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Evaluation of antiretroviral therapy results in Blantyre, Malawi

JJ van Oosterhout[1], N Bodasing[1], JJ Kumwenda[1], C Nyirenda[1,2], J Mallewa[1,2], PR Cleary[5], MP de Baar[6], R Schuurman[3], DM Burger[4], EE Zijlstra[1]

[1] Department of Medicine, College of Medicine, University of Malawi, Blantyre, Malawi
[2] Queen Elizabeth Central Hospital, Department of Medicine, Blantyre, Malawi
[3] Department of Virology, University Medical Centre Utrecht, the Netherlands
[4] Department of Clinical Pharmacy, University Medical Centre Nijmegen, the Netherlands
[5] Department of Community Health, College of Medicine, University of Malawi, Blantyre, Malawi
[6] Primagen, Amsterdam, the Netherlands

Abstract

We performed a cross sectional study to evaluate treatment results of the paying antiretroviral therapy clinic of Queen Elizabeth Central Hospital, Blantyre. The only antiretroviral therapy was a fixed drug combination of stavudine, lamivudine and nevirapine.

Methods:

Interviews, laboratory tests (CD4 count, viral load, nevirapine plasma levels, transaminases) and data extraction from files. 422 (59 %) of the patients who started antiretroviral therapy since 2000 were lost to follow up. The 176 patients enrolled in the study had good virological and excellent clinical treatment results. The most common side effect was peripheral neuropathy. Nevirapine plasma levels were remarkably high and associated with successful virological treatment results. Two simple adherence questions pertaining to the use of medication in the previous 8 days corresponded well with nevirapine levels. The most important reasons for non-adherence were shortage of drugs in the hospital pharmacy and personal financial constraints.

Conclusions:

1. Many patients were lost to follow up.
2. High nevirapine levels contributed to good therapy results in those studied.
3. Simple adherence questions predicted sub-therapeutic nevirapine levels.
4. Antiretroviral drug supply needs to be uninterrupted and free of charge, to prevent avoidable non-adherence.

Introduction

Malawi is among the countries with the highest burden of HIV infection in sub-Saharan Africa. Of a population of 10.5 million, 900,000 people were estimated to be infected with HIV by 2003 (National Tuberculosis Control Programme. 2004). The Government of Malawi introduced antiretroviral therapy (ART) in the central hospitals of the two major cities and QECH in Blantyre was the first to start an ART clinic in 2000. For the antiretroviral drugs patients paid on average USD 25 per month into a revolving fund, which was supported by the Ministry of Health and Population. Apart from the public sector, ART was also provided by some private hospitals to an unknown number of patients.

Initial ART activities in Africa generated some scepticism with warnings of "antiretroviral anarchy" (Harries AD et al. 2001). Inadequate health infrastructure, unavailability of skilled physicians and problems with drug procurement and delivery are considered major limitations to successful ART delivery. Other ART providers in Malawi include non-governmental organizations such as Medécins sans Frontières, who offer ART free of charge at two government district hospitals in a well-funded programme with adequate numbers of trained staff. Encouraging initial results were reported from one of these hospitals (Tassie JM et al. 2003). Others have also described favourable experience with ART from well-organised programmes in resource-poor settings (Farmer P et al. 2000, Weidle PJ et al. 2002). However, if successful scaling-up of ART is to be introduced throughout Malawi as indicated by the WHO 3 x 5 initiative (World Health Organisation. 2004), optimal use will have to be made of existing health structures as only limited extra funds and staff will be available. Clearly, previous experience from clinics that run under such circumstances may help to improve scaling-up activities both in terms of logistics as well as treatment results. To date there are few data available from ART clinics that are integrated in an existing hospital infrastructure and that run without additional human and financial resources. We undertook a cross sectional study to evaluate treatment results from the Queen Elizabeth Central Hospital ART clinic with emphasis on clinical response, adherence, drug side effects as well as drug levels.

