HIV antiretroviral therapy in Ethiopia

Overcoming implementation challenges

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Original Papers

This thesis is based on the following papers, which will be referred to in the text by their roman numerals.

I. ***Jerene D****, Lindtjørn B.* Disease progression among untreated HIV infected patients in south Ethiopia: Implications for patient care. MedGenMed 2005 Aug 30; 7(3):66.

II. ***Jerene D****, Næss A, Lindtjørn B.* Antiretroviral therapy at a district hospital in Ethiopia prevents death and tuberculosis in a cohort of HIV patients. AIDS Res Ther. 2006 Apr 7; 3(1): 10.

III. ***Jerene D****, Endale A, Hailu Y, Lindtjørn B.* Predictors of early death in a cohort of Ethiopian patients treated with HAART. BMC Infect Dis. 2006; 6:136

IV. ***Jerene D,*** *Endale A, Lindtjørn B.* Acceptability of HIV counselling and testing among tuberculosis patients in south Ethiopia. Submitted.

List of Abbreviations

3TC	Lamivudine
ABC	Abacabir
AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral Therapy
ARVs	Antiretroviral drug (s)
BMI	Body Mass Index
CBC	Complete Blood Cell Count
CDC	Centre for Disease Control and Prevention
D4T	Stavudine
DDI	Didanosine
DOTS	Directly Observed Treatment, Short Course
EFV	Efavirenz
FDA	Food and Drug Administration
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
IPT	Isoniazid Preventive Therapy
IRIS	Immune Reconstitution Inflammatory Syndrome
LPV/r	Ritonavir-boosted lopinavir
NLM	Norwegian Lutheran Mission
NORAD	Norwegian Agency for Development Cooperation
NRTI (s)	Nucleoside Reverse Transcriptase Inhibitor (s)

NtRTI (s)	Nucleotide Reverse Transcriptase Inhibitor (s)
NVP	Nevirapine
PI (s)	Protease Inhibitor (s)
PYO	Person-Years of Observation
SNNPRS	Southern Nations, Nationalities' and Peoples' Regional State
SQV/r	Ritonavir-Boosted Saquinavir
TB	Tuberculosis
TLC	Total Lymphocyte Count
VCT	Voluntary Counselling and Testing
WHO	World Health Organization
ZDV	Zidovudine

Executive Summary

Millions of people have died of HIV during the last 25 years. The highest number of deaths occurred in poor African countries where antiretroviral therapy was introduced only recently. Treating patients in settings with limited resources continues to be a challenge. The aim of this thesis was to improve antiretroviral therapy in Ethiopia. The main part of the thesis is based on a cohort of HIV infected patients at a district hospital in Ethiopia. We established the cohort before antiretroviral therapy was available in the country, and continued the follow-up afterwards. Additionally, we assessed the acceptability of HIV testing among tuberculosis patients.

In untreated patients, death and tuberculosis incidence rates were high. HAART improved survival and decreased tuberculosis to a level similar to that achieved by developed countries during early years. However, both death and tuberculosis continued to occur at higher rate especially during the early weeks of treatment. Patients die because of advanced clinical disease stage and very low levels of total lymphocyte count at presentation. We also found that HIV testing was not accepted by most of the tuberculosis patients, highlighting the need for alternative and additional methods of testing more patients.

Despite the limitations inherent in observational studies, the findings in this thesis provided useful data that can assist in the implementation of antiretroviral therapy in Ethiopia. To improve treatment outcomes, we need to treat patients before they progress

to advanced stages. This requires improved counselling and testing practices, and making treatment available even in settings with limited laboratory setup. Future studies should examine the underlying reasons for death and mechanisms should be in place to further improve treatment outcomes.

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1 INTRODUCTION

1.1 General

Outline of the HIV Epidemic

The AIDS epidemic is one of the most destructive epidemics in the history of humankind, claiming the lives of about 25 million people since it was recognized in 1981. [1] In 2005 alone, 3 million people died of AIDS, more than half a million of them being children. An estimated 40 million were living with HIV by the end of 2005, including 5 million new infections in the same year. Earlier projections had suggested that about 45 million people would be infected between 2002 and 2010 unless the world succeeded with concerted preventive effort.[2]

Sub-Saharan Africa is home to 25.8 million people living with HIV, accounting for two-thirds of the global figure. [1] This is higher than the 2003 estimate by almost 1 million. [3] An estimated 2.4 million people died of HIV in 2005, and a further 3.2 million became infected with HIV in the same year. [1]

According to the Ministry of Health of Ethiopia, about 4.4% of the population of Ethiopia live with HIV. [4] The country's epidemic is concentrated mainly in urban areas, where HIV prevalence among pregnant women has averaged at 12-13% since the mid-1990s'. More recent evidence, however, shows the epidemic is spreading to rural areas at faster rate than

ever before. [4] Given the high proportion of the rural population (about 85%), the current estimated rise of the rural prevalence from 1.9% to 2.6% is of great concern. [4]

With about 1.5 million people living with HIV in 2004 and at least half a million children orphaned by AIDS, there is a huge task of providing adequate treatment, care and support to the affected household. [4 5] By June 2005, only about 10% of eligible HIV infected patients were receiving antiretroviral therapy in Ethiopia. [6] According to the WHO, about 211000 people were estimated to be in need of ART by the end of 2004, and the country had declared to treat 93, 000 people by the end of 2005. [7] However, only about 34, 000 patients received treatment by the end of May 2006. [8]

Transmission and risk factors

HIV transmits through sexual exposure. Heterosexual intercourse accounts for about 70% of the overall sexual transmission in the developing countries. [9] In the Western countries, however, homosexual contact is the main mode of sexual transmission. [10] The risk of heterosexual transmission of HIV is 0.0003-0.0015 per coital act.[11] Presence of genital ulcers increases the risk of sexual transmission, as does sexual activity that is associated with infected blood .[11-14] Sexual transmission depends on the viral load as noted in serodiscordant African sexual partners, where transmission was rare at viral loads <1500 copies/ml. [15]

Blood and blood products account for a major share of nonsexual HIV transmission. Transmission can occur through blood transfusion, injection drug use, occupational exposure, or accidental needle sticks. The risk from occupational needle sticks to health care workers from known HIV-positive source patients was found to be 0.33-0.5%. [16 17] Deep injury, injury with a visibly bloody device, or injury with a device that had been previously

used in the source patient's vein or artery increase the risk of HIV acquisition. [17] The risk of HIV transmission through blood transfusion is a rare problem in the developed world but is significantly higher in developing countries. [18 19]

Mother-to-child transmission occurs in about 25% of live births to HIV-infected mothers.[20] Transmission occurs either during pregnancy, during delivery or during breast-feeding. Roughly one-third of cases of mother-to-child transmission result from breast-feeding, the risk increases with duration of breast-feeding, and the chance of breast-milk transmission of HIV is 0.00064 per litre of breast-milk ingested. [21] Other body fluids that are known to harbour HIV include seminal fluid, pre-ejaculate, vaginal secretions, cerebrospinal fluid, saliva, tears, and breast milk of infected individuals. [22-25] No cases of HIV infection have been documented to arise from contact with non-bloody saliva or tears.

Natural history and classification of HIV disease

Initial infection with HIV is non-specific, often without any symptoms or signs. Some infected persons experience a short, flu-like illness about 2-5 weeks after infection. This is known as acute viral syndrome of primary HIV infection. Because of the similarity of the syndrome with any acute febrile illness, diagnosis is often missed. Careful evaluation of prospective data, however, showed that about 87% of individuals who acquire HIV experience some of these symptoms. [26-32] Serologic diagnosis with routine HIV antibody tests is often delayed until several weeks or months after infection.[32]

After the period of acute HIV infection, a relative equilibrium between viral replication and the host immune response is reached, and individuals may have little or no clinical manifestations of HIV infection. This time between initial infection and the development of

AIDS--the chronic HIV infection period-- may be long, averaging 10 years, even in the absence of treatment. [33] There was initial speculation that annual progression rates from HIV infection to AIDS was shorter in females than in males, and in developing countries than in developed countries, but the available data suggest that no major differences exist. [34-41]

After variable period of chronic infection, patients eventually progress to AIDS and then to death. The US Centre for Disease Prevention and Control (CDC) defines AIDS by either diagnosis of AIDS-defining events, or by measurement of CD4 levels <200cells/mcL. The median survival time from AIDS to death is about 10 months, though it may vary according to the AIDS-defining event. [42]

In Africa, the natural course of HIV disease has not been well studied .[37] There is only one study from rural Uganda describing the natural history of HIV disease.[38] Since effective therapy is now available, studying the natural course of untreated HIV disease is unethical.

Currently, there are two systems of classifying HIV disease, the CDC classification and the WHO Staging. [43] The CDC classification has existed since 1982, before HIV was identified. [44] It has been updated several times and the most recent classification includes CD4 measurement as part of essential criteria. [45] The CDC classification was originally designed for surveillance purpose, but its use in clinical practice has increased over time.

The WHO staging system has existed since 1990 and it is mainly based on clinical parameters. [46 47] Both presumptive and confirmed clinical diagnosis is allowed in this staging. Resource-poor countries use the WHO staging routinely both in surveillance and in clinical practice.

