A follow-up study of HIV-infected adults in rural Tanzania after antiretroviral treatment failure

Abstract

Background: As access to and duration of antiretroviral therapy (ART) increase, the number of patients experiencing first-line ART failure, followed by the need for second-line treatment is rising. The availability of second-line ART drugs is, however, often insufficient in resource-limited settings. For this reason many patients remain on a failing first-line regimen instead of being given more efficient second-line treatment. The long-term consequences and outcomes of this practice in rural areas are uncertain. In rural Tanzania, we have therefore studied the clinical and immunological development of adults and adolescents after virological failure, both those remaining on first-line-, and those being switched to second-line ART.

Methods: 44 adults (≥15 years) who had been on ART for at least six months and had a confirmed virological failure (HIV-1 RNA ≥400 copies/mL) were included in the study. In the period December 2006 – September 2009, 27 patients (61 %), continued first-line ART, while 17 patients (39 %), changed to second-line ART because of a failing first-line regimen. Follow-up data was collected through February 2010. Data on clinical, immunological and virological outcomes for the two groups was compared and analysed by logistic regression analyses and paired samples T-tests.

Results: At the time of virological failure the median viral load (VL) was 28 000 copies/mL (IQR 2630-149 000) and the mean CD4 cell count was 321.2 cells/µL, standard deviation (SD) 224.88. At the same time samples from 33 patients were available for drug resistance testing, out of which 20 (60.6 %) had detectable resistance. Six patients died following virological failure, but there was no significant difference in the odds of death between patients on first-line ART compared to those on second-line ART, Odds Ratio 0.58; P = 0.54. The mean changes in CD4 cell count 6, 12 or 18 months after virological failure was -28.6, -80.5 and -48.3 cells/µL for those on first-line ART, while the mean changes was + 56.2, +83.2 and +90.9 cells/µL for those on second-line ART.

Conclusion: Patients who remained on a failing first-line regimen had a declining CD4 cell count and were at risk of opportunistic infections and death. Patients who changed to second-line ART had an increasing CD4 cell count. They were still at risk of opportunistic disease and death, which could be explained by late detection of virological failure. Improved access to VL monitoring and second-line ART could improve prognosis for HIV-infected patients in rural settings.

Background:

In 2009 1.2 million adults (aged 15 and up) were living with human immunodeficiency virus (HIV) in The United Republic of Tanzania in Sub-Saharan Africa. The Tanzanian population was in 2010 41.9 million people, and the HIV prevalence among adults aged 15 to 49 was 5.6 %, which is a 1.4 % decline from 2003-04 (1,2). National surveys in Tanzania have shown a significant reduction in HIV prevalence among young women and men, and the country is now listed as one of 33 countries where the HIV-incidence has decreased more than 25 % in the period 2001 – 2009 (3). Even though the total prevalence in the country decreases, it is still expected a relatively higher increase in rural areas compared to central ones in the forthcoming years. This is mainly due to ongoing spreading of the HIV-infection to these areas with the sheer number of people living there (2,4). Actually, approximately 75 % of the population in Tanzania reside in rural areas, most of which are characterized by poverty. HIV prevalence in rural areas was estimated to 5 % in 2010, compared to 11 % in urban areas (4,5).

Access to antiretroviral therapy (ART), drugs that inhibit the ability of HIV and other retroviruses to multiply, has increased in the developing world over the past few years. At the end of 2009, 5.25 million people were receiving ART in low- and middle-income countries worldwide. Nearly 200 000 of them were living in Tanzania, and the number of people receiving ART in the country has increased with 29 % 2008-2009. (6) As access to and duration of ART has increased, the need for second-line regimens has become increasingly necessary due to development of drug resistance and consequently treatment failure. Treatment failure is defined as detectable virus in plasma after six months or more on ART. Upon confirmed treatment failure WHO recommend changing to a second-line regimen (7,8). The availability of second-line drugs for ART is however often insufficient in resource-limited settings. For this reason, many HIV-infected patients remain on a failing first-line regimen, instead of changing to a second-line one.

Little is known about the long-term consequences and outcomes of not switching to second-line ART when recommended. The aim of this study was to evaluate long-term consequences of virological failure in rural Tanzania, and to compare those continuing on a virologically failing first-line regimen with those switching to a second-line regimen. There is limited access to further therapeutic options, therefore the evaluation of second-line ART in these settings is useful.

