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Antiretroviral Treatment at Ethiopian Health Centers

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Antiretroviral Treatment at Ethiopian Health Centers

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TRANSLATIONAL MEDICINE | FACULTY OF MEDICINE | LUND UNIVERSITY



Antiretroviral Treatment at Ethiopian Health Centers

Anton Reepalu



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DOCTORAL DISSERTATION

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<p>Abstract</p> <p>In 2017, for the first time, more than half of all people living with HIV (PLHIV) had access to antiretroviral treatment (ART). This expansion of ART has in part been achieved through decentralization of HIV care in resource-limited settings. There, many PLHIV now receive care at ART clinics integrated within the primary health care system. At these clinics, non-physician clinicians are responsible for all aspects of care. Concomitant tuberculosis (TB) is common among PLHIV in many high-burden countries. Therefore, clinicians at these clinics must be able to diagnose and treat concomitant TB in parallel with ART in order to achieve satisfactory outcome of care. Failing ART results in high mortality and risk of drug resistance development that can be detrimental both for the individual and for the community at large.</p> <p>The first three papers in this thesis explores aspects of ART outcome among patients receiving care at Ethiopian public health centers. In particular, the impact of concomitant TB on ART outcome is investigated. For this purpose, 812 ART-naïve adults eligible to start ART (CD4 count <350 cells/mm³ or WHO stage 4) were prospectively recruited and followed for up to four years after ART initiation. At study inclusion, all participants were investigated for concomitant TB by active case-finding. We found that concomitant TB, present in nearly 1/5 study participants, did not negatively impact short-term virological suppression (paper I), risk of short-term mortality (paper II), or long-term virological, clinical, or immunological outcome of ART (paper III). However, we did find men to have an increased risk of poor virological outcome of ART both in short- and long-term, and that they had an increased risk of becoming lost to follow-up. Additionally, subjects with malnutrition had an elevated risk of mortality both soon after study enrollment, and during the long-term follow-up. Furthermore, these individuals had high risk of poor long-term virological outcome of ART.</p> <p>In the fourth paper, a clinical algorithm for targeted viral load testing was constructed. Through the use of three simple criteria (current CD4 count, mid-upper arm circumference, and adherence to ART) subjects with high risk of virological failure 12 months after ART initiation could be identified. If the performance is validated, this algorithm could be used to guide virological monitoring of ART more efficiently in areas where viral load resources are limited.</p> <p>In conclusion, we found that most PLHIV – regardless of concomitant TB – have good outcome of ART at Ethiopian public health centers during up to four years of follow-up. These findings demonstrate the feasibility of health center-based ART in settings where concomitant TB is common. In addition, we identified factors associated with poor outcome of care. If these individuals are identified in time, outcome of care might be improved and drug resistance development prevented. Finally, we constructed an algorithm for targeted viral load testing.</p>		
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Antiretroviral Treatment at Ethiopian Health Centers

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For Stina, Emilia, and Mattis

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Sammanfattning på svenska

I början av 1980-talet insjuknade unga män i USA i infektioner som tidigare bara drabbade personer med kraftigt nedsatt immunförsvar. Inom några år lyckades man identifiera det humana immunbristsviruset (HIV) som orsakade den epidemi av förvärvat immunbristsyndrom (AIDS) som nu orsakade sjukdom och död runt om i världen. I mitten på 1990-talet introduceras effektiv kombinationsbehandling mot HIV, antiretroviral terapi (ART). Från att ha inväntat döden i AIDS, kunde de som fick tillgång till behandling nu förvänta sig att kunna leva nästan lika länge som de som inte blivit smittade av HIV. Dessvärre var det till en början bara personer som levde i de rikare delarna av världen som fick tillgång till behandling. I de delar som var svårast drabbade, framför allt Afrika söder om Sahara, fortsatte viruset att sprida sig och var kring millennieskiftet den ledande dödsorsaken i Afrika. Parallellt med den okontrollerade spridningen av HIV, skedde också en kraftig ökning av tuberkulos vilket ytterligare förvärrade situationen i drabbade länder. HIV och tuberkulos uppvisade en förödande synergism. HIV försvagar immunförsvaret hos infekterade individer vilket gör de mer benägna att utveckla tuberkulos. Tuberkulos å sin sida, driver på immunförsvaret på ett sätt så att HIV fortare leder till AIDS.

I början av 2000-talet började äntligen ART bli tillgängligt i de svårt drabbade låginkomstländerna. Eftersom behandlingen var komplicerad och dyr, fanns ART bara tillgänglig på en del sjukhus och specialiserade HIV-kliniker. Men för att kunna nå ut med behandling till fler insåg man snart att man måste förändra sättet man erbjöd HIV-vård. Därför påbörjades en omfattande decentralisering av ART till mer perifera kliniker och ansvaret för behandlingen gick från läkare till sjuksköterskor. Tidiga utvärderingar visade att man kunde uppnå likvärdiga behandlingsresultat och att man genom förbättrad tillgänglighet kunde behålla fler patienter i vården. Trots att man lyckades nå ut till fler än tidigare, var det fortfarande många som inte hade tillgång till behandling. Decentraliseringen fortsatte därför och man började integrera ART inom befintlig primärvård. Där blev det nu sjuksköterskors uppgift att diagnosticera, inleda behandling och följa upp HIV-patienter. Förutom HIV-patienter, ska sjuksköterskorna fortsätta att självständigt handlägga allt annat som faller inom primärvården i sådana länder såsom mödra- och barnhälsovård, förlossningar och trauma, infektioner som lunginflammation och diarré, samt tuberkulos.

Tuberkulos har visat sig vara en svår utmaning för kliniker som tar hand om HIV-patienter. Förutom att det är väldigt svårt att diagnosticera tuberkulos hos individer med försvagat immunförsvar, så måste man ofta genomföra tuberkulosbehandlingen parallellt med ART. Det finns stor risk att behandlingen kompliceras av det så kallade immunrekonstitutionssyndromet (IRIS), som kan vara en kraftig inflammatorisk reaktion som uppstår när immunförsvaret börjar återhämta sig efter att behandling med ART inletts. Det finns också flera besvärliga läkemedelsinteraktioner mellan antiretrovirala läkemedel och tuberkulosläkemedel. Ifall man inte lyckas uppnå en effektiv behandling mot både HIV och tuberkulos finns det stor risk för att läkemedelsresistens uppstår. Förutom att den drabbade individen löper stor risk att dö, innebär uppkomst och eventuell vidare spridning av resistent patogener en fara för samhället i stort.

Vi har studerat hur ART vid offentliga hälsocentraler i ett låginkomstland fungerar, både på kort och på lång sikt. Eftersom tuberkulos är vanligt i många länder drabbade av HIV, har vi särskilt studerat hur samtidig tuberkulos påverkar utfallet av ART.

För att studera detta rekryterades drygt 800 HIV-positiva individer vid fem etiopiska hälsocentraler. Studiedeltagarna skulle uppfylla kriterier för att påbörja ART, men inte redan ha startat sin behandling. I samband med att personerna gick med i vår studie undersöktes samtliga också med avseende på samtidig tuberkulos, vilket nästan var femte diagnosticerades med.

I den första delstudien såg vi att de med samtidig tuberkulos hade likvärdigt svar på ART efter 6 månaders behandling. Vi kunde också se att en relativt stor andel svarade bra på behandlingen, men att män hade större risk för behandlingssvikt.

I den andra delstudien visar vi att många av de som dör snart från det att de knutits till en hälsocentral inte har hunnit påbörja ART. Eftersom ART i många fall kan vara livräddande, är det viktigt att tidigt kunna identifiera personer med hög risk för sådan tidig död. I den justerade analysen sågs inte någon ökad risk för de med samtidig tuberkulos, men personer med nedsatt arbetsförmåga och undernärda hade en ökad risk.

Den tredje delstudien utgörs av långtidsuppföljningen – upp till fyra år efter påbörjad ART. Återigen kunde vi visa att de med samtidig tuberkulos inte svarar sämre på ART. Däremot kunde vi se att nästan var tionde studiedeltagare avlidit och lika många försvunnit från hälsocentralerna under studiens gång. Mer än var femte individ hade dessutom minst ett tillfälle med virologisk svikt under sin ART.

Slutligen konstruerade vi i den fjärde delstudien en klinisk algoritm som ska kunna användas för att identifiera personer med hög risk för virologisk svikt ett år efter påbörjad ART. Eftersom det fortsatt råder stor brist på resurser för att kontrollera

att alla som får ART verkligen lyckats uppnå virologisk suppression, skulle en sådan algoritm kunna användas för att rikta provtagningen och därmed använda befintliga resurser mer effektivt. För att säkerställa att algoritmen presterar lika bra i verkligheten som den gjorde på vårt studiematerial måste den utvärderas på en annan grupp människor innan man kan överväga att implementera den i vården.