Management of opportunistic infections

Because of improved treatment and prophylaxis of opportunistic infections, survival in HIV infected patients in the developed world had improved even before HAART was available. [48] Some studies in developing countries also showed the effectiveness of co-trimoxazole in preventing several opportunistic infections. [49-52] Based on the available evidence, the WHO has recently issued a guideline for the use of cotrimoxazole in resource-limited settings. [53] Unlike the CDC recommendation, which needs CD4 measurement, [54] the WHO guideline encourages the use of clinical criteria for starting and stopping cotrimoxazole prophylaxis.

However, the shortage of the data and other issues special to the resource-limited settings make implementing cotrimoxazole prophylaxis difficult. In settings with no facilities for CD4 testing, the time of beginning and discontinuation of cotrimoxazole prophylaxis is arbitrary. Even when the CD4 test can be done, there is no consensus on the timing of the initiation and discontinuation of cotrimoxazole. Other challenges in developing countries include high background resistance patterns, poor adherence, and overlapping toxicities with antituberculosis and antiretroviral drugs.

In Ethiopia, no study has evaluated the preventive effect of cotrimoxazole prophylaxis. The resistance pattern could be high given the wide availability of the drug on over-the-counter basis. Since decisions on the specific recommendations of cotrimoxazole prophylaxis are left to the individual countries, careful documentation of clinical data would be important.

Tuberculosis and HIV

Tuberculosis is the leading cause of sickness and death among HIV infected patients in sub-Saharan Africa. Unlike other HIV-related infections, tuberculosis occurs at all levels of CD4 count, is infectious, and its prevention is a major public-health priority. [55] In countries with high HIV burden, the current tuberculosis treatment has not been successful. As a result, efforts are under way to harmonize the management of the two diseases.[56-58] Ethiopia follows the directly observed treatment, short course (DOTS) strategy for tuberculosis treatment. However, Isoniazid (INH) preventive therapy (IPT) has not been carried out in the country. In the recently developed TB/HIV guideline, both co-trimoxazole prophylaxis and IPT are recommended. [59] Such effort would entail some change in the current HIV counselling and testing strategy. The traditional voluntary counselling and testing (VCT) is viewed as a cost-effective strategy that can serve as a gateway to most HIV-related services. [60-62] However, its acceptability has been low among different client populations. [63-65]

Some authors assessed the acceptability of VCT among TB patients [66], while others evaluated the acceptability of screening for TB among VCT clients . [67] In the former study in Malawi, authors concluded that VCT and adjunctive cotrimoxazole therapy is feasible, safe and reduces mortality rates in TB patients under routine programme conditions. [66] The group from Haiti reported high prevalence of active pulmonary tuberculosis among VCT clients and they stressed the need to integrate the two programmes. [67] Despite WHO recommendations, there is a need for creative application of rigorous scientific approaches to identify suitable ways of doing it. [68]

In Ethiopia, TB/HIV co-infection is high, with about 45% of sputum-smear-positive pulmonary TB patients being HIV positive. [69] The HIV epidemic is considered to be a major threat to the tuberculosis control efforts in Ethiopia . [70] According to the recently developed TB/HIV guideline, HIV counselling and testing will be part of the routine care for tuberculosis patients. [71] However, potential barriers to the successful implementation of the program such as low acceptability of HIV counselling and testing have not been studied.

1.2 Antiretroviral therapy

A decade ago, having AIDS was almost equivalent to a death sentence. Since 1996, with the introduction of combined antiretroviral treatment,AIDS has become chronic, manageable disease.[72] Currently, there are about 21 Food and Drug Administration (FDA) approved anti-retroviral drugs (ARVs) in the world. [73] Three classes of ARVs are now available: Nucleoside reverse transcriptase inhibitors (NRTIs), Non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs). Entry and Fusion Inhibitors have also emerged as additional class of antiretroviral drugs. [74]

When to start treatment

WHO recommends that in resource-limited settings HIV infected adolescents and adults should start antiretroviral therapy when the following conditions are met: [75]

- WHO stage 4 disease (clinical AIDS), regardless of CD4 count
- WHO stage 3 disease with consideration of CD4 count <350/mm3 in assisting decision making
- WHO stages 1 or 2 disease with CD4 count<200/mm3.

In settings where CD4 count is not available, the total lymphocyte count (TLC) can be used, and treatment is recommended for:

- WHO stage 3 or 4 disease (clinical AIDS) irrespective of the TLC
- WHO stage 2 disease with TLC <=1200/mm^3

Although the TLC predicts survival in untreated patients, the exact cut-off point for beginning treatment remains controversial. [47 76 77]This is a clear indication for a more rigorous scientific data in the appropriate setting.

Drug regimens

The WHO encourages a public health approach to ease the scale-up of ARV use in resource-limited settings. [75]This approach advises that ARV treatment programmes be standardized to reach as many patients as possible. Therefore, countries select their own first line regimens and a limited number of second-line regimens. Selection of the regimens depends on several reasons: potency, side effects, laboratory monitoring requirements, potential for maintenance of future treatment choices, expected patient adherence, coexisting conditions, pregnancy or the risk of it, availability and cost.[75 78 79]

WHO recommends a combination of 2 NRTIs+1NNRTI as first-line regimen for resource-poor countries. The use of five-formulary approach (d4T or ZDV) +3TC+ (NVP or EFV) gives four possible combinations and provides options for substitution of toxicity. Each of the possible combinations are equally potent . Protease inhibitors (PI) are suggested in case resistance to NNRTIs is known to exist. HIV-2 and HIV-1 group O are often resistant to NNRTIs-based regimens. [75]

In treatment failure, the entire first line drugs should be changed to a second line regimen. The second-line drugs should include at least three new drugs, one or more of them from a new class. This increases the likelihood of treatment success and minimizes the chance of cross-resistance. The second-line regimen requires a switch from (d4T or ZDV) +3TC+ (NVP or EFV) to (TDF or ABC) +ddI+ (LPV/r or SQV/r). [75]

Since publishing the first WHO guideline in April 2002, [80] more evidence has been obtained on the effectiveness of HAART in resource-limited settings. However, more work is needed to clarify its effectiveness under routine care conditions. As regional variations in viral subtypes and co morbid conditions may affect treatment outcomes, we need region-specific or country-specific information.

Monitoring Antiretroviral Therapy

Monitoring antiretroviral therapy starts before starting treatment. Once treatment is started, there are four essential aspects of monitoring: therapeutic response, drug toxicity, adherence to medication regimens, and monitoring for viral resistance. [81]

Monitoring for therapeutic response

Current North American and European clinical care guidelines incorporate measurements of plasma HIV RNA load and CD4 T-lymphocytes for overseeing response to antiretroviral therapy. [82 83] Since such techniques are not affordable in resource-limited settings, adapted version of these guidelines have been provided. WHO, for example, recommends that ART can be started where only HIV antibody test and Haemoglobin (Hgb) or Haematocrit (Hct)

can be done. [75] Others have suggested syndromic approach for monitoring response to antiretroviral therapy. [84 85]

Clinical monitoring alone might result in continued therapy despite emergence of drug resistance. Though viral load measurement allows for early detection of viral rebound, it is expensive and may compete with scarce resources in resource-limited settings. Identifying a balanced monitoring method suitable for the resource-limited settings is thus a priority. [81]

Monitoring for drug toxicity

Almost all antiretroviral drugs have some adverse effects. These toxicities could be specific to a single drug, shared by a class of drugs, or could be shared by all antiretroviral drugs. Also, some of the drugs have toxicities shared by other drugs commonly used in HIV infected patients. [86] In general, drug toxicity can be classified into four categories: mitochondrial toxicity, hypersensitivity, lipodystrophy syndrome, and other adverse events and special circumstances .[87]

NRTIs and nucleotide-analogue reverse-transcriptase inhibitors (NtRTIs) cause toxicity by inhibiting a mitochondrial enzyme responsible for energy synthesis. [88] The symptoms occur in organs rich in mitochondria such as muscles, nerves, liver and pancreas. The patient may thus complain of muscle aches (myopathy), pain in the nerve roots (neuropathy), or symptoms suggestive of liver or pancreatic injury. The clinical symptoms are often of gradual onset and offset.

Drug hypersensitivity could be up to 100 times more common in HIV-infected patients than in the general population. [89]Typically, patients present with an erythematous, maculopapular,

pruritic, and confluent rash with or without fever. The rash usually begins after 1-3 weeks' therapy. All NNRTIs, the NRTI abacabir, and the protease inhibitor amprenavir are commonly associated with drug hypersensitivity. About half of hypersensitivities associated with antiretroviral drugs resolve spontaneously. [87]

The main clinical features of the lipodystrophy syndrome associated with antiretroviral drugs include peripheral fat loss and central fat accumulation.[90] Other side effects include anaemia, gastrointestinal symptoms, and teratogenicity.

The best methods for monitoring drug toxicities are still unclear even for developed countries. [82] In resource-limited settings, both the size of drug toxicity and its monitoring methods are poorly documented. As with monitoring the response to therapy, there are trade-offs between intensive and less intense watching of toxicity. [81] Patients with moderate or even severe elevations in liver enzyme levels may have no detectable symptoms or signs. For such patients, relying on clinical evaluation may result in deaths that might be prevented by more intensive monitoring strategies.