Methods

Site

The ART clinic at QECH was integrated in the outpatient department for fee-paying patients. We report results from the time patients paid fees for antiretroviral drugs, since 2004 the clinic provides free of charge ART. As the clinic expanded with an annual fourfold increase in patient numbers, the number of physicians involved increased from 1 to 5. All were from the current establishment of the College of Medicine and QECH. The number of half-day clinics increased from 2 per week in 2000 to 9 per week in 2003. One nurse and one clerk were available to the clinic throughout this period.

Patients

Inclusion criteria were: age 18 years or older, naïve to antiretroviral drugs at the start of ART, and being on highly active ART at QECH for at least 6 months. Patients who had previously been on dual therapy were excluded. Patients were recruited at their scheduled visits to the ART clinic during a period of twenty weeks, from July to November 2003. All patients who were receiving treatment at QECH were expected to visit the clinic during this episode at least once, since the maximum number of prescribed tablets was for 3 months.

Antiretroviral drug treatment

Double nucleoside reverse transcriptase inhibitor (NRTI) therapy was the only ART available during the first months in 2000. Triomune® (Cipla Ltd., Mumbai, India [Cipla website. 2004]),

a fixed combination of stavudine, lamivudine and nevirapine, was introduced in 2001 and has since been the only form of highly active ART prescribed. Triomune® was chosen because it is inexpensive, effective (Kumarasamy N et al. 2003), and has a patient friendly dosing schedule of one tablet twice daily. No alternative regimens were available in case of treatment failure or side effects. To reduce hepatotoxicity and allergic skin reactions, nevirapine, but not the two NRTI's, should be taken at half the dosage during the first 2 weeks (Cipla website. 2004), a schedule Triomune® does not allow. Separate NRTI's were not available until 2003 and the patients in our population therefore started ART with a single tablet of Triomune daily, thus receiving only half of the required NRTI's during the first 2 weeks. Drug unavailability was common: Triomune® was regularly out of stock from the start of the ART programme. Laboratory monitoring of side effects and treatment results was rarely done due to financial constraints of patients and poor laboratory facilities.

Data collection

The following data were extracted from the ART clinic file of each enrolled patient: age, sex, WHO clinical stage of HIV infection (WHO 2004) at the start of ART, duration of ART, CD4 count at start of ART and AIDS defining events or pulmonary tuberculosis during ART. Height and weight, travel time from home to clinic as well as current medication other than Triomune® was recorded. We did not specifically ask for the use of traditional herbs. Laboratory tests and interviews for this study were performed only once for each enrolled patient. The study clinician and nurse, who were not members of the regular ART clinic staff, conducted interviews concerning side effects and adherence. Patients were encouraged to be open about possible non-adherence. They were assured that results of the adherence interview would be confidential, would not be communicated to the clinic physicians and would not affect their treatment in any way. We asked patients four short questions: did you miss a tablet the day before, the week before, the month before and did you never miss a tablet. We also asked for reasons for missing tablets. Interviews took place in either the local language or English, depending on the patient's preference. Blood was drawn to measure CD4 count (FacsCount, Becton-Dickinson, San Jose, CA, USA). Plasma viral load was determined by HIV-1 RNA Retina Rainbow Assay ™, from dried plasma spots on filter paper, stored at room temperature (Cassol S et al. 1997, de Baar MP et al. 2003). This assay is a single rapid real-time monitored isothermal RNA amplification assay that has a lower limit of detection of 50 copies / mm^3 (de Baar MP et al. 2001). Further plasma samples were stored at –80 °C for subsequent determination of nevirapine levels (Hollanders RM et al. 2000) and alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST). The laboratories performed internal quality assurance procedures for viral load tests and CD4 counts.

Virological treatment failure was defined as the presence of a detectable viral load after a minimum of 6 months of ART, immunological treatment failure as less than 25 % annual increase of the baseline CD4 count and clinical treatment failure as the occurrence of an AIDS defining event or pulmonary tuberculosis after being on ART for at least 6 months. Sub-therapeutic nevirapine plasma levels were defined as levels < 3.0 mg/L (HIV-pharmacology Website. 2004). Lastly, all files of the clinic were reviewed at the end of the study period to evaluate loss to follow up and possible causes thereof.