Methods:

Study setting, participants and treatment:

The study was conducted at Haydom Lutheran Hospital located in northern Tanzania, Manyara region. It is a 400-bed hospital owned by the Evangelical Lutheran Church of Tanzania, and serves a rural population of about 260 000 people. ART free of charge and in-patient care has been offered to eligible HIV-infected patients since October 2003. An HIV Care and Treatment Center was then established, and has offered ART in accordance with WHO guidelines (8-10). Patients come routinely every three months, and all clinical visits are conducted by clinical officers, who are trained by experienced HIV physicians to treat and follow-up patients. A community home-based care network was established to promote compliance. Patients started ART if they were in WHO stage IV irrespective of CD4 cell count, WHO stage III with CD4 </= 350 cells/µL, or had CD4 </= 200 cells/µL with any WHO stage. Reliable CD4 counts were however not available until September 2006. Earlier most patients in this study started ART based on clinical criteria only.

Patients who were aged 15 years or older, who had been on antiretroviral therapy for at least six months and who had a confirmed virological failure (HIV-1 RNA > 400 copies/mL) were eligible for inclusion in the study. The cohort study was conducted on 44 patients who had started ART at Haydom Luteran Hospital in the period 2003-2007. Follow-up data was collected in January and February 2010. First- line ART compromised stavudine or zidovudine, combined with lamivudine, and either nevirapine or efavirenz. A generic fixed-dose combination of stavudine, lamivudine and nevirapine was preferred whenever possible. Second-line ART was available from December 2006. 17 patients from the survey changed to second-line ART in the period December 2006 – September 2009. Second-line ART compromised lopinavir/ritonavir combined with either didanosine and abacavir, didanosine and tenofovir, combivir, abacavir and combivir, tenofovir and combivir, or didanosine and combivir. Treatment failure was defined as detectable virus (> 400 copies/mL) after 6 months or more on ART. Criteria for switching to second-line ART was viral load > 1000 copies/mL and detection of drug-resistance mutations. There were several reasons for not switching to second-line ART immediately, including delayed reporting of laboratory results, patient's refusal, non-compliance and clinician's judgment.

Of all the patients, six died during the follow-up period, August 2007 – February 2010. Four patients were transferred to another health facility and five were lost to follow up (LFU). Patients were considered LFU when they had not been in contact with the health services for more than 3 months, and could not be traced by the home-visitor. The remaining group of patients, alive and in care in 2010, consisted of 15 adults and adolescents on first-line ART and 14 adults and adolescents on second-line ART.

Laboratory investigations:

Standard laboratory investigations at baseline included: full blood cell count, erythrocyte

sedimentatation rate, creatinine, blood sugar, liver function tests, hepatitis B surface antigen and syphilis serology. Patients who started ART were followed up with laboratory investigations every three months. Hematology was measured using the Sysmex KX-21 Hematology Analyzer (Sysmex Corp. Kobe, Japan). CD4 cell counts were available from September 2006 using the FACS Count flow cytometer (Becton Dickinson, San Jose, California, USA).

Plasma specimens for virological analyses were stored at - 20°C until shipment. Manufacturer's instructions were followed with regards to sample collection and transport. HIV viral load was measured at Muhimbili National Hospital, Dar es Salaam, Tanzania, using the Cobas TaqMan 48 Analyzer (Roche Diagnostics, Branchburg, New Jersey, USA) with a lower detection limit at 40 copies/mL; however, due to equipment breakdown, a subset of the samples were analysed with the Cobas Amplicor HIV-1 Monitor v1.5 (Roche Diagnostics, Branchburg, New Jersey, USA) with a detection limit at 400 copies/mL. Samples were sent to Ulleval University Hospital, Oslo, Norway, for genotypic resistance testing. The ViroSeq HIV-1 Genotyping System (Abbott Molecular, De Plains, Illinois, USA) was used to determine HIV-1 subtype and mutations in the protease and reverse transcriptase genes. Resistance profiles to antiretroviral drugs were interpreted according to the Stanford University HIV Drug Resistance Database (11).