Monitoring for adherence to medication regimens

Poor adherence to medication is a common problem everywhere. Adherence to other chronic medications is about 50-75%.[91] Since treatment outcome (measured virologically) is sensitive to slight changes in adherence, about 95% adherence is recommended for antiretroviral therapy. [92-94]

Getting such high-level of adherence may be problematic. Poor adherence is not uncommon even in the industrialized world. [95] Both success and failure stories have been reported form

developing countries. [96] Speculations that poor adherence could lead to development of drug resistance have been challenged by some reports of high level of adherence in resource-poor settings. [97] Also, the relationship between adherence and resistance is bidirectional. [98] High-level of adherence in the presence of partial viral suppression in fact leads to higher chance of resistance. Among patients with complete viral suppression, development of viral resistance is less likely with high level of adherence.

Adherence to HAART is affected by the same reasons that are associated with adherence to other medications. [99 100] These factors could be related to the patient, the medication or health care. Interventions to improve adherence should take all these reasons into account. Such interventions could be patient-focused (education, reminders, rewards and reinforcement), provider-focused (continuing medical education, cues and instruments, etc.), or regimen-focused (decreasing frequency of dosing and pill burden, reduced cost). Often combinations of various strategies are used to improve adherence. [100]

Monitoring for drug resistance

Resistance to antiretroviral agents occurred soon after they were introduced. [101] Causes for viral resistance include poor drug absorption, drug-drug interactions, variable drug metabolism, and poor adherence. The baseline resistance pattern in resource-poor settings is unknown, and the best approach to resistance monitoring remains poorly documented. Some have suggested sentinel resistance monitoring for centrally designed programmes. [81]

1.3 Treatment versus prevention

Prevention is the most important strategy to halt the AIDS pandemic, and one of the final tools for prevention is introducing an HIV vaccine. However, the inconsistency of the success of prevention programmes and the little hope of having a vaccine in any near future provide grounds for looking more seriously at care for AIDS patients in resource-poor countries.[102 103]

Effective ways of interrupting the course of HIV disease progression have existed since shortly after the discovery of the virus. [104] However, antiretroviral therapy was limited only to the developed countries until recent years. The main barrier was the cost of antiretroviral drugs and laboratory expenses. [105-107] Because of political pressure, access to ART has now improved remarkably .[6 108 109]

However, only a small fraction of patients on the hardest-hit countries are now on ART. By June 2005, only one in ten Africans were getting treatment .[6] A more recent report shows a large increase (17%) in access to ART in sub-Saharan Africa. [110] Though the 3 by 5 initiative did not meet its target of treating 3 million people by the end of 2005, it provided the foundation for the Universal Access by the year 2010 as pledged by the international community. [111 112]

Debates about whether to prioritize treatment or prevention seem to be changing. [113 114] The current literature supports the synergistic effect of treatment and prevention .[115-117] Successful treatment makes prevention more acceptable and effective, and effective prevention makes treatment more affordable and sustainable. Widespread access to treatment

could bring many people into health-care settings, providing new opportunities for health-care workers to deliver and reinforce HIV prevention messages and interventions. [117] Improved access to HIV testing provides an entry point to both prevention and treatment services. Effective prevention leads to decline in new infections therefore finally will lead to a decrease in the number of people who will need treatment. [117] The current debate is not whether to prioritize treatment or prevention. The question is how to carry out both prevention and treatment in an effective way.

1.4 Rationale for the present study

There is no single proven model for delivering ART .[118] In resource-poor settings, little is known about the effect of treatment on patient survival and quality of life. Treatment guidelines are from the developed world. [75 78 80] This highlights the urgent need for generating regionally suitable data. We started our study when Ethiopia was planning for a large-scale ART programme. Successful implementation of such initiatives needs scientific evidence generated in the appropriate settings.

In this study, we tried to answer some questions that remain unanswered regarding implementation of ART in resource-poor settings. Our study on the natural course of disease progression (Paper I) defines pre-ART mortality and tuberculosis incidence rates, and provided useful data against which the clinical benefits of ART could be measured. It also identified a set of predictor models to guide clinical decision- making and economic analysis. The effectiveness of HAART could vary from region to region because of the difference in background disease burden (such as tuberculosis or intestinal parasites), viral

subtypes, and possible genetic differences in drug metabolism. However, such arguments are based on little data from the resource-limited settings. We set out to show the effectiveness of HAART under routine care conditions in south Ethiopia (Paper II). In further search for factors associated with increased mortality in treated patients, we evaluated a set of simple clinical and laboratory values as potential predictors of death (Paper III). As tuberculosis was an important infection in this setting (Paper I and II), we evaluated ways of integrating the management of the two infections using counselling and testing as entry point (Paper IV).

2 STUDY AIMS

Overall aim

The overall aim of this thesis was to improve antiretroviral therapy in Ethiopia.

Specific aims

1. To describe the pattern and predictors of HIV disease progression among untreated HIV infected patients (**Paper I**).

2. To assess the effect of antiretroviral therapy on patient mortality and tuberculosis incidence rate (**Paper II).**

3. To identify risk factors for mortality in patients treated with antiretroviral therapy (**Paper III**).

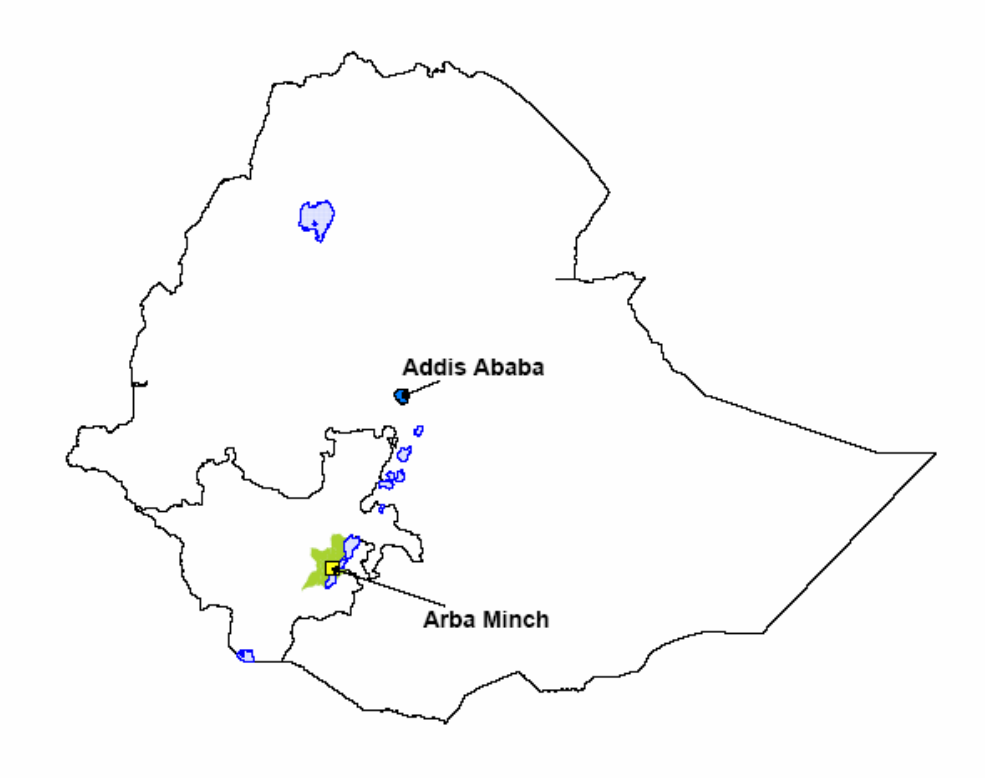
4. To identify appropriate ways of incorporating HIV screening as routine care for tuberculosis patients (**Paper IV**).

3 METHODS

3.1 Study area background

Ethiopia is located in Eastern Africa (See Map). The country has an estimated population of 75 million, with those aged 15-64 years accounting for 54% of the total population. The population growth rate is 2.3%. Ethiopia is a poor country with 50% of the population being below poverty line. [119]

Figure 1 Map of Ethiopia showing the study area, Arba Minch, and catchment area of Arba Minch Hospital (green)



Ethiopia has one of the worst health status in the world, with infectious and communicable diseases and nutritional problems accounting for 60-80% of health problems. [120] [121] Table 3-1 summarizes selected demographic and health indicators for Ethiopia.

Table 1 Selected demographic and health indicators for Ethiopia

Indicator	**Rate**
Life expectancy at birth in years (Male, Female)	53.4, 55.4*
Infant Mortality rate per 1000	77**
Under five mortality rate per 1000	123**
Adult HIV prevalence rate (%)	4.4*

* Source is reference [120] ** Source is reference [121]

The Southern Nations, Nationalities, and Peoples' Regional State (SNNPRS) is the third most populous region in Ethiopia with population of about 14 million. There are 13 zones (districts) and 52 woredas (sub districts) in the region. Most of the people are subsistence farmers, and over 93% of the population lives in rural areas. Infectious diseases account for the majority of health problems in the region. The HIV prevalence is estimated at 11.0 and 3.2% in the urban and rural areas, respectively. [122]

Gamo Goffa is one of the 13 districts in the SNNPRS with an estimated population of 1.5 million. Its capital, Arba Minch, is located 500 km south of Addis Ababa, and 275 Km from Awassa, the regional capital. Infectious diseases make up the major share of disease load in the district with malaria ranking first. [123]

Arba Minch Hospital

Arba Minch Hospital is a 158-bed general hospital serving a population of about 1.5 million. The hospital provides general outpatient service, surgical and obstetric emergency and regular services, and general medical and paediatric in-patient services. Staffed with over 200 technical and administrative staff, the hospital treats over thirty thousand patients a year. Infectious diseases account for the most of the in-patient and outpatient diagnoses, with malaria being the commonest cause. Tuberculosis was the third commonest cause of admission and the third cause of inpatient death in 2004/2005 (Arba Minch hospital statistics unit).