Sammanfattningsvis så har vi visat att man klarar av att hantera samtidig tuberkulos hos personer med HIV vid hälsocentraler och att de svarar lika bra på ART som de utan tuberkulos. Det är betryggande resultat eftersom fortsatt decentralisering av ART är ett måste ifall man ska uppnå målet med ART till alla människor som lever med HIV i låginkomstländer. Vi kunde också visa att inte alla svarar bra på ART och vi identifierade en rad faktorer som var associerade med sämre behandlingsutfall. Genom att lära sig mer om vilka personer med HIV som svarar bra på ART vid hälsocentraler så kan man anpassa hur pass intensivt dessa behöver följas under behandlingens gång. För de personer som sköter sin ART allra bäst, kan man troligen överväga att ytterligare decentralisera den uppföljande vården från hälsocentraler till så kallade *health posts*. Detta skulle då kunna frigöra resurser åt de som är allra sjukast eller löper stor risk att svikta på sin behandling. På samma sätt är det också tydligt att de allra sjukaste kanske borde hänvisas till en högre sjukvårdsinstans där man har mer resurser att undersöka och intensivt behandla HIV, tuberkulos och andra opportunistiska infektioner som är vanliga bland dessa individer.

Original papers

- I. Reepalu A, Balcha TT, Skogmar S, Jemal ZH, Sturegård E, Medstrand P, Björkman P. **High rates of virological suppression in a cohort of human immunodeficiency virus-positive adults receiving antiretroviral therapy in Ethiopian health centers irrespective of concomitant tuberculosis.** *Open Forum Infectious Diseases* 2014 Jun 19; 1(1): ofu039
- II. Reepalu A, Balcha TT, Skogmar S, Güner N, Sturegård E, Björkman P. **Factors Associated with Early Mortality in HIV-Positive Men and Women Investigated for Tuberculosis at Ethiopian Health Centers.** *PLoS ONE* 2016 11(6): e0156602
- III. Reepalu A, Balcha TT, Sturegård E, Medstrand P, Björkman P. **Long-term outcome of antiretroviral treatment in patients with and without concomitant tuberculosis receiving health center-based care – results from a prospective cohort study.** (under review)
- IV. Reepalu A, Balcha TT, Skogmar S, Isberg P-E, Medstrand P, Björkman P. **Development of an algorithm for determination of the likelihood of virological failure in HIV-positive adults receiving antiretroviral therapy in decentralized care.** *Global Health Action* 2017; 10: 1371961

Related papers

- Balcha TT, Sturegård E, Winqvist N, Skogmar S, Reepalu A, Jemal ZH, Tibesso G, Schön T, Björkman P. **Intensified Tuberculosis Case-Finding in HIV-Positive Adults Managed at Ethiopian Health Centers: Diagnostic Yield of Xpert MTB/RIF Compared with Smear Microscopy and Liquid Culture.** *PLoS ONE* 2014 Jan 22; 9(1): e85478
- Reepalu A, Balcha TT, Yitbarek T, Jarso G, Sturegård E, Björkman P. **Low prevalence of cryptococcal antigenemia in HIV-positive adults eligible for antiretroviral therapy (ART) in an Ethiopian regional hospital.** *BMC Research Notes* 2015; 8:702
- Wilhelmson S, Reepalu A, Balcha TT, Jarso G, Björkman P. **Retention in care among HIV-positive patients initiating second-line antiretroviral therapy: a retrospective study from an Ethiopian public hospital clinic.** *Global Health Action* 2016; 9: 29943
- Balcha TT, Skogmar S, Sturegård E, Schön T, Winqvist N, Reepalu A, Jemal ZH, Tibesso G, Björk J, Björkman P. **A Clinical Scoring Algorithm for Determination of the Risk of Tuberculosis in HIV-Infected Adults: A Cohort Study Performed at Ethiopian Health Centers.** *Open Forum Infectious Diseases* 2014 Oct 10; 1(3): ofu095

Abbreviations

AIDS	acquired immunodeficiency syndrome
ART	antiretroviral treatment
BMI	body mass index
CD4 cell	CD4 positive T cell
CDC	Centers for Disease Control and Prevention
CI	confidence interval
CPS	Clinical Predictor Score
DBS	dried blood spot
GALT	gut-associated lymphoid tissue
HIC	high-income countries
HIV	human immunodeficiency virus
INSTI	integrase strand transfer inhibitor
IQR	interquartile range
IRIS	immune reconstitution inflammatory syndrome
KPS	Karnofsky performance status
LIC	low-income countries
LMIC	low- and middle-income countries
LTFU	loss to follow-up
MSF	Médecins Sans Frontières
Mtb	Mycobacterium tuberculosis
MUAC	mid-upper arm circumference
NNRTI	non-nucleoside reverse transcriptase inhibitor
NRTI	nucleos(t)ide reverse transcriptase inhibitor

OIs	opportunistic infections
PEPFAR	President's Emergency Plan for AIDS Relief
PI	protease inhibitor
PLHIV	people living with HIV
PMTCT	prevention of mother-to-child transmission of HIV
RLS	resource-limited setting
SIV	simian immunodeficiency virus
SSA	Sub-Saharan Africa
TB	tuberculosis
UNAIDS	The Joint United Nations Programme on HIV/AIDS
VL	viral load (HIV RNA quantification)
VLTC	Viral Load Testing Criteria
WHO	World Health Organization

Introduction

It is believed that the human immunodeficiency virus (HIV) originated in the forests of west Central Africa [1]. Sometime in early 20th century, a strain of the simian immunodeficiency virus (SIV) defied the species barrier and infected humans [2], probably while they were manipulating chimpanzee meat. The newly created virus would remain unnoticed until decades later. In early 1980's, cases of Kaposi's sarcoma and a range of infections known to occur only in persons with severe immune suppression began to occur among young men in the USA [3,4]. Before long, it was obvious that this constituted a new disease syndrome, and the acquired immunodeficiency syndrome (AIDS) was recognized in 1982 [5]. By the time the causative agent was identified a few years later [6,7], patients with AIDS were reported from countries all over the world [8–10]. By the mid-1990's combination therapy based on several drugs targeting critical steps in the viral life cycle was introduced in Europe and the USA. Antiretroviral therapy (ART) transformed HIV from a deadly disease into a chronic infection. Shortly thereafter, decline in AIDS-related mortality was evident and the horrible saga of HIV as a deadly epidemic out of control had come to an end, at least in wealthy Western countries.

Whereas ART saved thousands of lives in rich parts of the world from the mid-90's and onwards, these new drugs were out of reach for millions of HIV infected in resource-limited settings (RLS), and in Sub-Saharan Africa (SSA) in particular. In contrast to Western countries where HIV was largely confined to high-risk groups, several countries in SSA suffered from a generalized HIV epidemic. At the same time, incidence of tuberculosis (TB) drastically increased and the deadly liaison of HIV/TB coinfection further deteriorated the situation. By the turn of the millennium, HIV/AIDS was the number one killer of people in Sub-Saharan Africa, home to more than two-thirds of all people living with HIV (PLHIV) globally.

Through efforts by international organizations and funders in co-operation with national governments, ART finally became available in the first years of the 21st century. Initial treatment programs were of small scale with treatment available to few of those in desperate need. This would change as an unprecedented scale-up of ART in RLS followed. Through expansion and decentralization of care away from central hospitals and HIV clinics, ART was made more accessible in high-

burden countries. By 2017, almost 20 million people in the world have access to ART – an impressive achievement. Yet, almost as many PLHIV still do not have access to treatment. Through further decentralization, treatment coverage can hopefully continue to improve, but many HIV programs already struggle to keep patients in care. Mortality remains high, in particular among individuals presenting to care with advanced disease. Coinfection with TB is common, complicates treatment, and constitutes the most common cause of death among PLHIV in SSA. Prevalence of both transmitted and acquired antiretroviral drug resistance is increasing, and has already reached high levels in several countries [11]. Furthermore, funding for HIV is decreasing, despite the call from the World Health Organization (WHO) that now is the time strengthen the grip on HIV/AIDS in order to eliminate it as a global threat. In all, this shows that the fight against HIV/AIDS is far from over.

This thesis explores aspects of decentralized ART, in a setting where coinfection with TB is common. More than 800 PLHIV receiving care at five Ethiopian public health centers were recruited, and followed for up to four years after starting ART. Through active case-finding before ART initiation, the impact of concomitant TB on ART outcome has been investigated. In particular, short-term virological outcome, early mortality both before and after starting ART, and long-term virological, clinical, and immunological outcome of ART in these subjects were investigated. Additionally, an algorithm for virological monitoring of ART was constructed.