Statistical considerations

Data were entered and analysed by SPSS version 11.0 and Intercooled STATA 7.0 for Windows. Proportions were compared by Chi-square test and means by student-t test (for normally distributed data) or Kruskal-Wallis test (for data that were not normally distributed). Spearman's correlation coefficient was used to test for associations with ordinal data. Odds ratios were calculated with 95% confidence intervals using the Mantel-Haentzel method.

Ethical considerations

The study was approved by the University of Malawi College of Medicine Research and Ethics Committee. Written informed consent was obtained from all patients at enrolment.

Results

A total of 717 patients had visited the ART clinic prior to 1 January 2003, and were therefore eligible for the study. However, 453 patients did not attend for a planned visit of the clinic during the four months study period. In 31 patient files we found a documented reason: 11 patients died, 11 patients stopped ART (7 due to side effects, 4 due to financial problems) and 10 patients were transferred to another ART clinic. For the remaining 422 (59 %) other patients no explanation was available. No detailed addresses or telephone numbers were recorded to trace patients, therefore they were considered lost to follow up. Of the 264 patients who started ART before 2003 and visited the clinic during the study episode, 88 did not fulfil the inclusion criteria (36 were not naïve to antiretroviral drugs at the start of ART, 19 were younger than 18 years and 33 did not give consent), thus 176 patients were enrolled into the study. Table 1 shows demographic, clinical and immunological data of the enrolled patients at the start of ART. Our study population had a median age of 39 years (range: 22 – 71). There was a preponderance of women (55 %).

Table 1: Demographic, clinical and immunological data at the start of ART of 176 patients studied

Age groups (years)	n	%
18 - 29	19	(11 %)
30 - 39	70	(40 %)
40 - 49	54	(31 %)
≥ 50	33	(19 %)
Sex		
male	80	(45 %)
female	96	(55 %)
Travel time home – clinic		
< 1 hour	113	(64 %)
1 – 2 hours	40	(23 %)
> 2 hours	23	(13 %)
WHO stage		
I	7	(4 %)
II	40	(23 %)
III	56	(32 %)
IV	22	(12 %)
Unknown	51	(29 %)

CD4 strata (cells/mm³)		
< 100	46	(26 %)
100 – 199	49	(28 %)
200 – 349	47	(27 %)
> 350	17	(10 %)
unknown	17	(10 %)

Twelve patients experienced a WHO stage IV clinical event or pulmonary tuberculosis after the start of ART (cryptococcal meningitis 2, oesophageal candidiasis 2, Kaposi's sarcoma 2, pulmonary tuberculosis 2, extra-pulmonary tuberculosis 2, Non-Hodgkin's lymphoma 1, chronic diarrhoea and wasting 1). In 9 patients these events occurred before the completion of six months of ART and therefore clinical ART failure was present in only three cases. Two of the three cases also had virological treatment failure; one patient had pulmonary tuberculosis after 1 year of ART despite an undetectable viral load at the time of the study. Whether this was due background tuberculosis prevalence in the community or inadequate immune reconstitution at the time of diagnosis is unknown, since a CD4 count at that point was lacking. Insufficient clinical details were available to determine if events that happened early during ART were due to immune reconstitution disease.

We found a low percentage of virological treatment failure: only 20 % of the patients had a detectable viral load. There was a significant trend of increasing virological treatment failure with increasing duration of ART (p for trend of odds = 0.002) (Table 2). Travel time from home to the ART clinic was not significantly associated with virological treatment failure (data not shown).

Table 2: Viral load in relation to duration of antiretroviral therapy

duration of ART		*total number* non-detectable viral load (%)		*number with* detectable viral load[1] (%)	
6 –12 months	95	84	(88 %)	11	(12%)
13 –24 months	79	56	(71 %)	23	(29%)
> 24 months	2	1	(50 %)	1	(50%)
Total	**176**	**141**	**(80 %)**	**35**	**(20%)**

1 Since all patients had a minimum of 6 months antiretroviral therapy, a detectable viral load indicates virological treatment failure

In 159 patients (90%) a CD4 count at the start of ART was available. Overall there was a mean rise of 68 CD4 cells/mm³ (95% confidence interval [CI]: 36 – 99) since the start of ART. Stratifying by treatment duration did not show a significant difference in mean rise in CD4 count in those on treatment < 1 year as compared to those who had been on ART > 1 year (p=0.58). Eighty-nine patients (56 %) had an increase of the CD4 count of more than 25 % per year, 15 patients (9 %) an increase of less than 25 % and 54 patients (34 %) had a decrease in the CD4 count. Therefore, 44 % met our criteria of immunological treatment failure.