The hospital has been providing HIV counselling and testing since the early 1990's. In 2002 a new HIV/AIDS project was started with financial support from the Norwegian Agency for Development Cooperation (NORAD)/ the Norwegian Lutheran Mission (NLM). The main objective of this project was to deliver antiretroviral drugs to the patients, and this was started in August 2003.There was some delay in providing drugs to the patients because of lack of national policy and guideline until recently. [124] [125] The Ethiopian free ART program was launched in January 2005. [126]

3.2 **Design of the specific studies**

The cohort study: Papers I-III

The study population

The specific aims 1, 2 and 3 were approached through a prospective cohort study design. A cohort of all HIV-infected individuals enrolled at the HIV clinic of the hospital between

January-August 2003 made up the study population for Paper I. All consecutive adult (age >15 years) HIV-infected patients who consented to participate in the study constituted the study participants. All symptomatic HIV positive adults (WHO stage II-IV), and those who were put on ART between August 2003 and August 2005 made up the study participants for Paper II. All patients put on ART between August 2003 and January 2005, who had at least one follow-up visit, and followed through August 2005 constituted the study participants for Paper III.

Data collection

The data collection for the cohort section of the study was a part of the routine work of the HIV Clinic. This work was according to the National treatment guideline. [125]

First visit

As part of a post-test counselling service, a counsellor referred all consenting HIV positive clients to a doctor after recording socio-demographic variables in a standardized form. The doctor recorded relevant history and physical findings and then classified the disease according to the WHO clinical staging . [45] We used a checklist of important symptoms and guidelines for staging. Following clinical staging, patients were subjected to complete blood cell count (CBC). Added investigations such as chest x-ray and sputum examination were done per clinical indication.

Treatment and follow-up plans

During the pre-ART period (before August 2003), all patients were scheduled to have 12 weekly follow-up. Also, they were encouraged to visit the clinic any time they felt sick. On each subsequent visit, a doctor asked them about new symptoms since the last visit and

relevant physical findings including body weight were recorded. Then CBC and other indicated tests were repeated. Patients were treated for identified illnesses according to the standard in the hospital.

After ARVs were made available in August 2003 (ART period), those with clinical indication for ART according to the National treatment guideline [125] were provided with adherence counselling. Once an agreement was reached to start ART, the doctor requested liver and renal function tests.

Availability and clinical indications dictated the exact drug regimen. After recording information, drugs were prescribed as advised by the Drug Administration and Control Authority (DACA). The following drugs were approved for district hospitals at the time of the study: Zidovudine (ZDV), Lamivudine (3TC), Stavudine (d4T), Nevirapine (NVP), Efavirenz (EFV), and a fixed combination of ZDV and 3TC. [127]The list of possible combinations included: ZDV+3TC/EFV, ZDV+3TC/NVP, d4T+3TC/EFV, and d4T+3TC/NVP. After starting ART, follow-up was scheduled at 4-weekly intervals to refill drugs. Patients were encouraged to report whenever they developed drug side effects or worsening of their health condition was noticed.

Monitoring treatment response and drug toxicity

Patients were asked about disease symptoms and drug side effects at each clinic visit. Body weight was also measured at each clinic visit. CBC and organ function tests (liver and renal) were done at weeks 4 and 12, 12-weekly after that and whenever clinically indicated.

Home visits

Two community agents followed the patients at community level. These were secondary school graduates with extra training on HIV and AIDS. We assigned each of them to the newly recruited patients with their contact details. They visited the patients each month at their home and provided home-based counselling and support during the pre-ART period (Paper I). During the ART period (Papers II and III), the community agents encouraged adherence to the medication and follow-up. They provided monthly reports on the status of each patient.

Outcome Measures

Time to death and time to diagnosis of tuberculosis were the main outcome measures for Papers I and II. Secondary outcome variables were time to WHO stage change (Paper I), loss to follow-up and transfer to other places. Time to death was the main outcome measure in Paper III.

The cross-sectional design: Paper IV

The study population

All adult (age>15 years) tuberculosis patients who attended the DOTS clinic of the hospital constituted the study population. Consenting patients who visited the clinic during January-June 2005 were the study participants.

Data collection techniques and tools

We interviewed all consenting patients. At the end of the interview, we asked the patients if they accepted a pre-test counselling. Those who accepted the invitation, were counselled and tested. We informed the patients on the same day or within the shortest possible time. If the

patient refused testing, it was regarded as non-acceptability of pre-test counselling. If the patient did not return for the results within 2 weeks, we labelled it as non-acceptability of post-test counselling.

3.3 Sample size and statistical power

In survival analysis, which was the main analysis technique in our study, the outcome variable (weeks of survival), is a continuous variable. In reality, however, what is actually being assessed is not time itself but the proportion of subjects who are still alive at each point in time. Thus, a reasonable approximation can be made by dichotomizing the outcome variable at the end of the anticipated follow-up period. [128] We used this principle in the calculation of sample size for Papers I-III.

Assuming WHO disease stage as main predictor of mortality rates at the end of the study, our null hypothesis was there would be no difference in mortality between stage I-II and stage III-IV (Paper I). Similarly, in Paper III, we assumed that the WHO clinical stage, categorized as Stage IV versus Stage II-III, would be the main predictor of mortality in treated patients. The alternative hypothesis was there would be difference in mortality. From pilot analysis, the mortality rate was 37% (P1=0.37) in stages III-IV, and 20% (P2=0.20) in stages I-II at about 20 weeks of follow-up. Assuming alpha (two-sided)=0.05 and power 90%, we determined a minimum sample size of 91 for each group both for Papers I and III. [128]

In order to compare the mortality rate between the pre-HAART and the HAART group (Paper II), we assumed that 30% and 12% of patients in the pre-HAART and HAART cohorts, respectively, would die at the end of the follow-up. Using the same alpha and

power, we arrived at a minimum sample size of 91 in each cohort. [128] Assuming that about 20% of the patients will be lost to follow-up, we adjusted for the loss by a factor of 1/ (1/1-0.20)=1.25.

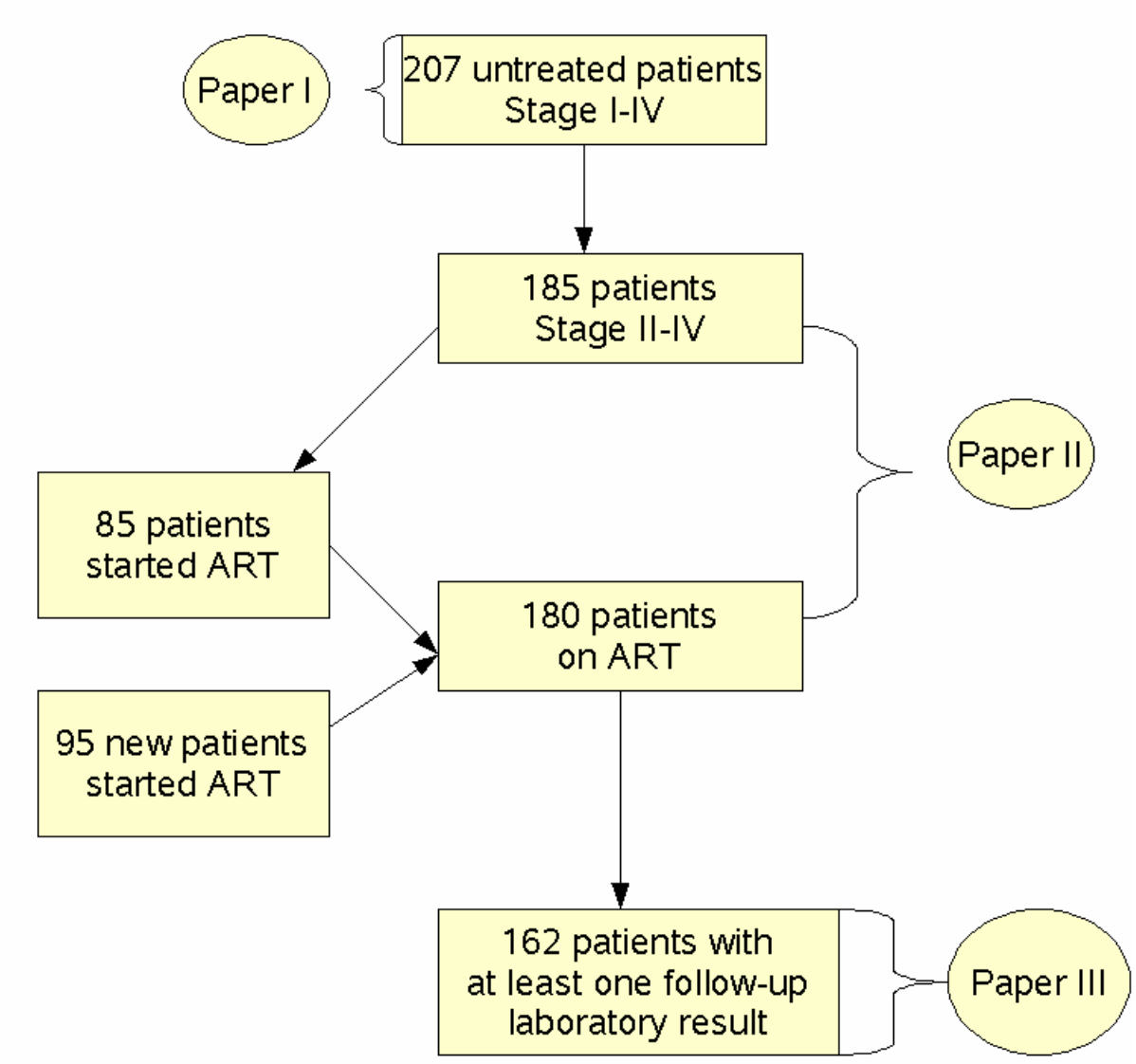
Therefore, the minimum sample size for each exposure group in the cohort part of this study (Papers I-III) was 134 (91x1.25=134). However, we recruited more patients in stage III than the other stages, as the study was done under routine care conditions. As a result, we had unbalanced sample sizes for Papers I and III. To check if we had adequate statistical power to distinguish between the hypotheses, we calculated statistical power analysis using OpenEpi computer software. [129] The results are summarized in Table 2.