Human Immunodeficiency Virus (HIV)

The virus and its replication

HIV is a member of the lentivirus genus of the *Retroviridae* family. The lentivirus genus contains another human lentivirus, HIV type 2. Compared with HIV type 1 (referred to as HIV throughout this thesis), HIV type 2 is a less virulent virus that is largely confined to West Africa [12,13]. Other known human pathogenic retroviruses are the Human T-lymphotropic viruses 1 and 2 (HTLV-1 and HTLV-2), with HTLV-1 conclusively associated with hematological and neurological disorders [14].

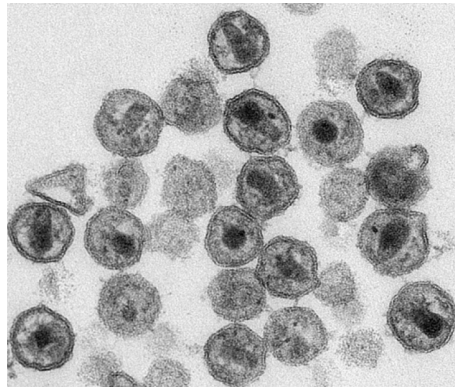


Figure 1.
Transmission electron microscopic image of numerous HIV virions. Credit: CDC/ Maureen Metcalfe, Tom Hodge

An HIV virion has a spherical shape with a lipid bilayer membrane that surrounds the core containing genomic RNA molecules and enzymes required for the initial stages of replication (Figure 1). These include the reverse transcriptase, integrase, and protease [15]. HIV infects human cells by attaching to the cell surface via an interaction between viral envelope glycoproteins and certain cellular receptors. The primary receptor for HIV is CD4, which is found on the surface of T helper lymphocytes, macrophages, and dendritic cells, the main target cells for HIV [16]. Following binding to the CD4 receptor, binding to co-receptors, most commonly the chemokine receptors CCR5 or CXCR4 [17], initiate fusion of viral and cellular

membranes and the viral core is released into the cytoplasm of the cell. The viral reverse transcriptase then transcribes the viral RNA into double-stranded DNA. Since the reverse transcriptase is error-prone and lacks proofreading activity, genetic mutations and recombination events are common at this stage [18,19]. The transcribed DNA is then integrated into the host chromosome by viral integrase. The integrated virus can remain quiescent for a long period of time. Yet, as infected cells are activated, viral transcription is initiated making use of the host cell transcription components. The viral protease then cleaves the transcribed polyproteins into new structural proteins and enzymes, an essential step in the maturation of new HIV virions that bud off from the infected cell (Figure 2) [20].

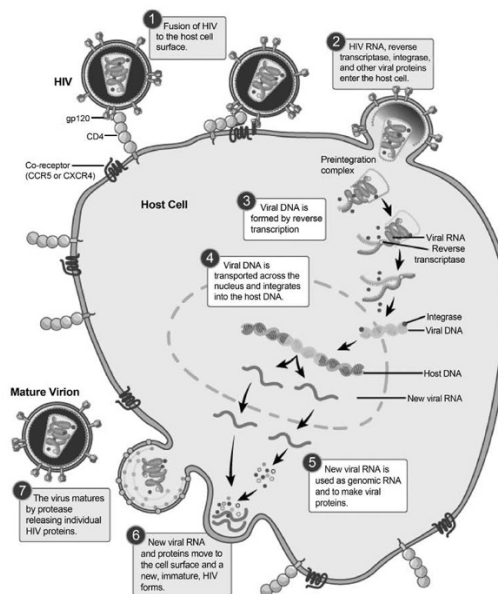


Figure 2.

HIV replication cycle involving a host cell within the human body, beginning with a virion attaching to the host cell wall, ending with a newly created virion budding off the cell wall. Credit: National Institute of Allergy and Infectious Diseases (NIAID).

As new HIV virions bud off the infected cell, further spread of HIV within the body of the infected individual occurs. Additionally, HIV can also infect new cells through direct cell-to-cell transfer [21].

Pathogenesis

Transmission of HIV from one individual to another can occur if body fluids that harbor HIV (blood, semen, pre-seminal fluid, rectal mucous, vaginal fluids, and breast milk) are exposed to mucosal surfaces or enter the body of another individual [22,23]. Common modes of transmission are unprotected vaginal or anal intercourse, mother-to-child transmission (during pregnancy, at childbirth, or while breastfeeding), contaminated blood transfusions, and sharing of injecting equipment [24].

Immediately after exposure and transmission, HIV replicates in the mucosa and draining lymphoid tissues. As the infection progresses, rapid expansion of HIV in gut-associated lymphoid tissue (GALT) occurs, followed by systemic dissemination [23]. As HIV disseminates, HIV DNA is integrated into resting CD4⁺ T cells (CD4 cells) and HIV latency is established.

The acute phase of HIV infection is characterized by a high degree of viral replication and viremia. At the same time, there is a rapid and profound depletion of CD4 cells in extra-lymphoid effector sites, especially the intestinal mucosa [25]. As host immune responses are triggered, the level of viremia declines and stabilizes at a lower level compared with the acute phase. This so-called 'set-point' differs between individuals and is often correlated with subsequent prognosis and duration of the chronic phase of HIV [26]. During the chronic phase, there is a gradual loss of CD4 cells, which is mainly mediated through chronic persistent immune activation. This immune activation varies over time and is caused both by HIV in itself, but also by mechanisms of microbial translocation over the gut mucosa and by reactivation of other chronic viral infections such as cytomegalovirus and Epstein-Barr virus [27,28]. If left untreated, the cellular immune system of the HIV-infected individual will gradually be depleted, leading to the final phase of HIV – AIDS. In this final stage of acquired cellular immunodeficiency the body cannot protect itself against a range of opportunistic infections and tumors, nor with HIV itself which replicates unhindered. Without effective treatment, death ensues shortly thereafter.

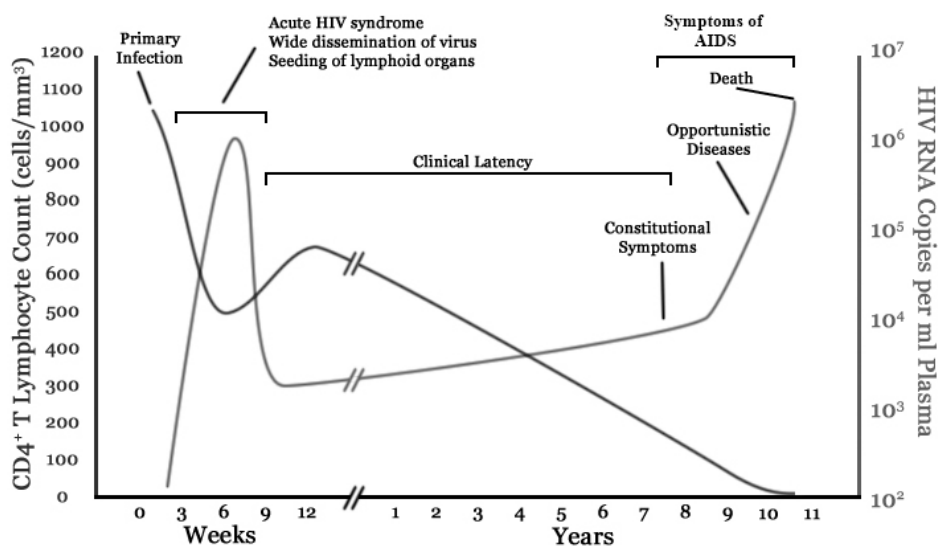


Figure 3.
The natural course of HIV infection.
(https://commons.wikimedia.org/wiki/Category:HIV_time_course#/media/File:Hiv-timecourse.png)

HIV/AIDS – clinical presentation

Individuals infected with HIV can experience flu-like symptoms within days to weeks after initial exposure [29]. The most common signs and symptoms include fever, fatigue, lymphadenopathy, rash, and myalgia. Previously considered to commonly occur in acute HIV infection [29], more recent studies indicate that only around 50% of individuals experience such symptoms [30]. Additionally, these symptoms are far from pathognomonic for HIV, and usually dissipate within two weeks.

The duration of the subsequent asymptomatic stage of HIV is on average 10 years [31,32], yet with large inter-individual difference with both rapid progressors [33], and elite controllers [34].

Infection with HIV, over time leads to an increasingly weakened immune system (Figure 3). The extent of this immunosuppression is usually estimated through analysis of CD4 cells in peripheral blood, expressed either as number of CD4 cells/mm³ (CD4 cell count) or as percentage of total lymphocytes (CD4%).

As immunosuppression advances, infections caused by organisms of low pathogenicity, so called opportunistic infections (OIs), and certain malignancies occur more frequently and more severe. At CD4 counts below 200 cells/mm³ all

HIV-related infections and malignancies escalate in frequency, yet some AIDS-defining illnesses have been shown to occur more frequently in individuals with CD4 counts up to 750 cells/mm³, compared with those with higher CD4 counts [35]. The risk of some manifestations, such as thrombocytopenia, herpes zoster, and even TB, is elevated compared with HIV-negative even before CD4 starts to decline [36–38].