Statistical considerations:

The main outcomes of interest were death and CD4 evolution after virological failure. We compared risk of death between groups using logistic regression analysis. Changes in CD4 cell count and BMI following virological failure were studied using paired samples T-tests. Mean with standard deviation (SD) was used to describe normally distributed data, and median with interquartile range (IQR) or range, to describe non-normally distributed data. Data was analysed with SPSS version 16.0 for Windows (SPSS Inc., Chicago, Illinois, USA). All tests were two-sided and level of significance was set at $P < 0.05$.

Ethical considerations:

Ethical approval was obtained from the National Institute for Medical Research in Tanzania and Regional Committee for Medical Research in Norway, and all patients gave written consent to participate in the study.

Results:

Baseline characteristics at start ART:

Of the material of 44 patients starting ART in the period 2003-2007, 10 patients (22.7 %) started the combination zidovudine, lamivudine and nevirapine, 23 (52.3 %) stavudine, lamivudine and nevirapine, 9 (20.5 %) stavudine, lamivudine and efavirenz and two patients (4.5 %) started with the combination zidovudine, lamivudine and efavirenz.

Median age at initiation of ART was 31.5 years (range 15-68 years) considering all 44 patients. The patient material consisted of 80 % women. Mean body mass index (BMI, kg/m^2) at initiation of ART was 19.5, SD 3.39. Results were missing from two patients. Mean CD4 cell count at initiation of ART was 206.4 cells/µL, SD 104.07. Results were missing from 14 patients.

15.9 % of the patients were classified to have HIV-infection at WHO stage I when initiating ART. None were classified as WHO stage II, 47.7 % as WHO stage III and 36.4 % as WHO stage IV.

Patient characteristics at first virological failure

Median VL was 28 000 (IQR 2630-149 000) copies/mL at first virological failure. Mean BMI was 21.0, SD 3.45. Data were missing from one patient. Mean CD4 cell count was 321.2 cells/µL, SD 224.88. Data were missing from seven patients. For those continuing first-line ART, mean CD4 cell count was 381.1 cells/µL, SD 220.43, seven data were missing. For those on second-line ART, mean CD4 cell count was 250.8 cells/µL, SD 215.25. Of all the patients, 20 (61 %) had developed drug resistance at the time of virological failure. 13 (39 %) had none detectable drug resistance, and data was missing from 11 patients.

Patient evolution after virological failure

In the period December 2006 – September 2009, 27 patients (61 %) continued first-line ART, while 17 patients (39 %) changed to second-line ART because of a failing first-line regimen. Out of these 17 patients starting second-line ART, 5 received lopinavir/ritonavir, didanosine and abacavir, 2 lopinavir/ritonavir, didanosine and tenofovir, 1 lopinavir/ritonavir and combivir, 6 lopinavir/ritonavir, abacavir and combivir, 2 lopinavir/ritonavir, tenofovir og combivir and 1 patient lopinavir/ritonavir, didanosine and combivir. Out of the 17, 14 (82 %) were women, and 3 (18 %) were men. Mean duration on first-line ART before changing to second-line was 1026 days. Mean duration on ART overall, was 1604 days.

From August 2007 – February 2010, out of the 44 patients in this study, six died, five were lost to follow-up and four were transferred to another health facility. The number of dead patients on first- and second-line ART was the same, with three dead patients in each group. Recorded causes of death were, for the patients on first-line ART; TB-meningitis, dehydration due to diarrhoea and toxoplasmosis of

the brain. For the patients on second-line ART the reported death-causes were; lactoacidosis, possible malaria and dehydration due to diarrhoea. Two of the dead patients were men.

In a logistic regression analysis there was no statistically significant difference in the odds of death between patients on second-line ART, compared to those on first-line ART (Odds Ratio 0.58; 95% confidence interval 0.10-3.29; *P*=0.54). The mean time from virological failure to death were 306 (381, 257, 279) and 444 (282, 405, 645) days for the patients on first-line ART and second-line ART, respectively. The time spent on second-line ART before death of the three patients was 219, 336 and 582 days. The values for last measured viral load before death of the patients on first-line ART were: 1886, 229 000 and 3198 copies/mL, and for the patients on second-line ART: 434 131, < 40 and 1 157 345 copies/mL. The last measured mean CD4 cell count before death, all within six months before death, was 193 cells/µL (125, 281, 174) for the patients on first-line ART. For the patients on second-line ART the last mean CD4 cell count was 97 cells/µL (46, 233, 11). Last measured BMI-value, all within nine months before death, was 18.3 (16.4, 20.4, 18.0) for the patients on first-line ART, and 18.2 (16.9, 22.4, 15.4) for the patients on second-line ART. At first virological failure, resistance against ART had developed in one of the three patients remaining on first-line ART, and in one of the three patients on second-line, but data were missing from the other two patients in this group.