Table 2. Statistical power for the cohort study (Papers I-III)

Data input categories	Paper I	Paper II	III
Number of exposed	157	185	35
Number of unexposed	50	180	117
Risk of death among exposed (%)	28	25.4	30
Risk of death among unexposed (%)	6	13.9	10
Two-sided confidence interval (%)	95	95	95
Definition of exposure category			
Exposed	Stage III-IV	Pre-HAART	Stage IV
Unexposed	Stage I-II	HAART	Stage II-III
Risk ratio detected	4.7	1.8	3
Power based on normal approximation	90.5	79.1	84.3

For the cross-sectional study (Paper IV), we planned to include all the patients who visited the clinic during the data collection period. We were able to recruit 190 patients. While study size determination can be aided by conventional formulas, such decision must also incorporate unquantified practical constraints such as time and finances. [130] In our case, it was time which limited us from including more patients.

Figure 2. Diagrammatic representation of the cohort part of the study (Papers I-III)



3.4 Data Management and statistical analysis

Statistical methods

We used SPSS (SPSS Inc, Chicago, USA) software for data processing and analysis. Data analysis was performed when data collection was completed for each study. Our main method of analysis for the cohort part of the study was survival analyses. We used the Kaplan-Meier (KM) method for event-free survival, and the Cox Regression (CR) method to evaluate the relative effect of selected variables. The Log-rank test (for the KM) and the hazard ratios (for the CR) were used to test for statistical significance. For the cross-sectional part (Paper IV), we used the Logistic regression method to assess factors affecting acceptability. All statistical tests were considered significant if the two-sided P-value <0.05. Table 4 summarizes the main statistical methods.

Data safety and quality assurance

Patient records were kept in the clinic in hard-cover file holders in a lockable shelf. Only authorized personnel had the keys to the rooms and to the shelves. Access to databases was restricted by passwords known to the principal investigator and to the data clerk. Back-ups were taken on a daily basis and kept in the investigator's home. Completed questionnaires (Paper IV) were archived in the investigator's office in a lockable shelf.

In order to assure consistency of information gathered in the clinic, checklists of important symptoms and signs were posted in front of the doctor's desk and on the walls in the offices. The doctors, nurses, and laboratory technicians working with the HIV unit completed the necessary national courses. The doctors also attended a one-month course in comprehensive HIV care at Makerere University in Uganda.

Table 4. Summary of study design, population, tools and main statistical methods

Paper	Study population	Study design	Data collection tools	Main statistical analysis
I	207 untreated HIV patients	Prospective cohort	Clinical records Home visits	Kaplan Meier and Cox-regression
II	185 untreated patients 180 treated patients	Prospective cohort	Clinical records Home visits	Kaplan Meier Cox regression
III	162 treated patients	Prospective cohort	Clinical records Home visits	Kaplan Meier Cox regression
IV	190 tuberculosis patients	Cross-sectional	Questionnaire, HIV counselling and testing	Logistic regression

Refresher courses were arranged when areas of weakness were detected. The principal investigator made unannounced visits to homes of randomly selected patients every 2-3 months. Field reports were checked for consistency on a monthly basis.

3.5 Ethical Considerations

Ethical approval was obtained form the Regional Committee for Medical Research Ethics in Bergen, Norway, and from the National Ethics Review Committee in Ethiopia. All patients gave informed written consent for HIV testing and for participating in the study. Treatment of patients was according to the National treatment guideline. Prompt medical treatment was given to all patients irrespective of their consent. All patient records were kept in a confidential way as described above.

4 SYNOPSES OF THE PAPERS

Paper I: Disease progression among untreated HIV infected patients in south Ethiopia: implications for patient care

Understanding the natural course of HIV disease progression is important for clinical decision making, for modelling the epidemic, and for planning interventions at programme level. In resource-limited settings, such information is poorly documented. In this study, we described the short-term disease progression pattern among Ethiopian patients under routine clinical care conditions.

Recruitment into the cohort started in January 2003, as a part of the preparation to start ART. After initial assessment and WHO staging patients were reassessed every 12 weeks. We documented new stage-defining events and measured CBC at each visit. Patients received the standard care in the hospital. Death, diagnosis of tuberculosis and change in disease stage were the outcome measures. Two community agents reported patient status every month. We used the Kaplan-Meier and Cox-regression methods to assess predictors of disease progression.

We followed 207 patients for a median duration of 19 weeks.132 (64%) patients were in WHO stage III. The Mortality rate was 46 per 100 person-years of observation (PYO). Mortality increased with advancing disease stage. Diarrhoea, oral thrush and low total lymphocyte count were significant markers of mortality. Tuberculosis incidence rate was 9.9 per 100 PYO. Easy fatigability and fever were strongly associated with tuberculosis (HR=15.6, 95%CI=2.8-87.5 for weakness; HR=9.2, 95%CI=1.9-44.5).

Oral thrush, diarrhoea and total lymphocyte count predicted mortality, and easy fatigability and fever predicted tuberculosis. In settings with scarce resources, patients with oral thrush, diarrhoea or low total lymphocyte count should be prioritized for treatment. Patients with history of prolonged fever and easy fatigability are at higher risk of developing tuberculosis and thus require close attention.

Paper II: Antiretroviral therapy at a district hospital in Ethiopia prevents death and tuberculosis in a cohort of HIV patients

In resource-poor settings, the effectiveness of HAART has not been fully studied. Our aim was to assess the effect of HAART on patient mortality and tuberculosis incidence rate under routine clinical care conditions in a resource-limited setting in south Ethiopia. Starting in January 2003, we followed all consecutive adult HIV infected patients who visited the HIV clinic. Since August 2003, we treated patients with HAART. Only basic laboratory services were available.

We followed 185 patients in the pre-HAART cohort and 180 patients in the HAART cohort. The mortality rate was 15.4 per 100 person-years of observation (PYO) in the HAART group and tuberculosis incidence rate was 3.7 per 100 PYO. In the pre-HAART group, the mortality rate was 58.1 per 100 PYO and the tuberculosis incidence rate was 11.1 per 100 PYO. HAART resulted in a 65% decline in mortality (adjusted hazard ratio [95%CI] =0.35 [0.19-0.63]; P<0.001). Tuberculosis incidence rate was lower in the HAART group (adjusted hazard ratio [95%CI] =0.11 [0.03-0.48]; P<0.01). Most of the deaths occurred during the first three months of treatment.

HAART improved survival and decreased tuberculosis incidence to a level similar to that achieved in the developed countries during the early years of HAART. However, both the mortality and the tuberculosis incidence rate were higher in this resource-limited setting. Attention should be paid to the early weeks of treatment when mortality is high. The high tuberculosis incidence rate, when coupled with the improved survival, may lead to increased tuberculosis transmission at the community level. This highlights the need for strengthening tuberculosis prevention efforts with the scale-up of treatment programmes.

Paper III: Predictors of early death in a cohort of Ethiopian patients treated with HAART

Although the survival of HIV infected patients has improved following the introduction of HAART, patients in resource-poor countries have higher mortality rates especially during the first weeks of treatment. In this study, we examined factors predicting mortality in Ethiopian patients treated with HAART.

We treated patients using the WHO clinical stage and the TLC as criteria for beginning treatment. We measured body weight and CBC at baseline, 4 weeks later, then repeated weight every month and CBC every 12 weeks. Time to death was the main outcome variable. We used the Kaplan Meier and Cox regression survival analyses to identify prognostic markers. We also calculated mortality rates for the different phases of the follow-up.