Two major classification systems for staging of HIV disease are currently in use. The US Centers for Disease Control and Prevention (CDC) definition, intended for public health surveillance and not as a guide for clinical diagnosis, is based on current CD4 count and on certain HIV-related conditions (category A-C) [39]. The WHO Clinical Staging and Disease Classification System was created for use in resource-constrained settings without access to CD4 cell count measurements [40]. In this system, the clinical stages are categorized from 1 through 4, progressing from asymptomatic infection to advanced HIV/AIDS (Table 1). Although the WHO system was intended for use in resource-constrained settings, many of the included diagnoses can only be based on clinical suspicion in such settings since the sometimes-required sophisticated diagnostic methods for confirmation are usually not available.

Table 1. The 2007 WHO Clinical Staging and Disease Classification System

Stage 1	Asymptomatic Persistent generalized lymphadenopathy
Stage 2	Moderate unexplained weight loss (<10%) Recurrent respiratory tract infections Herpes zoster Angular cheilitis Recurrent oral ulceration Papular pruritic eruption Fungal nail infections Seborrhoeic dermatitis
Stage 3	Unexplained severe weight loss (>10%) Unexplained chronic diarrhea (>1 month) Unexplained persistent fever (>1 month) Persistent oral candidiasis Oral hairy leukoplakia Pulmonary tuberculosis Severe bacterial infections (such as pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteremia) Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis Unexplained anemia, neutropenia and/or chronic thrombocytopenia
Stage 4	HIV wasting syndrome Pneumocystis pneumonia Recurrent severe bacterial pneumonia Chronic herpes simplex infection Esophageal candidiasis Extrapulmonary tuberculosis Kaposi's sarcoma Cytomegalovirus infection Central nervous system toxoplasmosis HIV encephalopathy Extrapulmonary cryptococcosis Disseminated nontuberculous mycobacterial infection Progressive multifocal leukoencephalopathy Chronic cryptosporidiosis Chronic isosporiasis Disseminated mycosis Lymphoma (cerebral or B-cell non-Hodgkin) Symptomatic HIV-associated nephropathy or cardiomyopathy Recurrent septicaemia Invasive cervical carcinoma Atypical disseminated leishmaniasis

Diagnosis of HIV-infection

The diagnosis of HIV is usually made by detection of HIV-specific antibodies. Serological testing can detect antibodies within 2-4 weeks after primary HIV infection [23]. Enzyme-linked immunosorbent assays (ELISA) is a common method for antibody detection in resource-rich settings. Fourth generation serological assays that can detect both HIV p24 surface antigen and antibodies have the potential to identify infected individuals earlier following infection since p24 antigen is detectable about one week before antibodies. In most RLS, rapid diagnostic tests form the mainstay diagnostic. These assays rely on detection of antibodies and are therefore not able to identify infected individuals as early as the combined antibody/antigen ELISA assays. The rapid tests do have high sensitivity

beyond the initial diagnostic window, but also a certain low level of false reactivity [41]. Testing algorithms using these as first-line assay should therefore include more specific second- or third-line assays to verify the diagnosis [42].

In case of suspected acute HIV infection, polymerase chain reaction (PCR) that detects HIV RNA in plasma can be an additional diagnostic tool. Although it further reduces the time from infection until a positive diagnostic test, there is still a period of 10-14 days, known as the eclipse period, before HIV RNA levels in plasma are high enough for detection using commercial PCR assays [43].

Antiretroviral treatment (ART)

Antiretroviral drugs

The replication cycle of HIV has been elucidated in great detail (Figure 2). This made it possible to construct molecules that specifically interfere with different steps in this cycle. Currently, 25 molecules are approved by the US Food and Drug Administration (FDA) for treatment of HIV infection. These drugs comprise six mechanistic classes: nucleos(t)ide reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI), integrase strand transfer inhibitors (INSTI), fusion inhibitors, and chemokine receptor (CCR5) antagonists.

During the natural course of HIV infection 10^{9-14} new virions are created each day. Since mutations and recombinations are common events during HIV replication a swarm of highly-related but genotypically different viral variants exist within an infected individual [44]. The clinical significance of this was evident shortly after treatment for HIV became available and replicating virus was exposed to antiretroviral drugs.

In 1987, the first antiretroviral drug was approved, zidovudine (AZT), belonging to the NRTI class. It had a dramatic effect on suppression of viral replication which resulted in both increased short-term survival and reduced frequency of OIs [45]. Unfortunately, it was soon obvious that monotherapy was not a sustainable treatment. HIV variants resistant to AZT rapidly emerged in treated patients [46]. In 1995, trials of dual NRTI therapy with the newly approved lamivudine (3TC) in combination with AZT showed promising results [47]. But the real paradigm shift occurred in 1996, as the NNRTI and PI classes were introduced and triple combination therapies showed durable and effective suppression of viral replication [48,49]. By interfering with different components of the HIV replication cycle the barrier for resistance development was raised greatly. In

2007, the first drug in the INSTI class was approved. By then all four classes (NRTI, NNRTI, PI, and INSTI) that comprise the components of currently recommended standard therapies were available.

Some antiretroviral drugs are associated with severe toxicities such as pancreatitis, hyperlactemia, and lipodystrophy (stavudine and didanosine), rhabdomyolysis (AZT), drug reaction with eosinophilia and systemic symptom (DRESS; nevirapine), and to some extent ischemic heart disease (abacavir and lopinavir) [50]. In addition, drug-drug interactions are common, making concomitant treatment for comorbidities and ART complicated, in particular if using ritonavir-boosted PIs or NNRTIs [51].

Despite the use of combination therapies, antiretroviral drug resistance can emerge, in particular if uninterrupted adherence to the medication is not achieved. The vulnerability to resistance differs between drug classes, and also between drugs within the same class. This vulnerability is determined by the intrinsic antiviral potency of the drug, together with the genetic barrier to resistance. The genetic barrier depends on the number of mutations necessary to confer resistance, and the ease or frequency at which they develop [52]. Cross-resistance within drug classes are common, while there is essentially none between drug classes [53]. Whereas INSTI and ritonavir-boosted PIs are considered less prone to resistance development, NRTIs and NNRTIs are more vulnerable.

Currently, the recommended 1st line ART regimens are based on a combination of two NRTIs with either a boosted PI, a NNRTI, or an INSTI [50,54]. These triple combination ART regimens have proven long-term efficacies and limited toxicities compared with earlier regimens [55,56]. Due to the high costs associated with several of these drugs, 1st line ART in RLS is usually limited to a combination of two NRTIs and one NNRTI, with PIs reserved for 2nd line treatment [57,58].

A problem with the widespread use of NNRTIs is the relatively low genetic barrier to resistance together with a plasma half-life of several weeks [59,60]. Indeed, the prevalence of resistance to NNRTI-based regimens has increased in RLS [61], reaching worryingly high levels in some settings [62,63].

When to start ART

Indications for when to start ART have changed as a result of less toxic regimens becoming available and of more data on benefits of starting ART early. These benefits include a reduction in HIV-associated inflammation [64], and improved immunological recovery [65,66]. More importantly, two large, randomized controlled trials demonstrated about a 50% reduction in death or serious HIV-

related illness among those with CD4 counts >500 cells/mm³ randomized to receive ART immediately versus later [67,68]. Additionally, the risk of HIV transmission is markedly decreased for subjects on effective ART compared with those not on treatment [69,70].

In 2006, WHO recommended ART for all with CD4 counts below 200 cells/mm³, in addition to subjects at advanced clinical stages [71]. In 2010, the CD4 count threshold was raised to 350 cells/mm³ or less [72], and in 2013 to 500 cells/mm³ or less [73]. Since 2015, WHO recommend ART initiation for all PLHIV regardless of CD4 count or clinical stage [74].

Once ART has been initiated, it should be maintained indefinitely. If treatment is interrupted, reactivated HIV DNA integrated in host cells will soon cause a rapid rise in plasma HIV RNA levels.

Treatment monitoring

Establishing and maintaining virological suppression during ART is key in achieving favorable outcomes, reducing the risk of drug resistance, and to limit HIV transmission.

There are currently three different methods to monitor treatment response: clinically, immunologically, and virologically. Correspondingly, WHO has three definitions for ART failure [57].

Virological failure is usually defined as two consecutive viral load (VL) determinations above 1000 copies/mL after at least 6 months of ART and with adherence support in between the two measurements. The threshold of 1000 copies/mL is used since the risk of resistance development and disease progression is low below this level [75,76]. Intermittent low-level viremia below this threshold (viral blips) can occur during effective treatment, but the clinical significance of such blips on treatment outcome remain uncertain [57,77–79]. Persistent low-level viremia, however, has been associated with accumulation of drug resistance mutations and virological failure, raising concerns whether the current threshold is too high [80,81].

