years,  $p < 0.001$ ) (Table 1). The majority of the patients in both groups were in WHO-stage 3 or 4 at the time of ART initiation; however, significantly more patients were in WHO-stage 4 in the VR group.

Twenty-five of the 56 patients with VF (45%) switched to second-line treatment after recognition of virological failure at baseline. None of the patients in the VR group changed to second-line ART during the follow-up. Median time before treatment switch after virological failure was 7 months (IQR, 4-11). Reasons for late switching and for not switching treatment included delayed reporting of laboratory results, patients' refusal and clinician's discretion. All patients switched to a regimen based on a boosted protease inhibitor (lopinavir/ritonavir).

### **Clinical outcome**

During the follow-up time 9 patients died (7.6%), 3 were lost to follow-up for more than 12 months (2.5%) and 35 were transferred to other clinics (29.4%). Seventeen patients (14.3%) still in care did not show up during collection of blood samples. The study profile is presented in Figure 2.

The proportion who died in the group with VF (8 of 56, 14.3%) was significantly higher than in the VR group (1 of 63, 1.6%),  $p=0.009$  (Table 2). Odds ratio (OR) of mortality was 10.3 higher given virological failure at baseline compared to virological response (95% CI 2.1-85). Multivariate adjustments are not justified due to small sample size. However, the odds ratios were practically unchanged after adjustment for age and gender; OR=15.1 (1.7-138) and 9.7 (1.2-80) respectively. If patients lost to follow-up were assumed dead the difference was even more striking: 11 of 56 (19.6%) with VF compared to 1 of 63 (1.6%) in the VR group ( $p=0.001$ ).

Median follow-up time from baseline to death was 12 months; ranging from 8 to 23 months. Three of those who died in the VF group had changed to second-line ART regimen after detection of virological failure. The presumed causes of death were related to HIV/AIDS: dehydration of diarrhoea and enteritis ( $n=3$ ), renal failure ( $n=1$ ), pulmonary tuberculosis ( $n=1$ ), meningitis from tuberculosis ( $n=1$ ) cytomegalovirus infection ( $n=1$ ) and toxoplasmosis of the brain ( $n=1$ ). For one of the patients, the cause of death was not registered.

### **Immunological outcome**

Median CD4 T-cell count was continuously lower in the VF group compared to the VR group every sixth month from baseline up to 3.5 years later. However, the difference was not significant at baseline ( $p=0.158$ ), 1 year ( $p=0.266$ ), 1.5 years ( $p=0.149$ ) and 2 years ( $p=0.133$ ). After 2.5 years ( $p=0.017$ ), 3 years ( $p=0.018$ ) and 3.5 years ( $p=0.036$ ) the difference in median CD4 count was significant.

### **Virological outcome**

An additional HIV-RNA viral load was measured in the 55 patients who showed up for their routine check-up in July/August 2011; 30 with VF and 25 with VR. Most patients received two nucleoside reverse transcriptase inhibitors (NRTI) and one non-nucleoside reverse transcriptase inhibitor (NNRTI), either efavirenz or nevirapine; however, 19 of the patients with virological failure received second-line treatment including lopinavir/ritonavir.

Virological failure at baseline predicted subsequent virological failure: Eighteen of 30 patients with previous failure (60.0%), compared to 5 of 25 VR patients (20.0%) had subsequent virological failure ( $p=0.003$ ) (Figure 2).

Thirteen of the 18 patients in the VF group with subsequent virological failure were patients who had switched to second-line treatment before the last HIV-RNA measurement. These patients were neither subjects to randomly nor routinely consistent implementation of second-line treatment when failure was first recognised. Because of this selection-bias, statistical comparative analysis of the effect of second-line ART was not conducted.

### **Resistance analyses**

Forty-seven patients in the VF group had isolates successfully sequenced at baseline, of whom 34 (72%) had resistance against first-line ART (Table 1). The most frequent mutations were M184V, conferring resistance to lamivudine and emtricitabine, and Y181C, B190A/S

and K103N, conferring resistance to NNRTIs. Twenty-two of the patients with detected mutations switched to second-line treatment. Resistance at baseline did not predict death. Six of the 34 patients (17.6%) with baseline resistance died during follow-up, compared to 2 of 13 patients (15.4%) without baseline resistance ( $p=1.00$ ).

## **V. DISCUSSION**

In this study from rural Tanzania, virological failure predicted a poor long-term outcome.

Among those with virological failure from the first-line ART regimen, 14.3% died during the follow-up time, compared to only 1.6% of the virological responders. This demonstrates the excellent prognosis of HIV-infected patients in rural Africa if they achieve full viral suppression, which has also been demonstrated earlier [14]. However, it also shows that patients who fail treatment might die prematurely in the absence of timely detection of viral failure and prompt action to secure proper treatment and compliance.

In settings without access to viral load monitoring, the WHO recommends to detect treatment failure by clinical (new or recurrent WHO stage 4 condition) or immunological (impaired CD4 cell response) criteria [15]; however, recent studies have shown that these criteria have poor sensitivity and specificity in detecting true virological failure [16, 17]. Using the WHO criteria, the majority of patients with treatment failure will not be detected until they develop severe immunodeficiency or opportunistic disease, at which stage widespread resistance is likely [18]. Furthermore, many patients will be misclassified as treatment failures despite adequate virological response and risk prematurely switch to complex and expensive second-line therapy. Thus, there is an urgent need for point-of-care viral load kits designed for use in rural, resource-limited settings, like our study site in Haydom.