Out of 162 recruited, 152 treatment-naïve patients contributed 144 person-years of observation (PYO). 86 (57%) of them were men and their median age was 32 years. The overall mortality rate was 16.7 per 100 PYO (24 deaths/144.1 PYO). The highest death rate

occurred in the first month of treatment. Compared to the first month, mortality declined by 9-fold after the 18th week of follow-up. Being in WHO clinical stage IV and having TLC<=750/mcL were independent predictors of death. BMI <=18.5 kg/m2 at baseline was associated with death in univariate analysis. Weight loss was seen in about a third of patients who survived up to the fourth week, and it was associated with increased death. Decline in TLC, HGB and BMI was associated with death in univariate analysis only.

The high early mortality associated with advanced disease stage highlights the need for identifying and treating patients early. Underlying causes for the early death should be investigated.

Paper IV: Acceptability of HIV counselling and testing among tuberculosis patients in south Ethiopia

To benefit from the available care and treatment options, patients need to know their HIV status. Many tuberculosis patients are also HIV infected and they represent patients with advanced disease. Our aim was to assess the acceptability of HIV testing among tuberculosis patients under routine care conditions in south Ethiopia.

We asked adult tuberculosis patients who were treated at Arba Minch Hospital between January and August 2005 if they were willing to be counselled and tested. Those who showed willingness were counselled and tested, and told about their results. Using the logistic regression method, we assessed factors associated with willingness and acceptability.

Among 190 ptients (52% men), 49(26%) were previously tested including 29 (59%) HIV positive. Of 161 remaining patients, 118 (73%) were willing to be tested and 58% (68/118) of those willing accepted the test, making the overall acceptability rate 35% (56/161). Fourteen (20.6%) were HIV positive and women were more likely to be HIV infected. Unemployment and self-perceived high risk of HIV infection were associated with initial willingness. However, only unemployment was associated with accepting the test.

The low acceptability of HIV counselling and testing among tuberculosis patients poses a challenge to the scale-up of TB/HIV collaborative efforts. Added methods such as routine asking of the patient's previous history of HIV testing should be encouraged.

Additional results

Between December 2005 and March 2006, baseline CD4 count was available for 105 treatment naïve patients (44 men and 61 women). Their median (range) age was 33 (15-60) years. Baseline WHO disease stage was available for 82 patients (77%) including 59/82 (72%) in stage III, 13/82 (16%) in stage IV, 7/82 (8%) in stage II, and 3/82 (4%) in stage I. Their median CD4 and TLC values were 210 cells/mcL (95% range; 7, 1003) and 1500 cells/mcL (95% range; 332, 3735), respectively. The CD4 count was positively correlated with TLC (r=.518, P<.001, n=98), BMI (r=.311, P=.002, n=98) and WT (r=.231, P=.022, n=98).

TLC<=1200/ml predicted CD4 <=200/ml with sensitivity of 59%, specificity of 79%, PPV of 52% and NPV of 73%. Addition of BMI<=18.5 kg/m2 increased the sensitivity to 61% and the PPV to 70%, but the NPV decreased to 71%. TLC<=1200/ml predicted CD4<=100

with sensitivity 73%, specificity 77%, PPV 56%, and NPV 88%. Addition of HGB and/or BMI did not improve the models.

5 DISCUSSION

5.1 Discussion of methods

Study design

Our main study design was a cohort study. Cohort studies track people forward in time from exposure to one or more outcomes. Data collection may occur in the same direction (prospective cohort study), in the opposite direction (retrospective cohort study), or in both directions (ambidirectional). [131] In this study (Papers I-III), we collected prospective data under routine clinical care conditions.

Cohort studies have several advantages. They are useful to ascertain both incidence and natural history of a disease. They are also important in investigation of multiple outcomes that might occur after a single exposure. The cohort study design is suitable for studying rare exposures, and it reduces the risk of survivor bias. Cohort studies also allow calculation of incidence rates, relative risks, and other outcome measures such as survival analyses. [131]

Cohort studies have disadvantages too. [131] Selection bias is built into cohort studies. Loss to follow-up can be a problem, and cohort studies are not suitable for studying rare diseases. Before-after cohort studies have important limitations. Here, an investigator takes a measurement, exposes participants to an intervention, repeats the measurements, then compares them. First, regression to the mean is often ignored. If admission to the cohort includes extreme measurements, such as high laboratory values, then lower mean values will arise at follow-up, irrespective of treatment. Second, secular trends, such as seasonal

changes in disease frequency, can affect results. Third, washout periods are often needed to avoid carryover effect of drugs given during the initial observation period. [131] In our study, loss to follow-up was minimal. Because the follow-up period crossed both rainy and dry seasons, the secular trends are unlikely to affect the outcome. Since we compared no treatment with treatment situation, there was no need for washout period.

In principle, a cohort study could be used to estimate average risks, rates, or occurrence times. This requires that the whole cohort remain at risk and under observation for the entire follow-up period. [130] In practice, patients may be lost, transferred, or die of competing causes. When losses or competing risks do occur, one may still estimate the incidence rate using survival methods. [130] In our cohort, loss to follow-up was minimal and we used survival methods to estimate predictors and rates of death and disease.

An individual can contribute person-time to one or more exposure groups in a study, because each unit of person-time contributed to follow-up by a person possesses its own classification with respect to exposure. [130] Thus an individual whose exposure changes with time may contribute follow-up time to several exposures. This explains the design in Paper II, where 85 patients contributed person-time to both the pre-HAART and the HAART cohorts. These are patients whose exposure experience (pre-HAART versus HAART) changed with time.

Validity

The validity of a study is classified into two: internal validity and external validity (or generalizability).

Internal validity

Internal validity is the ability of a study to measure what it sets out to measure. It shows the accuracy of inferences that can be drawn from study participants. Internal validity is a pre-requisite for external validity. In clinical research, extrapolation of invalid results to the broader population is worthless and potentially dangerous. [132]

Internal validity implies accurate measurement of effects apart from random errors. However, all observational studies have built-in bias, and bias undermines the internal validity of a study. The three major types of bias include: selection bias, information bias, and confounding. [130 132]

Selection bias stems from lack of comparability of groups studied. It results from procedures used to select subjects and from factors that influence study participation. The common element of such biases is that the relation between exposure and disease is different for those who participate and those who should be theoretically eligible for study, including those who do not participate. The result is that associations observed in the study represent a mix of factors affecting participation, as well as factors determining disease. [130] In our cohort (Papers I-III), we included all adult HIV infected patients who willingly came to our clinic for medical care following post-test counselling. Therefore, selection bias is unlikely to occur in this study.

Information bias, also called measurement bias, results from incorrect determination of exposure or outcome, or both. In a cohort study, information about outcomes must be obtained the same way for those exposed and unexposed. The main outcome measures in our

study (Papers I-III) were death, diagnosis of tuberculosis, and change in specific laboratory values. Lack of information about specific causes of death might have resulted in incorrect determination of outcome. Since diagnosis of extrapulmonary and smear negative pulmonary tuberculosis was dependent on the clinical judgement of a doctor, difference in clinical experience might have resulted in measurement bias. The same is true for the WHO clinical staging. Failure to recognize a stage-defining sign or symptom may result in variations in disease stage. Although the haematological parameters were determined by automatic machines, differences in the handling of the samples might have resulted in measurement bias.

Confounding is a mixing or blurring of effects. A researcher attempts to relate an exposure to an outcome, but actually measures the effect of a third variable, termed a confounding variable. A confounding variable is associated with the exposure and it affects the outcome, but it is not an intermediate link in the chain of causation between exposure and outcome. [130]

Confounding is correctable provided potential confounders are anticipated. It can be avoided by restriction, matching, stratification, and by multivariate techniques. In our study, we used various regression models to control for the relative effect of potential confounders. In Paper I, for example, we included oral thrush, TLC, HGB, WHO clinical stage, Diarrhoea, and BMI in a multivariate Cox regression analysis to see if these variables were independent predictors of death. Only TLC<=1200/ml and oral thrush were found to be independent markers of death.

In Paper II, we examined if the observed difference in mortality between pre-HAART and HAART cohorts was the effect of treatment. As shown in Table 3 in Paper II, treatment with

HAART was the strongest predictor of improved survival. We further checked for multicolinearity between HAART and TLC, and HAART and BMI. The interaction term was not significant (P>0.1 in both), suggesting that HAART prevents death irrespective of the patient's TLC and BMI values.

In Paper III, we made detailed analysis of factors predicting mortality for the different phases of the follow-up. Here, we found that that the TLC<=750/ml and WHO clinical stage were independent predictors of mortality. We demonstrated that the WHO clinical stage was a better predictor of deaths that occur in the first month of the follow-up, while the TLC<=750 was a better predictor of overall mortality.

External validity

External validity refers to the validity of the inferences as they apply to people outside that population. In other words, external validity gauges the applicability of study results under routine care conditions. [130 132] Applicability of study results outside the study setting depends on the feasibility, coverage, and acceptability of the interventions. [133] Feasibility of health interventions depends on capacity of care providers, has some cost dimensions, and may require the existence of other health services. To be applicable, an intervention must achieve adequate coverage which in turn depends on the overall comprehensiveness of health coverage. Acceptability determines the recipients' level of adherence to treatment and follow-up plans, and it may vary between populations depending on cultural norms and cost of the interventions. [133]

Although our study was observational, results of observational studies may approximate those of randomized trials if conducted properly. [134] Therefore, factors that affect results of

randomized trials are likely to affect the generalisability of our findings. For example, the presence of community agents as additional service providers might have introduced some differences in the outcome of treatment. The doctors had also received advanced trainings on clinical management of HIV which might have created a better capacity of care providers than in most other district hospitals in Ethiopia.