Immunological failure is currently defined as a CD4 count persistently below 100 cells/mm³, or below 250 cells/mm³ following clinical failure [57]. For immunological failure, it is pertinent to rule out other concurrent or recent infections which can cause a transient decline in CD4 count [82].

Clinical failure is defined as a new or recurrent clinical event indicating severe immunodeficiency after at least 6 months ART. Events indicating severe immunodeficiency include all WHO clinical stage 4 conditions. Additionally,

certain stage 3 conditions are also considered as indicative of clinical failure, such as pulmonary TB and severe bacterial infections [57].

Both the clinical and immunological failure criteria are recognized as having low sensitivities and positive predictive values for identifying individuals with virological failure [83]. Furthermore, the delay before clinical and/or immunological failure is obvious can result in disease progression, the accumulation of drug resistance mutations, and HIV transmission [84–86]. Virological monitoring of ART is therefore strongly recommended as preferred monitoring approach to diagnose and confirm treatment failure [50,54,57].

Whereas virological monitoring has been used in HIC since ART became available [50,54,87], implementation of this method in RLS is hampered by the requirements for laboratory infrastructure, supplies, the need for skilled staff, cold-chains, and high costs [88,89]. To overcome the cold-chain requirement, VL can also be performed on dried blood spots (DBS), instead of on plasma. These DBS can be sent to centralized laboratories for batch testing [90]. This strategy has been in clinical use for early infant diagnosis for several years [91]. Whereas HIV RNA can be quantified in plasma at levels around 20-50 copies/mL on most VL assays currently in clinical use, the sensitivity is lower when performed on DBS [92]. Although still inferior compared with analysis on plasma, the sensitivity has been improved in recent years, and WHO included VL on DBS for ART monitoring in the 2016 ART guidelines [57,93]. This recommendation was conditional, due to low-quality evidence, and VL on DBS is only recommended in settings where logistical, infrastructural or operational barriers prevent VL monitoring using plasma specimens. Several point-of-care VL assays have been under development for some years [94]. Some, such as the Xpert HIV-1 Viral Load assay (Cepheid, Sunnyvale, CA), have become available and evaluations show promising results regarding its performance on clinical samples [95]. Such point-of-care assays do not, however, have sufficient throughput capacity to enable routine monitoring of all patients on ART in most RLS [95,96].

The timing and frequency of virological monitoring has been debated for several years [97]. In HIC, VL is usually performed quarterly and later biannually on all patients on ART [50,54]. WHO recommend routine VL at 6 and 12 months after starting ART, and annually thereafter if the patient is stable on treatment [57].

In settings with insufficient resources for routine testing, targeted VL testing has been suggested as an alternative approach. With this strategy, patients are categorized according to their individual risk of having failed treatment, thereby making more efficient use of existing VL resources. For such an approach to be meaningful, algorithms used to categorize patients must have high sensitivity and acceptable specificity in order to misclassify few individuals with virological failure while reducing the number needed to be tested. Such algorithms have been

constructed, both in SSA [98–102] and in Cambodia [103]. A clinical predictor score developed by Lynen et al. in Cambodia [103], did perform well in a validation in Cambodia [104], but sensitivity was low when validated in Uganda [98]. Its use of trends in laboratory data requiring calculations by the clinicians was also shown to be error-prone [105]. Validation of a predictor score developed in South Africa reported sensitivity of 65% in detecting patients with virological failure 6 months after ART initiation [106]. It is questionable whether an algorithm that misses one of three patients with failing treatment should be considered for clinical use. If so, and then only for settings with severely limited access to VL facilities, close clinical monitoring is needed for those not eligible for a VL. The need for better performing algorithms will remain until resources and infrastructural improvements enables routine virological testing of all PLHIV.

Although studies have reported both high levels of virological suppression in settings without access to virological monitoring [107], and similar rates of NNRTI resistance at 48 weeks when comparing sites without VL to those performing VL every 3-6 months [108], virological monitoring of ART remains the preferred monitoring approach. It can also serve as a powerful tool in adherence counselling [109,110], minimize the delay until failing ART is noticed (depending on monitoring strategy used), and be cost-saving through viral-load-informed differentiated care [85,111,112]. Importantly, virological confirmation of patients with clinical and/or immunological failure also prevents unnecessary switches to 2nd line ART. Subjects with virological suppression despite indications of failing treatment should instead be investigated and treated for concurrent OIs or other comorbidities. A minority of patients on ART also exhibit an immune-virological discordant response to treatment – a suboptimal immune recovery despite virological suppression. Such individuals do not benefit from switching ART, but should be monitored closely as they are at higher risk of both death and HIV-related illness [113,114].

A drawback of all three monitoring strategies (clinical, immunological and virological) is that the reason for treatment failure is not clear, i.e. whether drug resistance has developed or not. Without drug resistance testing, which is rarely available in RLS, the choice of alternative treatment regimen, for those with suspected drug resistant viruses, will have to be based on data from resistance surveillance and drug sequencing strategies [53,115]. It is not obvious, however, whether the best approach would be to allocate resources for large-scale drug resistance testing on clinical samples, or using more robust, but more expensive, 1st line regimens to tackle the growing problem of drug resistance [116–118].

Management of OIs and other HIV-associated conditions

Prophylactic treatment with co-trimoxazole leads to reduced incidence of a number of OIs such as *Pneumocystis jirovecii* pneumonia and toxoplasmosis [119]. Recent data also show the effectiveness of co-trimoxazole prophylaxis in reducing mortality, severe bacterial infections, and malaria in PLHIV [120]. Therefore, WHO recommend prophylactic treatment with co-trimoxazole to all PLHIV at advanced stages or with CD4 counts below 350 cells/mm³ [57].

Along with treatment directed at the opportunistic pathogen, if such treatment is available, prompt initiation of ART with subsequent immunologic recovery is an effective treatment for many OIs. However, the immunologic recovery after ART initiation can sometimes be overwhelming, resulting in the immune reconstitution inflammatory syndrome (IRIS) [121]. IRIS is characterized by a transient inflammatory response directed either against an opportunistic pathogen, or as an autoimmune disease [122]. First reported in 1992, it has subsequently been associated with several pathogens (cytomegalovirus, hepatitis B and C, *Cryptococcus neoformans*, and mycobacteria), with cancers (Kaposi's sarcoma and non-Hodgkin's lymphoma), and autoimmune diseases [123]. Whereas IRIS is often self-limiting, it can be severe and even life-threatening. In particular, IRIS in cryptococcal and TB meningitis is associated with high mortality [124–127]. For both these conditions, it is recommended to defer ART initiation for a period in order to reduce the risk of IRIS and IRIS-associated complications [57,128].

Today, many PLHIV have access to ART, and patients on stable and effective ART have a greatly reduced risk of OIs and AIDS-defining events. However, it has become clear that PLHIV, despite effective ART, have excess risk of several non-AIDS conditions, such as cancer, liver disease, renal disease, neurocognitive decline, and osteoporosis [129–131]. Although multiple factors are likely to contribute to this excess risk, such as comorbid conditions and toxicity from ART, chronic inflammation that persists despite virological suppression of HIV are considered by many to be an important factor [132]. Consequently, the disease panorama associated with HIV in settings where effective ART is available has shifted with causes of death no longer being limited to AIDS-related conditions [133]. This poses a new challenge for HIV treatment and care [129,134].

Keeping these conditions in mind, the most common cause of death in PLHIV in RLS is TB [135].

HIV/TB coinfection

TB is caused by *Mycobacterium tuberculosis* (Mtb), a human pathogen since thousands of years [136]. A marked decline in TB incidence occurred in industrialized countries in early 20th century. Improved living conditions with less crowding and improved general health and nutritional status are believed to be responsible for this decline in TB disease. Further reduction followed as the BCG vaccine became available [137], and by anti-mycobacterial agents being introduced in mid 20th century [138]. But in the final decades of the 20th century, TB again became common in settings where it almost had been forgotten [139]. This dramatic turn was due to several factors, in particular the emergence of HIV.

Whereas TB in industrialized countries re-emerged in subpopulations severely affected by the growing HIV epidemic, TB had never been successfully controlled in many RLS even before the advent of HIV. In such settings, in particularly in Southern Africa, TB incidence started to rise explosively as HIV prevalence increased (Figure 4) [140].