Results of interviews on side effects indicated that 76 % of the patients experienced at least one side effect at any time during ART. Numbness and pain in the lower extremities were the most common complaints (Table 3). Only 3 patients (3 %) mentioned side effects as a reason for non-adherence. There was no statistically significant difference in the frequency of side effects between men and women (data not shown). A minority of patients had increased transaminase levels but none had a rise of more than 5 times the upper limit of normal (Table 3).

Table 3: Number (%) of patients who experienced side effects[1] at any time during ART and transaminase levels at the time of study enrolment

	n	(%)
Numbness and/ or pain of lower extremities	99	(56)
Rash	45	(26)
Headache	40	(23)
Nausea	26	(15)
Vomiting	23	(13)
Dizziness	17	(10)
Diarrhoea	18	(10)
Abdominal pain	14	(8)
Jaundice	1	(1)
Other	27	(15)
ALT (Units/L)		
< 45 (normal)	172	(98)
45 - 134	4	(2)
135 - 224	0	(0)
AST (Units/L)		
< 40 (normal)	147	(84)
40 - 119	27	(15)
120 - 199	2	(1)

1 patients may have reported more than one side-effect
ALT = alanine-aminotransferase; AST = aspartate-aminotransferase;

During adherence interviews, 92 patients (52 %) reported never having missed a single tablet (and one did not know). However when subsequently asked for reasons of interruptions of ART, a total of 124 reasons were mentioned by 106 patients. The most frequent reason was unavailability of Triomune® in the hospital pharmacy (46 patients [43 %]). Financial constraints and forgetting to take the tablets were mentioned by 34 (32 %) and 29 (27 %) patients respectively. 15 patients (14 %) gave other causes of non-adherence. 132 patients (75 % of the study population) were unable to obtain Triomune® from the pharmacy because of inadequate supplies on at least 1 occasion, but 86 managed to access alternative sources, as they did not indicate it as a cause for missing tablets.

148 (84%) of the study patients had nevirapine plasma levels in the therapeutic range [15] or higher (Table 4). From the 4 adherence questions, there was a significant association of sub-therapeutic nevirapine levels with having missed a tablet of Triomune® the day before (odds ratio [OR] 15.1; CI 4.6 – 50.0; p < 0.0001) or the week before (OR 6.5; 95% CI 2.2 – 19.1; p=0.0001). Those with a sub-therapeutic nevirapine level had an OR of 6.0 (95% CI 2.4 – 15.3) for virological failure, as compared to those with therapeutic or higher levels. There was a borderline significant association between a high therapeutic or potentially toxic level and a higher risk of side effects (OR 2.0;

95% CI 1.0 - 4.1; p=0.05). There was no difference in the proportion of males and females with sub-therapeutic nevirapine levels (17.5 % and 14.6 % respectively; OR 1.24; 95% CI 0.55 – 2.80; p=0.60) or with high or potentially toxic nevirapine levels (57.5 % and 63.5 % respectively; OR 1.3; 95% CI 0.7 - 2.4; p=0.42). There was no correlation between body mass index and nevirapine plasma level (rs = 0.80; p=0.1).

Table 4: Number (%) of patients per nevirapine plasma level group

< 0.15 mg/L	(undetectable)	14	(8 %)
0.15 – 2.9 mg/L	(sub-therapeutic)	14	(8 %)
3 – 6 mg/L	(therapeutic)	41	(23 %)
6.1 – 8 mg/L	(high therapeutic)	47	(27 %)
> 8 mg/L	(potentially toxic)	60	(34 %)
total		176	(100 %)

Discussion

We conducted a cross sectional study to evaluate treatment results of the first government ART clinic established in Malawi, which operated in a resource-poor health care setting. The main features of this study were the high loss to follow up and the good treatment results in those who were adherent, probably due to the higher than expected nevirapine levels.