The findings of this study can be generalized to adult HIV-infected treatment-naïve patients in public health settings in Ethiopia, or even in other resource-limited settings in Sub-Saharan Africa for a number of reasons. First, we did the study under routine care conditions in a typical district hospital in Sub-Saharan Africa. Second, no strict exclusion criteria were applied except in the analysis of the laboratory data in Paper III where 10 patients with previous exposure to ARVs were excluded. Third, loss to follow-up was minimal. Moreover, our results agree with findings from other studies in similar settings. The decline in mortality rate and tuberculosis incidence rates in patients treated with HAART is consistent with reports from other African countries. [135 136] The increased early mortality in treated patients concurs well with a large multicentre study and consistent with reports from Senegal and South Africa. [137-139]

5.2 Discussion of main results

Disease progression among untreated patients

Natural history studies of untreated HIV disease are useful for clinicians, public health experts and policy makers. Such information is important for developing treatment guidelines, for modelling the epidemic, and for prioritizing and allocating resources. Since effective treatment for HIV infection has existed since early in the epidemic, the natural

course of HIV disease progression has been poorly documented. Most of the studies were conducted in developed countries. We have limited information from the resource-limited settings. Some of the earlier studies reported faster disease progression in African patients than in patients from the developed world. [140 141] However, recent evidence suggests that no difference exist. [41] The apparently faster disease rate among Africans could be due to late presentation to the health service, higher rates of co morbid clinical conditions, and lack of prophylaxis for opportunistic infections. The difference in disease classification could be additional factor.

In Paper I, we presented the pattern of short-term disease progression among untreated patients. The short follow-up and lack of information about the date of HIV infection limited us from describing the full picture of the natural history of HIV infection. However, we identified short-term prognostic markers that are of practical importance in resource-limited settings.

The WHO staging system, which has clinical and laboratory components, has rarely been used in routine clinical practice in Ethiopia. It has been in use in selected research projects in the country including a validation study which described the clinical usefulness of the clinical axis of the WHO staging system. [142] In their cross-sectional validation study, which included both clinical cohorts and participants from a cohort of factory workers, Kassa et al demonstrated that the WHO clinical staging correlated positively with the CD4 count, and negatively with the plasma viral load. Moreover, they found that the hospital cohorts were different from the factory cohorts, the former group presenting with advanced disease, as is in our study (Paper I). Our study further showed the prognostic value of the WHO clinical staging under routine clinical care conditions in a prospective cohort of patients.

Moreover, our findings suggest that some stage-defining events such as the oral thrush and diarrhoea can be useful in further stratification of patients for clinical decision-making. Some modifications in the WHO staging system were suggested by some authors. [143 144] Lifson et al, for example, suggested addition of oral thrush, pulmonary tuberculosis and chronic oral or genital ulcer, and substitution of BMI for weight loss as Stage IV-defining diseases. [144] Their suggestion was based on findings from a cohort of Rwandan women who were followed for four years. In their study, the WHO stage III and IV were indistinguishable after 12 months of follow-up, but provided clear distinction during the first year. Since ART is now widely available, pre-ART follow-up is likely to be much shorter than 1 year in symptomatic patients, perhaps even shorter than the 19 weeks' of follow-up in our cohort. In the most recent version of the WHO staging system, oral thrush is categorized under stage III. [47]

The main purpose of staging in clinical practice is to time the initiation, changing, or stopping of chemoprophylaxis and antiretroviral therapy. In resource-limited settings, this is an important issue. Mekonnen et al described a set of simple markers (TLC, HGB, and BMI) as important prognostic markers that can be used in timing of treatment beginning. [145] They did this study among a cohort of HIV infected factory workers who were likely to be in less advanced clinical stage than our patients (Paper I). [142] We also used slightly different cut-off points for haemoglobin and TLC. In both studies, however, the direction of association between these markers and mortality was the same, i.e., low TLC, anaemia, and low BMI predicted death, suggesting their potential utility in deciding when to start or stop treatment and prophylaxis.

Like the mortality, tuberculosis incidence rate was high at 9.9 per 100 PYO; twice as high as in HIV positive factory workers in Ethiopia. [145] In Cape Town, tuberculosis incidence rate was 10.4 per 100 PYO in HIV positive adults, and being in WHO stage III and IV was a strong risk factor. [146] The non-significant association between disease stage and tuberculosis in our study could partly be because of the smaller sample size. The striking finding in our study was the significant association between easy fatigability and fever, and tuberculosis. Undiagnosed tuberculosis could be a possible explanation, suggesting the need for improved diagnostics. The high tuberculosis incidence rate also implies the need for some prophylactic measures.

Effect of HAART on mortality and tuberculosis incidence rate

Most early publications on the effectiveness of HAART originated from industrialized countries and the comparisons were population based.[147-151] Moreover, some were based on selected group of patients, making the generalizability to other group of patients difficult. The reduction in mortality also varied remarkably, ranging from 20.7% in Brazil to 79% in Australia .[150 151]

Several factors may result in variations in population based reductions in AIDS-related mortality and morbidity. These include, but perhaps not limited to, variation in study design, recruitment criteria, years analyzed, extent of HAART use and adjunctive medical treatments. Despite the difference in the magnitude of change in mortality and morbidity, the direction of change was consistent in all these studies, i.e., reduction in death or illness.

Some studies have measured the direct effect of HAART. In patients with advanced HIV disease, for example, Pallela et al reported a 4.5 times higher risk of death in untreated

patients compared to patients who received a PI-containing antiretroviral therapy. [152] In Taiwan, use of HAART was associated with a 75% reduction in mortality in patients with a CD4 count less than 100/mcL. [153] In a more recent study from China, a 79% reduction in mortality was achieved for patients with CD4 count less than 200/mcL. [154]

A 65% reduction in mortality associated with HAART in our patients (Paper II) is consistent with findings from the developed world during the early years of treatment. A protease inhibitor-containing HAART reduced mortality by 64% in an Italian cohort .[155 156] Similarly, a multicentre HAART trial reported a 62% decrease in mortality among patients with advanced HIV. [156] A study from Kenya reported a very low mortality rate (5.4%), but 24.5% of their patients were lost to follow-up. [157] We had accurate information and little loss to follow-up because of the community agents and the prospective nature of the data. Therefore, ours is likely to represent the actual situation at a primary health care setting in Africa.

Most of the deaths in our patients occurred during the early weeks of treatment (Paper II). When compared with patients from high-income countries, patients from low-income countries were about four times more likely to die during the first months while on HAART, and the mortality rate was 147 per 1000 PYO. [139] The mortality rate of 15.4 per 100 PYO in HAART treated patients correlates well with this finding. Increased mortality in the early weeks of mortality appears to be associated with the advanced disease stage at presentation in resource-poor settings. Tuberculosis, drug side effects, and the immune reconstitution syndrome could be contributing causes. [158] Strategies aimed at early recognition and management of tuberculosis, drug side effects and immune reconstitution syndromes are likely to improve the high early mortality.

In South Africa, the tuberculosis incidence rates in the HAART and in the pre-HAART cohort were 2.4 and 9.7 per 100 PYO, respectively. Tuberculosis was more likely to occur among patients with advanced HIV disease suggesting the need for early initiation of antiretroviral therapy. [136] In our study (Paper II), tuberculosis incidence rates were 3.7 and 11.1 per 100 PYO in the treated and untreated patients, respectively. Tuberculosis incidence rate declined from 3.5 per 100 PYO in the first year to 1.01 per 100 PYO in the fifth year. [135] Higher risk of relapse was reported in patients with previous history of tuberculosis in a western African cohort. [159] Though this has not been confirmed in other studies, the authors suggested the use of secondary prophylaxis in patients with previous history of tuberculosis. The high early mortality possibly associated with tuberculosis and the high incidence rate of tuberculosis in patients treated with HAART highlights the need for coordinating tuberculosis control programs and HIV treatment efforts. [160-162]

Predictors of early mortality in treated patients

Compared to patients in high-income countries, patients in low-income countries have higher mortality rates especially during the first months of treatment. [137-139] In Paper III, we made a more detailed examination of factors associated with early death in Ethiopian patients.

Being in WHO clinical stage IV and having the TLC<=750/mcL at the beginning of treatment were the two most important predictors of death in this cohort. This highlights the need for starting treatment before patients progress to such advanced stages. Patients need to be identified early through improved counselling and testing strategies.

The TLC<=1200/mcL is often recommended as the cut-off point for beginning treatment. However, we found a much lower cut-off point, TLC<=750/mcL, as significant predictor of death. Similarly, HGB<=10g/dl and BMI<=18.5 kg/m2 were not significantly associated with increased death. This could be because of the small number of patients and events with TLC, HGB, and BMI above the stated cut-off points.