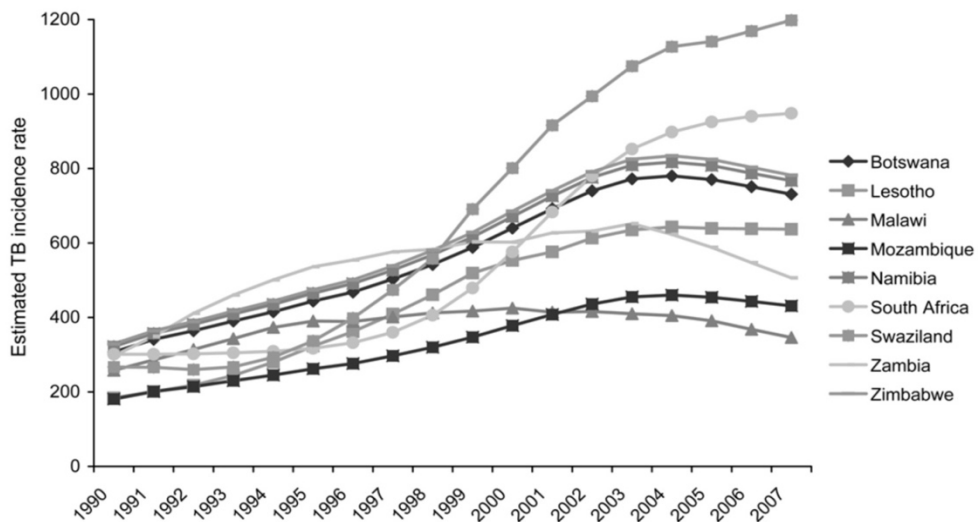


Figure 4. Increased incidence of TB in countries with high prevalence of HIV [141]. Reprinted with permission of the International Union Against Tuberculosis and Lung Disease. Copyright The Union.

In 2015, there were more than 10 million new TB cases globally, with nearly 2 million of them dying [135]. TB thereby surpassed HIV as a leading cause of death due to infectious diseases, second only to diarrheal diseases. Worldwide, it is estimated that 11% of new TB cases are coinfecting with HIV. But the burden of HIV/TB coinfection disproportionately affects SSA. In parts of Southern Africa >50% new TB cases are coinfecting with HIV [135].

Pathogenesis

Mtb is a human pathogen that exists in humans either in a latent, non-infectious state, or active and potentially infectious. Transmission occurs primarily by aerosol from an individual with active, pulmonary TB [142]. As Mtb enters a new host, innate immune defense can either clear the infection, or, with assistance from the adaptive immune defense and its CD4 cells in particular, contain Mtb in pulmonary granulomas [143]. The infection can be controlled in this latent stage indefinitely, but 5-10% of infected individuals will develop active disease with potential onward spread of TB [143]. Factors that increase the risk of developing active disease, include, but are not limited to: diabetes mellitus [144], immunosuppressive agents [145], malnutrition [146], tobacco smoking [147], and in particular HIV.

HIV infects and effectively depletes a part of the adaptive immune system, the CD4 cells, which are important in controlling TB. The risk of developing active TB is thereby markedly increased, with annual progression from latent to active disease in around 10% [148]. Additionally, active TB increases HIV replication which can accelerate the natural course of HIV infection [149,150]. Whereas the risk of developing TB increases gradually as immune depletion is exacerbated during the course of HIV infection, this risk has been found to be elevated already soon after infection. This was shown in a cohort of South African mine workers that had an increased risk of TB within one year after seroconversion [36]. Immune reconstitution through ART is associated with reduced risk of active TB, yet the risk remains elevated compared to HIV-negative persons despite effective ART [68,135,151,152]. In a study from South Africa, subjects on ART and with substantial immune recovery (median CD4 count of 821 cells/mm³) still had a more than 4-fold higher risk of TB compared with HIV-uninfected individuals living in the same community [153].

TB is predominantly a disease of the lung, but all organs can be affected, such as lymph nodes, pleura, bones/joints, and the central nervous system [154]. Besides increasing the risk of active TB, the immune suppression in HIV infection can impact the disease manifestations of TB. Especially in advanced HIV disease,

atypical manifestations are common. These individuals more often present with extrapulmonary and disseminated TB disease (miliary TB) [155]. A proportion of HIV/TB coinfecting individuals can also be asymptomatic, despite having active TB disease (subclinical TB) [156,157].

Although TB-related deaths among PLHIV have fallen by 32% since 2004, TB remains the leading cause of death among PLHIV [158]. This large burden of both recognized and unrecognized TB disease in deceased PLHIV in SSA has also been shown in several autopsy studies [124,159,160].

Diagnostic aspects

Identification and treatment of TB early in the course of TB disease leads to improved survival and reduced risk of transmission [161]. Therefore, WHO recommends screening for active TB in all PLHIV in TB-endemic areas using the WHO TB symptom screen [162]. This tool consists of a 4-question panel: current cough, fever, night sweats, and weight loss. If any symptom is recorded, further TB investigations are mandated. But the availability of further diagnostic tools highly depends on the setting.

In most RLS, sputum smear microscopy is the only additional tool available [135]. Unfortunately, this cheap and simple method has low sensitivity, especially in HIV-associated TB [163]. Consequently, a large proportion of TB diagnoses in HIV-positive subjects, is based on clinical suspicion without bacteriological confirmation [135].

Mycobacterial culture has high sensitivity and is required for drug susceptibility testing [156,164]. Whereas culture is routinely used in HIC for investigation of suspected active TB, this method is seldom available in RLS due to high costs and requirement for sophisticated laboratories with high level of biosafety [165].

In recent years, a new diagnostic tool has been introduced – the Xpert MTB/RIF (Cepheid, USA). This is a modular-based, automated PCR assay that can detect Mtb DNA in a variety of sample types [166]. It can also detect presence of mutations associated with rifampicin resistance, a marker for MDR TB. This technique has drawbacks such as relatively high costs, and the requirements for controlled ambient temperature and uninterrupted power supply. In addition, the Xpert MTB/RIF has lower sensitivity than culture [167]. Nonetheless, WHO endorse this assay for initial diagnosis of HIV-associated TB since 2010 [168].

Chest X-ray has a role as an adjunct method for TB diagnosis. Its usefulness in HIV-associated TB is limited due to low sensitivity and the frequent lack of classic radiographic TB signs, such as apical lung cavities, in this population [169,170].

The challenges of HIV-associated TB are not limited to the above mentioned unusual clinical manifestations of the disease making diagnosis difficult [171]. Treatment of concurrent HIV and TB can also be challenging due to overlapping side effects and drug toxicity, adherence issues and drug-drug interactions, especially between rifampicin and various antiretroviral drugs, and TB-IRIS.

Treatment of concomitant TB and HIV

Short-course treatment of drug susceptible TB usually consists of a 2-month intensive phase with rifampicin, isoniazid, pyrazinamide, and ethambutol (ethambutol can be omitted if drug susceptibility is confirmed), followed by a 4-month continuation phase with rifampicin and isoniazid [172]. Extended treatment duration is recommended for some forms of extrapulmonary TB. Treatment of HIV-associated TB follows the same principle, but is complicated by the need for concomitant treatment of HIV. Rifampicin, a core TB drug, is one of the most potent inducers of the cytochrome P450 (CYP) system in clinical use [173]. Three of the most important classes of HIV drugs are metabolized by the CYP3A4 hepatic isoenzyme: PIs, NNRTIs, and INSTIs [51].

Among available HIV drugs, efavirenz has been shown to be effective despite the drug-drug interaction with rifampicin [174]. This co-administration has been extensively studied, and although rifampicin does decrease levels of efavirenz, standard dosing of efavirenz at 600mg once daily is considered sufficient [51]. This does not apply for the other widely used NNRTI, nevirapine, which should not be co-administered with rifampicin. Profound decrease in PI concentrations, which can result in virological failure and resistance development, makes co-administration with rifampicin contraindicated. Finally, INSTI concentration is also affected by rifampicin, yet by doubling the dose of INSTI this effect could be overcome. Such co-administration has not been adequately studied however, and co-administration is not recommended if other choices are available [51].

An alternative to rifampicin is rifabutin, which is a less potent inducer of the CYP system. This enables co-administration of rifabutin and INSTI. Co-administration with PIs is possible, but ritonavir-mediated inhibition of CYP3A4 cause substantial increase in exposure to rifabutin and its active metabolite, increasing the risk of toxicity [51]. Additionally, rifabutin is seldom available in RLS due to high costs.

Consequently, the recommended ART for HIV/TB coinfectd is a combination of two NRTIs and efavirenz [57].

Despite the additional complexity of concomitant ART and TB treatment, survival is increased if ART is initiated during the course of TB treatment [175]. Shown in several randomized controlled trials, additional survival benefits can be achieved if ART is started within 8 weeks of TB treatment, especially in those with severe immunosuppression [176–178]. Therefore, current WHO recommendations state that TB treatment should be initiated first, followed by ART as soon as possible within the first 8 weeks of treatment [57]. Additionally, individuals with profound immunosuppression (CD4 counts less than 50 cells/mm³) should receive ART within the first two weeks of TB treatment [179]. Initiation of ART soon after start of TB treatment increases the risk of IRIS; however, the benefit of early ART outweigh this risk [57].