Nearly 60 % of the patients who started ART were lost to follow up and could not be evaluated in our study. The reasons for this large loss to follow up could not be ascertained. One quarter of these patients never returned after a first visit and we therefore have no confirmation that they actually started ART. Financial constraints are likely to have been an important cause of loss to follow up. Many patients were dependent on their income as small-scale entrepreneurs for buying antiretroviral drugs and they, as well as others who were employed, would have had no source of income on becoming chronically ill - in the absence of social security and medical insurance. Others were financially dependent on relatives for buying drugs. Another related reason was self initiated transfer to other ART centres. Two well-organised clinics in neighbouring districts hospitals, coordinated by Medécins sans Frontières, offered free ART, which attracted many Blantyre residents (A. Jeannini, personal communication). Lastly, a number of people may have died; as there are no death registers in Malawi, no information concerning mortality was available.

The patients enrolled in our study generally started ART when they reached an advanced stage of HIV infection: nearly all were symptomatic and had CD4 counts below 350 / mm^3. Almost two thirds of those in whom a WHO stage was documented were in stage III or IV. Despite this they had good virological and excellent clinical treatment results. Half of the detectable viral loads were below 500 copies / mL and since we only had one measurement, some of these patients may have had so called blips, temporarily detectable viral loads during otherwise successful ART (Karlsson AC et al. 2004). Therefore the virological treatment failure is likely to be lower than the 20 % we found. There are several explanations for these good results. Clearly, the cross sectional design of our study led to selection bias: among those lost to follow up may have been more treatment failures and deaths. On the other hand, all our patients were antiretroviral drugs naïve and were treated with a simple, effective and adherence promoting ART regimen. Importantly, large numbers of patients had higher than expected nevirapine plasma levels. Our study describes nevirapine levels in the largest series of African patients to date. High nevirapine levels have been associated with good virological treatment results in developed countries (Veldkamp AI et al. 2001), which was confirmed in our study. Nevirapine plasma levels in cohorts in the developed world on average showed lower values than in our study population (Donnerer J et al. 2003, Zhou XJ et al. 1999). Lower body mass index and a preponderance of females (Regazzi M et al. 2003) could have explained the higher nevirapine levels in our study, but neither was associated with high nevirapine levels. We hypothesize that genetic factors involving nevirapine metabolism in the liver may lead to higher nevirapine levels, similar to what was recently described for efavirenz in non-Caucasians (Ribaudo H et al. 2004). The high nevirapine levels may have implications for nevirapine usage during rifampicin containing tuberculosis treatment. If confirmed, it improves the prospect that nevirapine in combination with rifampicin, despite their interaction, may still lead to adequate nevirapine levels and good ART results in African patients, as was found by others in Europeans (Oliva J et al. 2003). Our good virological and clinical treatment results are in contrast with the immunological findings; one third of the patients had a decrease in CD4 count compared to the start of treatment value. These first CD4 count results need to be regarded with caution. They were done in at least 7 different laboratories; the majority were done by manual CD4 count assays, a group of methods more sensitive to subjective interpretation (Crowe S et al. 2003). Furthermore, CD4 counts can vary over time and we were only able to compare two measurements, mostly with long intervals in between. Our definition of immunological failure was strict and several other definitions are in use (Department of Health and Human Services 2004, World Health Organisation 2004), but these assume regular CD4 counts or a known peak value on ART and neither was available. In view of our results, we do not recommend the use of isolated immunological treatment failure as an indication for second line ART in resource poor settings, particularly if regular and reliable CD4 counts are not available.

The side effects we observed showed an expected pattern for patients treated with Triomune®. The high level of suspected peripheral neuropathy may have been influenced by the fact that 32 % of the patients had a bodyweight below 60 kg, while Triomune® containing stavudine 30 mg (the advised dosage for persons below 60 kg, instead of 40 mg) was unavailable for most of the study period. Having a high nevirapine plasma level appeared to be associated with clinical side effects but this did not reach statistical significance. It did not seem to lead to abnormal transaminases levels.