There are several possible explanations for the negative long-term outcome after first-line failure in our study. A delayed switch from failing ART regimen is associated with increased mortality [19, 20]. In our study viral failure predicted both death and further viral failure, despite the fact that many of the patients with viral failure received second-line treatment. The switch of regimen was delayed for many of these patients due to logistical constraints and prolonged time before virological failure was known to the clinicians. This result stresses the importance of early detection of treatment failure with identification of possible reasons. In rural and resource-limited settings where access to virological monitoring is scarce, this finding is of particular interest.

Resistance and low adherence are other causes of negative long-term outcome [21]. However, resistance at baseline does not predict death in our study. This strengthens the theory that low adherence is likely to explain the subsequent virological failure and mortality. Those who exhibited poor adherence before viral failure might also have a sub-optimal adherence after detection of viral failure. Therefore, an important intervention to prevent premature switch to second-line treatment and subsequent failure, is to implement strategies to secure better adherence.

In the present study there were more children  $\leq 15$  years in the viral failure group than in the viral suppression group. Hence, the poorer prognosis observed in the viral failure group could have been just a marker of poorer prognosis in children than adults. However, only one child died during the follow-up. This is therefore unlikely to explain the difference in fatality.

Our study has some limitations. First, the sample size is small, and the groups differ in age and WHO stage. Second, the patients who failed to attend for viral load measurements might have biased the study. It is possible that this group represents patients with low compliance and worse clinical outcome, and that the negative clinical and virological outcome therefore might be underestimated. Third, we have not been able to assess adherence. Lack of

adherence could have explained the continuous failure in the virological failure group. Fourth, some of the patients in the virological failure group received second-line therapy, which is a confounding variable that makes the group less comparable. The outcome might have been better if all of the patients in the virological failure group had switched to second-line treatment.

Despite the limitations of this study, the results indicate that virological failure predicts mortality and subsequent virological failure in patients on ART in a resource-limited setting. Only 1.6% of the virological responders died during follow-up, which confirms the success of ART for HIV-infected patients if viral suppression is achieved. On the other hand, the high mortality observed among patients who failed to achieve virological suppression, underscores the need to identify virological failure at an earlier stage and intensify adherence counselling and monitoring of these patients, before they develop life-threatening complications. Access to viral load testing and better follow-up of patients with virological failure is warranted to improve survival in rural, resource-limited settings.



## **VI. ABBREVIATIONS**

ART = antiretroviral therapy

VF = virological failure

VR = virological response

HLH = Haydom Lutheran Hospital

## **VII. COMPETING INTERESTS**

We declare that we have no conflicts of interest.

## **VIII. AUTHORS' CONTRIBUTIONS**

IKB and PSP did the collection and interpretation of data, statistical analyses and drafted the manuscript. AJ designed the study and collected the baseline data, participated in interpretation of data and drafted the manuscript. AMDH participated in interpretation of data and drafted the manuscript. MT participated in interpretation of data, statistical analyses and drafted the manuscript. EN was responsible for clinical follow-up of the patients and helped organizing the collection of data. JB designed and initiated the original cohort study. All authors have read and approved the final manuscript.

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## XI.

**Table 1. Patient characteristics at baseline,**

**i.e. time of first HIV-RNA viral load assessment.**

	Total	Virological failure	Virological response	p-value <sup>3</sup>
Included patients, N	119	56	63	-
Female, N (%)	85 (71)	36 (64)	49 (78)	0.104
Age at baseline, N				
≤15	21 (18)	18 (32)	3 (5)	<0.001
>15	98 (82)	38 (68)	60 (95)	
WHO stage at initiation of ART				
1 to 2	16	9	7	0.449
3	40	26	14	0.006
4	62	21	41	0.002
Median CD4 count at baseline, (IQR) <sup>1</sup> cells/ul	432 (252-638)	358 (223-635)	499 (290-636)	0.158
Median HIV-RNA at baseline, (IQR) copies/ml	-	13,573 (2326-129,736)	0	-
Resistance analysed, N (%)	47	47 (84)	0	-
Mutations detected (%)		34/47 (72)	0	-
Number of patients on 2nd line ART <sup>2</sup> , N (%)	25 (21)	25 (45)	0	-
Median time on ART at baseline (IQR), months	28 (16-35)	29 (22-36)	25 (14-34)	0.054
<sup>1</sup> IQR = interquartile range, <sup>2</sup> ART = antiretroviral treatment, <sup>3</sup> Chi-squared test, Fishers' exact test and non-parametric test as appropriate.				

## XII.

**Table 2. Virological failure predicts mortality; proportion of patients who died with and without virological failure at baseline.**

	Virological failure (n=56)	Virological response (n=63)	p-value <sup>1</sup>
Lost to follow-up excluded	8/56 (14.3%)	1/63 (1.6%)	0.009
Lost to follow-up included	11/56 (19.6%)	1/63 (1.6%)	0.001

<sup>1</sup>Chi-squared test or Fischer`s exact test as appropriate. Analyses were performed with and without three patients lost to follow-up.