In addition to ART, prophylactic treatment with isoniazid for PLHIV found to have latent TB has been recommended by the WHO since 1998 [180]. Isoniazid preventive therapy has a protective effect on progression to active TB [181]. It is nevertheless crucial to rule out active TB before such preventive therapy can be considered. Failure to do so results in inadvertent isoniazid-monotherapy for active TB with subsequent treatment failure and high risk of isoniazid resistance development [182,183].

HIV care in resource-limited settings

Whereas HIV, within a decade after its discovery, was gradually transformed from an inevitably fatal disease into a chronic, manageable infection in HIC, the situation in RLS at the time continued to deteriorate. The high costs of ART and lack of health care infrastructure in many RLS were considered insurmountable obstacles for sustainable ART delivery by many stakeholders [184]. Competing health care needs such as diarrheal and childhood diseases, TB, and malaria further hindered the allocation of resources from already overstrained domestic budgets [185,186]. Consequently, by the year 2000, Africa was home to nearly 70% of adults and 80% of children living with HIV in the world, and to three-quarters of the more than 20 million who had died of AIDS since the epidemic began [187]. In 2002, HIV/AIDS was recognized as the leading killer of people in SSA.

The *Joint United Nations Programme on HIV/AIDS* (UNAIDS) was launched in 1996 to strengthen the way in which the UN was responding to the HIV pandemic. Through its efforts, the *Drug Access Initiative* was launched in 1997, providing ART to patients in Uganda and Cote d'Ivoire in early 1998 to pilot the concept of durable ART in Africa [188]. During these years, drug-related costs were substantially lowered as generic drugs were starting to be manufactured in several countries. In 2000, the *Accelerating Access Initiative* was launched and contributed to additional reduction in costs of 1st line ART through negotiations between pharmaceutical companies and international organizations such as UNAIDS, WHO, and the World Bank [189]. The *Global Fund to Fight AIDS, Tuberculosis and Malaria* was created in January 2002, and its decision to make generic drugs eligible for funding was an important milestone. In 2003, the US President George W. Bush announced the launch of the *Presidential Emergency Plan for AIDS Relief* (PEPFAR) with an initial budget of US\$15 billion to the worldwide AIDS response over the next 5 years. Shortly thereafter, the WHO launched the '3 by 5' program in order to speed up access to ART in RLS, aiming for 3 million PLHIV on ART by 2005 [190]. Although this initiative fell short of its projected goal, around 1.3 million PLHIV in low- and middle-income countries (LMIC) had initiated ART by the end of 2005 [191].

By this time, the feasibility of ART in LMIC had been shown in several piloting programs and cohort studies [192–197], with similar response to ART as in HIC

but with a high early mortality attributed to the advanced stages of HIV and high prevalence of concomitant TB in those started on treatment [175]. Many of these programs received great support from non-governmental organizations, in particular *Medecins Sans Frontières* (MSF) [198].

Despite these advances in provision of ART in RLS, treatment coverage remained insufficient with only 30% of patients eligible for ART on treatment by the end of 2007 [199]. Shortage of human resources for health and the reliance on physician- and hospital-based ART constituted major barriers to achieve universal access to HIV care and treatment in many countries [200]. In response, the WHO, invoking its *Alma Ata Declaration* of 1978, recommended a task shifting approach to decentralize HIV care making it more accessible and equitable for all PLHIV [201]. These recommendations included redistribution of tasks related to HIV care from highly qualified health workers to those with shorter training and less qualifications to make more efficient use of the available human resources for health. Furthermore, by decentralizing ART delivery from central hospitals and clinics into peripheral health centers within primary health care, ART could be within reach for more PLHIV. Evaluations of programs piloting this new approach showed promising results [202], but also indicated difficulties in managing those with severe immunosuppression and concomitant TB [203].

Ethiopia, one of Africa's most populous countries, was also severely affected by the epidemic with HIV prevalence rates reaching double-digit figures by the year 2000 [186]. As in many other LIC at the time, ART was out of reach for the majority of those in need. But within a few years this would change. Through immense efforts by domestic and foreign organizations, ART would become available. At first only to a selected few, but answering to the call of WHO, decentralization and further ART roll-out would soon follow.

Ethiopia

Health situation in Ethiopia

Located in the Horn of Africa, the Federal Democratic Republic of Ethiopia, is the most populous landlocked country in the world. With a population exceeding 100 million (2016 estimate), this second most populous country in Africa is predominantly agricultural [204]. Whereas a rapid population growth is putting increasing pressure on land resources, the economy has experienced a strong and broad-based growth over the past decade with an average growth of 10.8% per year [205]. As a result, the proportion of Ethiopians living in extreme poverty decreased from 55% in 2000 to 34% in 2011. In line with this, Ethiopia is among the countries that have made great progress toward achieving the *Millennium Development Goals*, with great improvements in education, reduction of child mortality, increased access to clean water, and a strengthened fight against malaria, HIV/AIDS, and TB [206].

In 2016, an estimated 722,000 people were living with HIV in Ethiopia. HIV prevalence among adults was estimated at 1.2%, but with large regional variations [207]. Whereas the capital, Addis Ababa, had an estimated HIV prevalence of 5.0%, the most populous region, Oromia, with the largest number of PLHIV in Ethiopia (184,000) had a prevalence of 0.8%. Nevertheless, even at regional level there are substantial variations in prevalence with recognized hot-spot corridors where HIV transmission is high, such as along the Addis Ababa-Djibouti highway and in some areas with large scale development projects. Among commercial sex workers, HIV prevalence was around 25% according to estimates from 2013-2015 [208].

Ethiopia is one of 14 countries recognized by the WHO as highly burdened by both TB, HIV/TB coinfections, and MDR-TB [135]. In 2015, there were around 140,000 new TB cases, of which 8% were known as HIV/TB coinfections. The most recent estimates from 2016, indicated a lower number of new TB cases (approx. 126,000) with 35% of these being bacteriologically confirmed pulmonary TB [209]. The proportion of extrapulmonary TB was high at 32%.

Non-communicable diseases are increasingly being recognized as a rapidly growing burden in Ethiopia, with increased prevalence of obesity, diabetes mellitus, cardiovascular disease, and cancer [209].

In 2014, 4.9% of gross domestic product (GDP) was spent on health expenditures, which corresponds to about US\$83 per capita [205]. The HIV/AIDS program heavily rely on external funding with more than 86% coming from PEPFAR and the Global Fund [210]. Similarly, only 11% of the national TB budget is covered by domestic resources (51% from international donors and 38% unfunded) [135].

Public health care in Ethiopia

In 2009, there were 3 physicians/100,000 population in Ethiopia [205]. In response to this shortage of physicians, several new medical schools have recently been opened throughout the country. Despite this, the numbers of physicians remain at a comparably low level with 7 physicians/100,000 population in 2016, with large regional variations throughout the country [209]. In comparison, the corresponding ratio are 77 for South Africa and 411 for Sweden [211,212].

Consequently, health care in Ethiopia is based around services managed by non-physician clinicians, such as health officers, nurses and health extension workers [213]. The most peripheral part of Ethiopian health services are the more than 16,000 health posts situated in rural areas across the country. Each health post has two health extension workers assigned to it. In addition to health promotion and disease prevention, these provide basic curative services, particularly for malaria, diarrheal diseases and common infections, serving around 3,000-5,000 people living in the surrounding area. Health centers constitute the second level of health services. The more than 3,500 health centers in Ethiopia are managed by nurses and health officers which are non-physician clinicians with 3-4 years of academic training. Health centers usually consist of several different sections, such as an outpatient department, antenatal clinic, delivery service, and TB clinic. During the last decade HIV clinics have also been incorporated into several health centers. One health center, together with five health posts, form a primary health care unit in rural Ethiopia. District hospitals, serving around 100,000 residents, are staffed by physicians and form the most peripheral physician-run health service in Ethiopia. Finally, regional and federal hospitals constitute the final level of public health care in Ethiopia. In 2016, 241 regional hospital were operational with an additional 153 under construction [209].

ART in Ethiopia

In 2003, ART was available to few through a user fee-based program [214]. Supported by PEPFAR, the Global Fund, and others, this was replaced in 2005 by a free national ART program. Because of the above-mentioned shortage of physicians, Ethiopia adopted the task-shifting approach and partly decentralized the ART program to health centers in 2006. Since then, the number of health facilities providing ART has increased from 72 to more than 1,000 by 2015 [214]. During this period the number of patients receiving ART increased from 40,000 to more than 375,000 [214]. ART coverage has correspondingly increased, but large regional disparities remain. As of 2016, national estimates report that around 65% of PLHIV were on treatment [209], and PEPFAR report provision of ART through their support for 404,000 (56% of 722,000) in Ethiopia that same year [215].