We used a simple questionnaire to assess adherence to ART. Two questions pertaining to having missed a tablet a day before or a week before the clinic visit correlated with sub-therapeutic nevirapine plasma levels. Virological treatment failure was not associated with a positive response to any of the four questions. The results of the interviews indicated good adherence in our population, but it was not possible to extrapolate the result of the 4 questions into an individual adherence of more than 95 %, which is a much quoted target in the literature. Whether this target is as relevant in our population is uncertain, since many of the patients in the western cohorts from which this percentage originated, used protease inhibitor-based ART (Turner BJ. 2002). Pill counts and more detailed interviews could be used to

generate an individual adherence percentage, but these methods are elaborate, time-consuming and may not be feasible in many African ART clinics. More studies are required to validate methods of adherence testing in resource poor ART settings. Meanwhile, we advise the use of the two simple questions mentioned above, to identify persons who are more likely to have low antiretroviral drug levels - and are therefore at higher risk to develop virological treatment failure - for additional adherence counselling. The reasons for non-adherence in our study were very different from those found in developed countries: unavailability of drugs and personal financial problems accounted for two thirds of the non-adherence. Despite the fact that many patients managed to access Triomune® from an alternative source, more than one third of the total reported non-adherence in our study resulted from the unreliable drug supply.

Our study demonstrates the difficulties encountered in a fee-paying ART clinic in a resource-poor setting, largely unsupported by international donors. While it was possible to run the clinic with the limited resources available, there was a large loss to follow-up, most likely related to drug costs. The high percentage of loss to follow-up does not allow extrapolation of the results to all the patients who visited the clinic to start ART. Nevertheless, the patients studied showed good virological and excellent clinical outcome. Unexpectedly high nevirapine plasma levels in most patients may have contributed to this. Provision of free of charge ART with reliable drug supply will result in better access to ART, reduction of loss to follow up and promotion of adherence and these are prerequisites for successfully scaling-up ART programmes in resource-poor settings.