VL was measured in 2010, but only eight analyses were successful. None of the four patients receiving first-line ART with successful analyses had achieved virological suppression (HIV-1 RNA < 40 copies/mL). One of the four patients on second-line ART with results was suppressed.

Mean CD4 cell count for those on first-line ART decreased from 381.1 cells/µL at virological failure with 28.6, 80.5 and 48.3 after 6, 12 and 18 months, respectively, see Figure 1. The changes were not statistically significant (P= 0.539, 0.263 and 0.378). Mean CD4 cell count for those on second-line ART increased from 250.8 cells/µL at virological failure, with 56.2, 83.2 and 90.9 after 6, 12 and 18 months, respectively. One of these three changes was statistically significant (P= 0.047, 0.120, 0.086).

Mean BMI for those on first-line ART increased from virological failure with 0.9, 0.5 and 2.0 after 6, 12 and 18 months, respectively. The changes were not statistically significant (P=0.405, 0.788, 0.229). Mean BMI for those on second-line ART had decreased 1.5, 2.2 and 3.3 compared to the value at virological failure. One of these changes was statistically significant (P=0.122, 0.137, 0.038).
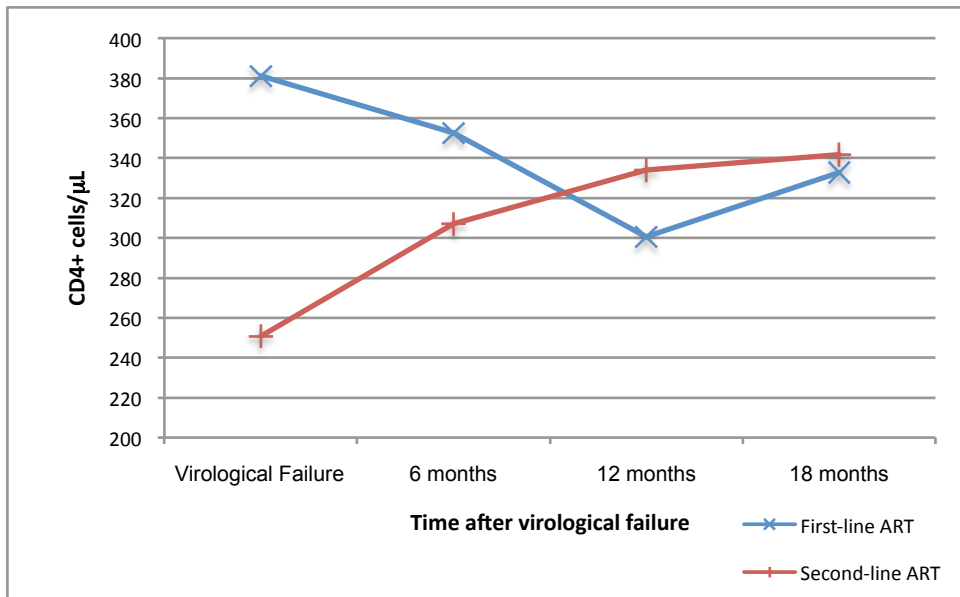
Figure 1. Mean CD4 cell count following virological failure compared for those on first- and second-line ART, using paired samples T-test. CD4 values were measured 6, 12 and 18 months after antiretroviral treatment failure.

Discussion:

In this study CD4 cell count decreased for patients continuing first-line ART following virological failure, while CD4 cell count increased for those who had changed to second-line ART. These data are comparable with other studies conducted in resource-limited settings, and they reveal that it is possible to achieve good treatment results also in these settings (12,13). The data highlight the need for increased and better access to second-line therapy also in rural settings, and this should be a priority in WHO´s upscaling of ART.