In some Asian and African populations including Ethiopians, the normal CD4 range is low and therefore the same TLC may not be used as a surrogate marker for CD4 count. [163-165] In Chinese patients, the TLC<=1200/mcL predicted the CD4<=100/mcL with better sensitivity and specificity than CD4 <=200/mcL. [166]

Although the availability of ARVs has improved during the last few years, lack of suitable monitoring approaches has continued to be a barrier to access. [81] The majority of patients residing in rural villages and smaller towns could not access these services. Even if the equipment is made available, lack of trained personnel and poor infrastructure such as frequent power failures represent major obstacles. [85]

Several approaches can be proposed to overcome this challenge. Inventing simplified, low-cost technology is one possibility. Some have suggested the use of dried whole blood spots to measure CD4 counts as alternatives to the standard flow cytometry technique which requires fresh blood samples. [167] A manual bead assay technique was also shown to correlate well with the flow cytometry technique. [168] Such initiatives should be viewed as long-term strategies, but they are still far from reach in the settings where they are most needed.

The more possible alternative seems the use of simple clinical and laboratory markers such as the TLC, HGB, body weight and BMI. Several authors both from low-income and high-income countries have demonstrated that these simple markers correlate fairly well with CD4 count, and thus could be used to monitor immunological response to ART. [166 169-172] However, most of these studies were done among untreated patients and utilized cross-sectional data. Future prospective studies should examine the utility of these simple markers in treated patients.

Weight loss continues to be a significant problem even among patients treated with HAART. [173] The aetiology of weight loss is complex and multifactorial. [174] The underlying reasons could be infectious diseases (example, tuberculosis), nutritional disorders, immune reconstitution inflammatory syndrome (IRIS), or drug side-effects. There is little information from resource-limited settings regarding weight loss in treated patients. In our study, weight loss was present in a third of patients who survived up to the fourth week of treatment. Future prospective studies should, therefore, define the magnitude, causes and consequences of weight loss among resource-poor patient populations. Such observational studies will provide useful data for planning possible interventions.

HIV testing and counselling among tuberculosis patients

Historically, tuberculosis and HIV/AIDS prevention and treatment have been approached differently. This made the TB/HIV collaborative efforts difficult. [175] Tuberculosis control programmes have used a public-health approach of case finding, name-based case notification, and at times contact tracing. The focus was on control of tuberculosis transmission and prevention of drug resistance. Little attention was paid to patient-centred goals such as reduction in death. [176] On the other hand, HIV/AIDS programmes have focused on individual approach to HIV testing which is private, confidential, and voluntary. [177]

In an effort to narrow the gap between the control programs for tuberculosis and HIV, the WHO now encourages closer collaboration between the two programs. [178] Routine counselling and testing of tuberculosis is a component of such an effort. With the improved access to antiretroviral therapy, there is a greater need to test more people for HIV. This cannot be achieved with the traditional VCT alone and alternative testing strategies are needed. [177 179]

The low acceptability rate of HIV testing and counselling in our study (Paper IV) shows part of the challenge we may be facing in counselling and testing as many people as who need care and treatment. Only a third of patients who needed counselling and testing utilized the service. Moreover, unemployed people who are likely to need financial support because of their HIV status were more likely to be tested. A delay in providing the service could occur at any stage of the counselling and testing process, turning more people away from getting the service as evidenced by much lower acceptability rate of testing among patients who had some kind of employment. Short counselling session followed by rapid HIV testing at the

DOTS clinic itself could have improved the acceptability rate. Whether such approaches would work requires further evaluation in the appropriate setting.

De Cock et al emphasized the importance of introducing routine diagnostic HIV testing in Africa: “If the benefits of antiretroviral treatment and prevention of opportunistic infection are to reach the people who need them, routine diagnostic testing will have to become standard practice in medical care. Routine HIV testing should initially be concentrated in general medical and tuberculosis patients, but in the long term, testing and provision of follow-up information should become a routine component of all health care interactions.” [177] Current evidence, however, shows that only about half of in-patients with HIV related illnesses are counselled and tested. [180]The situation could be worse in Ethiopia.

HIV is a life-long and potentially fatal condition for which there is no cure at the moment. However, the advent of the HAART changed the way people used to view HIV infection. HIV is now viewed as a chronic manageable condition like any other chronic disease such as diabetes. At the same time HIV is an infectious disease which is mainly transmitted through sexual route. It is an accepted practice that any medical information is confidential. But HIV is probably the only infectious disease which required counselling before and after testing. We are not arguing that HIV counselling should be abandoned. Our point is that it should be adapted to the changing environment of the disease itself.

In conclusion, the low acceptability of HIV counselling and testing among tuberculosis patients shows the need for alternative counselling and testing approaches. The current HIV counselling and testing strategies should be adapted to the changing environment of the

disease itself. More patients can be identified by routine asking of their previous history of testing.

5.3 Policy implications

Many patients need antiretroviral treatment in Ethiopia. This study evaluates the antiretroviral therapy programme that started at Arba Minch Hospital in 2003. We show that it is possible to achieve good treatment results within the existing health institutions. The treatment reduced deaths among AIDS patients by 65%. Over 90% of the patients followed the treatment. Few patients experienced serious side effects of the antiretroviral drugs.

Unfortunately, many patients presented themselves with advanced disease. We encourage telling the public so patients may start treatment earlier. This thesis provides information that is important in carrying out antiretroviral therapy in Ethiopia.

Many health centres and district hospitals in Ethiopia do not have the capacity to do CD4 tests. Patients should not be denied treatment because of lack of such laboratory facilities. We identified simple clinical and laboratory markers that can be used in the follow-up of patients in settings with limited laboratory setup.

The main challenge is whether this way of providing ART at district hospitals in Ethiopia is sustainable. The Ethiopian Health Policy focuses on thorough and integrated primary health care with emphasis on community based services. [181] In 2004, the Government of Ethiopia introduced the Health Service Extension Programme which intends to bring the health service close to people. According to the package, both health centres and district hospitals

should provide HIV care and treatment, including ART. [182] It is thus encouraging to note the Integrated Management of Adolescent and Adult Illness (IMAI) developed by the WHO, provides practical guidance on decentralised, integrated delivery of HIV care. [183] Ethiopia is now training many health workers on the use of the IMAI guideline. Through this approach, nurses and clinical officers shall provide ART to patients. Lay workers and patients will further have a greater role in care delivery.

The successful implementation of our project was a result of both local and international collaboration. The community agents were helpful not only for securing complete data for the investigation, but also for the day-to-day follow-up of patient care. They acted as a liaison between the community and the hospital. Patient groups, such as Tesfa Goh in Arba Minch, also played an important role in the care delivery. We advise the Ministry of Health to strengthen patient organisations and community workers in the ART delivery.

We found many new tuberculosis cases among patients receiving ART. Also, patients with tuberculosis often refused to be HIV tested. Therefore, tuberculosis control should be linked to HIV treatment and care. Patients with tuberculosis need to be told about ART, and HIV counselling and testing strategies need to be improved.

We were able to conduct this research in a remote part of the country. Such research should be viewed as an opportunity for strengthening research capacity at the newly established universities in South Ethiopia. The sustainability of such efforts needs both enabling policy environment and resources.

6 CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. We found high mortality and high tuberculosis incidence rates among untreated HIV infected patients in south Ethiopia. Simple clinical and laboratory markers predicted both mortality and tuberculosis occurrence in this cohort.

2. HAART improved survival and decreased tuberculosis incidence to a level similar to that achieved in the developed countries during the early years of HAART. Most of the deaths occurred during the early weeks of treatment.

3. The baseline WHO clinical stage, change in body weight and change in total lymphocyte count predicted mortality in treated patients in a complementary way and therefore can be used in the follow-up of patients in resource-limited settings.

4. The acceptability of HIV counselling and testing was low among tuberculosis patients in Arba Minch. This poses an important challenge to the scale-up of TB/HIV collaborative efforts, and highlights the need for alternative counselling and testing strategies.

Recommendations

For clinical practice:

1. In settings with limited capacity to do CD4 tests, patients with advanced HIV infection, particularly those with oral thrush, diarrhoea and low total lymphocyte

count should be treated promptly. Patients with baseline history of prolonged fever and easy fatigability should be followed more closely for tuberculosis.

2. In order to prevent the many deaths that occur during the early weeks of antiretroviral therapy, health workers should plan for more frequent contact with patients during the early phase of treatment. Patients should also be informed about the need for early diagnosis of HIV infection and associated illnesses.

3. Documenting the WHO stage and body weight should be viewed as important component of the routine clinical care for patients on antiretroviral therapy.

4. Health workers in the TB/HIV treatment units should be prepared to practice HIV counselling and testing as routine part of their work.

For research:

1. Since tuberculosis is a common problem even among patients receiving HAART, the feasibility, acceptability and effectiveness of INH preventive therapy should be evaluated in patients treated with HAART.

2. Factors that lead to increased mortality during the early weeks of treatment should be identified and interventions should be planned for. Particular attention should be paid to infectious and potentially treatable or preventable causes such as tuberculosis, drug toxicities and nutritional disorders.

3. The mechanism and magnitude of weight loss among patients treated with HAART needs further research.

4. Whether our model of care would work in other settings (example, health centres) under the leadership of primary health care workers needs further research.

For policy:

1. Simple clinical and laboratory markers should be incorporated in the revision of antiretroviral treatment guidelines.

2. Our model of integrating research into the routines of the primary health care settings should be encouraged. The sustainability of such models requires strengthening the existing links and developing new partnerships between the academia and disease control/treatment efforts.

3. The Ethiopian HIV counselling and testing policy should be adapted to the changing environment of HIV treatment and care.

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7.1 Papers I-IV