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References

1. Cassol S, Gill MJ, Pilon R, Cormier M, Voight RF, Willoughby B, *et al* (1997). Quantification of human immunodeficiency virus type 1 RNA from dried plasma spots collected on filter paper. *J Clin Microbiol*; 35:2795-2801.
2. Cipla website (2004). Product information Available at: http://www.cipla.com/ourproducts/Antiretrovirals/triomune1.htm Accessed 13 September 2004.
3. Crowe S, Turnbull S, Oelrichs R, Dunne A (2003). Monitoring of human immunodeficiency virus infection in resource-constrained countries. *Clin Infect Dis*; 37(Suppl 1): S25-35.
4. de Baar MP, van Dooren MW, de Rooij E, Bakker M, van Gemen B, Goudsmit J, *et al* (2001). Single rapid real-time monitored isothermal RNA amplification assay for quantification of human immunodeficiency virus type 1 isolates from groups M, N, and O. *J Clin Microbiol*; 39:1378-1384.
5. de Baar MP, Timmermans EC, Buitelaar M et al (2003).Evaluation of the HIV-1 RNA Retina™ Rainbow Assay on Plasma and Dried Plasma Spots: Correlation with the Roche Amplicor HIV-1 v1.5 Assay [abstract no. 1231]. Presented at: 2nd IAS Conference on HIV Pathogenesis and Treatment, Paris, France.
6. partment of Health and Human Services (2004). *Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents.* Available at: http://AIDSinfo.nih.gov Accessed 13 September 2004.
7. Donnerer J, Kronawetter M, Kapper A, Haas I, Kessler HH (2003). Therapeutic drug monitoring of the HIV/AIDS drugs abacavir, zidovudine, efavirenz, nevirapine, indinavir, lopinavir, and nelfinavir. *Pharmacology*; 69:197-204.
8. Farmer P, Leandre F, Mukherjee JS, Claude M, Nevil P, Smith-Fawzi MC, et al (2000). Community-based approaches to HIV treatment in resource-poor settings. *Lancet.*; 358:404-409.
9. Harries AD, Nyangulu DS, Hargreaves NJ, Kaluwa O, Salaniponi FM (2001). Preventing antiretroviral anarchy in sub-Saharan Africa. *Lancet*; 358:410-414.
10. Kumarasamy N, Solomon S, Chaguturu SK, Mahajan AP, Flanigan TP, Balakrishnan P, Mayer KH (2003). The safety, tolerability and effectiveness of generic antiretroviral drug regimens for HIV-infected patients in south India. *AIDS*; 17:2267-2269.
11. HIV pharmacology Website 2004. www.HIVpharmacology.com Accessed 13 September 2004.
12. Hollanders RM, Van Ewijk-Beneken Kolmer EW, Burger DM, Wuis EW, Koopmans PP, Hekster YA (2000). Determination of nevirapine, an HIV-1 non-nucleoside reverse transcriptase inhibitor, in human plasma by reversed-phase high-performance liquid chromatography. *J Chromatogr B*; 744:65-71.
13. Karlsson AC, Younger SR, Martin JN, Grossman Z, Sinclair E, Hunt PW, *et al* (2004). Immunologic and virologic evolution during periods of intermittent and persistent low-level viremia. *AIDS.*; 18:981-989.
14. Malawi Ministry of Health and Population Website (2004). Available at: http://www.malawi.gov.mw/health/health2edoc.htm Accessed 13 September 2004.
15. National Tuberculosis Control Programme, HIV/AIDS Unit, Department of Clinical Services, National AIDS Commission, Ministry of Health and Population (2004). *Report of a countrywide survey of HIV/AIDS services in Malawi (for the year 2003).*
16. Oliva J, Moreno S, Sanz J, Ribera E, Molina JA, Rubio R, *et al* (2003). Co-administration of rifampicine and nevirapine in HIV infected patients with tuberculosis. AIDS; 17:637-638.
17. Regazzi M, Villani P, Seminari E, Ravasi G, Cusato M, Marubbi F, *et al* (2003). Sex differences in nevirapine disposition in HIV-infected patients. AIDS; 17:2399-2400.
18. Ribaudo H, Clifford D, Gulick R, Shikuma C, Klingman K, Snyder S, et al (2004) Relationships between Efavirenz Pharmacokinetics, Side Effects, Drug Discontinuation, Virologic Response, and Race: Results from ACTG A5095/A5097s. [Abstract 132] Presented at: 11th Conference on Retroviruses and Opportunistic Infections; San Francisco.
19. Tassie JM, Szumilin E, Calmy A, Goemaere E (2003). Highly active antiretroviral therapy in resource-poor settings: the experience of Medecins Sans Frontieres. *AIDS*; 17:1995-1997.
20. Turner BJ (2002). Adherence to antiretroviral therapy by human immunodeficiency virus-infected patients. *J Infect Dis*; 185 (Suppl 2): S143-151.
21. Veldkamp AI, Weverling GJ, Lange JM, Montaner JS, Reiss P, Cooper DA, et al (2001). High exposure to nevirapine in plasma is associated with an improved virological response in HIV-1-infected individuals. *AIDS*; 15:1089-1095.
22. Weidle PJ, Malamba S, Mwebaze R, Sozi C, Rukundo G, Downing R, et al(2002). Assessment of a pilot antiretroviral drug therapy programme in Uganda: patients' response, survival, and drug resistance. *Lancet*; 360:34-40.
23. WHO (2004). *Staging system for HIV infection and disease in adults and adolescents* Available at http://www.who.int/docstore/hiv/scaling/anex1.html Accessed 13 September 2004.
24. WHO website (2004). Available at: www.who.int/3by5/about/en/ Accessed 13 September 2004.
25. World Health Organisation (2004). *Scaling up antiretroviral therapy in resource poor settings: guidelines for a public health approach. 2003 revision.* Available at: http://www.who.int/hiv/pub/prev_care/en/arvrevision2003en.pdf Accessed 13 September 2004.
26. Zhou XJ, Sheiner LB, D'Aquila RT, Hughes MD, Hirsch MS, Fischl MA, *et al* (1999). Population pharmacokinetics of nevirapine, zidovudine, and didanosine in human immunodeficiency virus-infected patients. The National Institute of Allergy and Infectious Diseases AIDS Clinical Trials Group Protocol 241 Investigators. *Antimicrob Agents Chemother*; 43:121-128.