The statistics performed in this study showed no statistically significant difference in the odds of death in the two subgroups of patients. This even though the subgroup of patients switching to second-line ART, did this in a critical clinical condition. Delayed reporting of laboratory results, poor adherence and in some cases patient´s refusal, delayed the therapy changing, and the patients were often seriously immunosuppressed and at high risk of death, at the time they switched to second line ART. The mean time from virological failure to death, was 138 days longer for those receiving second-line ART than for those that died in the first-line group. There were no obviously differences in last measured VL, CD4 cell count, BMI and resistance in the two subgroups before death. Our results agree with an analysis of several ART programmes in sub-Saharan Africa which concluded that there was not a higher risk of death after switching to second-line ART, compared to staying on first-line ART after virological failure (14).

Measurements on compliance were not a priority in this study, but are of course of great value considering efficacy of ART. Many of those who have failed virologically and switched to second-line therapy may have had suboptimal adherence to their first-line regimens and may have continued to be less adherent than those who have remained on first-line ART. This can be a factor that underestimates the efficacy of second-line therapy. To optimize adherence, and thereby reduce the risk of jeopardizing future treatment options, is important. This in settings where there is limited access to further therapeutic options. It can be more difficult to maintain adherence to therapy with second-line ART, which contains more pills and can be complicated to understand. WHO guidelines recommend using fixed-dose combinations when starting ART, because of the benefits shown reducing the high pill-burden (7). In WHO´s guidelines from 2010 they have placed high value on using simpler second-line regimens and the availability of fixed-dose combinations also for second-line ART (7). It is important that this goal is also achieved in resource-limited settings, since access most certainly is of great value in these areas. The optimal second-line regimen in rural settings is still not found, but it should be simple, affordable and potent. More research is needed to reveal this optimal regimen in resource-limited settings.

Detection of early treatment failure will ensure that patients are able to switch regimens before the occurrence of severe opportunistic infections and death. One strategy for improving the diagnosis of treatment failure, is to expand the role of VL monitoring, and to make this a routine analysis in the

follow-up of HIV-infected patients receiving ART. It is however necessary to include technologies that are suitable for rural Africa. VL monitoring is rarely available in these settings to day, due to costs associated with monitoring, insufficient trained personnel and infrastructure issues such as the availability of reagents and laboratory facilities, among other things.

The current WHO adult and adolescent guidelines recommend use of a boosted protease inhibitor (bPI) for second-line ART (7). The relative costs of bPIs, are greater than the cost of recommended first-line combinations. Even if access to second-line ART will be achieved, the drugs need to be affordable. Despite guideline recommendations, few developing countries can offer statesubsidized access to second-line therapy for their HIV-infected populations (15). It is crucial with ART free of charge in these settings. Studies have shown that provision of free medication is associated with a significantly higher proportion of patients with suppressed viral load, than is the requirement of partial or full payment of therapy (16-18). Reduction in the price of boosted PI-based regimens must be prioritized to achieve effective treatment of patients in resource-limited settings. Allowing access to competitive generic drug versions should also be a future goal.

There were several limitations to this study. The study was limited by the lack of testresults, few successful analyses in the latest follow-up period and thereby limited statistical power. There is also a need for longer follow-up periods, beyond 18 months, after confirmed virological failure. The study was small, with relatively few patients included. Another limitation was the high percentage of patients LFU or transferred to another health facility, thereby missing pairwise and comparable data throughout the study. Furthermore, the number of deaths is likely to have been underestimated since some of the patients classified as LFU probably died.

Conclusion:

Patients who remained on a failing first-line ART regimen had a declining CD4 cell count and were at risk of opportunistic disease and death. Patients who changed to second-line ART had an increasing CD4 cell count. They were still at risk of opportunistic disease and death, which could be explained by late detection of virological failure. As access to and duration of ART increases, the demand for second-line ART increases as well, due to the development of drug resistance. It is important to meet this demand, and increase the access to second-line ART and virological monitoring in resource-limited settings. There should be a focus on more affordable diagnostic tests and low-cost second-line drugs. Efficient affordable laboratory analyses, fixed-dose combination drugs for second-line ART and improving compliance, should be priorities in resource-limited settings. Earlier detection of treatment failure and confirmation of virological failure could be obtained, leading to improved prognosis for HIV-infected patients in rural settings.

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