Consequently, there has been a marked reduction by 63% in AIDS-related deaths between 2005 and 2013 [216]. Within the same time span, the median CD4 count at the time of ART initiation increased from 125 cells/mm³ to 231 cells/mm³, and the proportion of patients with CD4 counts above 350 cells/mm³ at ART start increased from 15% in 2013 to 34% in 2015 [214]. Achievements have also been made regarding the prevention of mother-to-child transmission (PMTCT) with 95% of pregnant women tested for HIV during their pregnancy. However, only 62% of HIV positive mothers were reported having started ART as part of PMTCT in 2016 [209].

Health center-based ART in Ethiopia

In Ethiopia today, thousands of PLHIV receive ART through public health centers. As mentioned above, health centers are managed by health officers and nurses. They are responsible for all aspects of patient care, including management of OIs such as TB.

Diagnosis

Diagnosis of HIV has been simplified through the introduction of rapid diagnostic tests, and can be performed by persons with minimal training [217]. Due to the risk of false-positive reaction with newer, highly sensitive tests, a tiebreaker algorithm consisting of three different tests are used. As a first screening test, the *HIV (1 + 2) Antibody Colloidal Gold* (KHB) is used, followed by *HIV 1/2 STAT-*

PAK if screening positive. Where the result of STAT-PAK is discordant with KHB, a third test, *Unigold HIV*, is used as a tiebreaker to determine the result.

Two major models for HIV testing are used: health facility-based testing and community-based testing [58]. Community counsellors have been recruited to have a supportive role in community-based testing after an initial short training. At the health centers, voluntary counselling and testing (VCT) is an integrated part where patient-initiated testing is performed. Furthermore, provider-initiated testing is used on eligible patients, such as pregnant women, family planning clients, all children below 5 years of age, subjects with TB or sexually transmitted infections, and those with clinically suspected HIV/AIDS, by an opt-out approach. In 2016, it was estimated that around 64% of all PLHIV in Ethiopia were aware of their HIV status. Shortages of diagnostic tests have been reported as an obstacle for further expansion of testing services [209].

ART eligibility criteria

When ART roll-out started in 2005, only individuals with severe immunosuppression were eligible for treatment. In the 2008 National ART guidelines, ART initiation was recommended for all patients with CD4 counts below 200 cells/mm³, patients with WHO clinical stage 3 and CD4 counts below 350 cells/mm³, and patients with clinical stage 4 irrespective of CD4 counts [218]. In 2012, eligibility criteria were expanded to include all patients with CD4 counts below 350 cells/mm³, or those with WHO stages 3 or 4 [219]. In 2014, the CD4 threshold level was raised to 500 cells/mm³ or below [58]. Furthermore, ART was recommended irrespective of CD4 count for HIV/TB coinfecting patients, pregnant and breastfeeding women, HIV positive partner in serodiscordant couples, and for all subjects below 15 years of age. As of September 2016, Ethiopia officially adopted the WHO test-and-treat recommendation. Since then, all PLHIV are eligible for ART in Ethiopia (Taye Balcha, personal communication, Aug 30, 2017).

Besides ART, co-trimoxazole preventive therapy is recommended for all PLHIV with CD4 counts below 350 cells/mm³, and 6 months of isoniazid preventive therapy for those without active TB [58].

Assessment before ART initiation

Not all patients will be able to initiate ART at first visit to the HIV clinic. It is important to assess each patient's willingness and ability to initiate lifelong ART. Support for disclosure and partner notification should be provided, in addition to

psychosocial counseling and support regarding treatment adherence [58]. Patient education on risk reduction and family planning should be given. Furthermore, besides nutritional assessment, investigations for and management of concurrent OIs, in particular TB, and other comorbidities should be performed. The extent of these investigations depends on the available resources at the health facility and the frequency of the respective condition in that geographical area. Health centers in Ethiopia often have their own laboratory for basic hematological investigations, sputum and stool microscopy, and urine analysis. The WHO TB symptom screen is recommended to be used on all PLHIV, and those reporting any symptom suggestive of TB should undergo further investigations. In most health centers, these further investigations consist primarily of sputum smear microscopy and physical examination [219]. Sputum smear negative patients with uncertain diagnosis can be referred for chest X-ray (if available, and at an upfront cost for the patient) and/or to a facility, often a regional hospital, for further investigations with Xpert MTB/RIF (available at 110 health facilities in 2016 [209]).

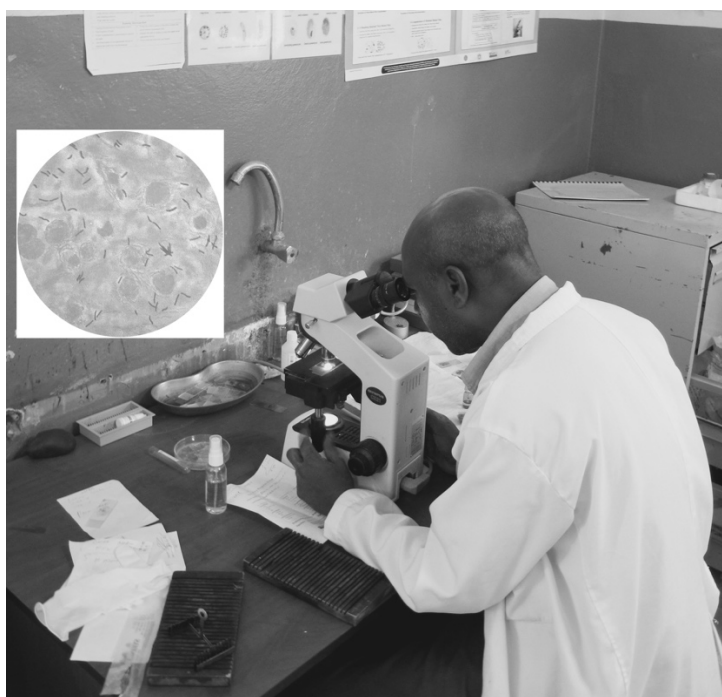


Figure 5. Laboratory technician performing smear microscopy on sputum samples at a health center in Ethiopia. Inserted picture of acid-fast bacilli (Mtb) as seen under the microscope. Credit inserted picture: CDC.

ART regimens

In HIC, ART regimens are usually tailored for each patient based on patient characteristics and preference, level of HIV RNA viremia, results from drug resistance analysis, and, to a lesser extent, cost. In contrast, ART in Ethiopia, and other RLS, consists of a 1st and a 2nd line regimen. Since 2012, the 1st line ART regimen in Ethiopia is a fixed-dose combination of two NRTIs: tenofovir (TDF) and lamivudine (3TC), and the NNRTI efavirenz (EFV). This combination, which is preferred by WHO, is potent and simple to use, cheap, can be given regardless of pregnancy, and can be combined with TB drugs without severe drug-drug interactions. For some, intolerable side effects or other medical reasons necessitate substitution of one or more components of the 1st line ART regimen. Alternative 1st line drugs include the NRTIs zidovudine (AZT) and abacavir (ABC), and the NNRTI nevirapine (NVP). Phasing out of stavudine (d4T) began in 2010, and it is rarely used.

The number of patients switched to 2nd line ART in Ethiopia remains low, at around 1.5% [214]. This likely reflects the difficulty in determining treatment failure, and barriers in access to 2nd line regimens that seldom are available at health centers. The recommended 2nd line regimen consists of switching the first NRTI component (TDF to AZT, or vice versa), and replacing the NNRTI component for a boosted PI (either lopinavir/r or atazanavir/r) [58]. Before switching to 2nd line, treatment failure should be confirmed by VL, with a VL above 1000 copies/mL considered as treatment failure. Drug resistance testing on patient samples is currently not available in Ethiopia. Importantly, through the addition of a boosted PI, concurrent treatment for TB is no longer possible due to drug-drug interactions.

Treatment monitoring

Once started on ART, patients are followed up after two weeks, at months 1, 2, and 3, and quarterly thereafter unless medical complications occur. Since 2015, Ethiopian ART guidelines state that treatment monitoring with VL should be performed for all patients six months after ART initiation and annually thereafter. However, due to lack of available resources for VL testing, treatment monitoring is still based on clinical and immunological monitoring [58,209,220]. Point-of-care CD4 count assays, such as the *Alere Pima CD4 test* (Alere, Germany), are available at an increasing number of peripheral health centers, facilitating immunological monitoring of patients on ART. Immunological monitoring is performed biannually.

Decentralized ART – achievements and obstacles

When a person living with HIV starts ART, a life-long commitment between that individual and the health care system begins. On behalf of the patient, adherence to the medication and return visits for regular medical check-ups are required. The health care system commits to monitor the treatment response and to make sure that drugs are continuously available. Unfortunately, neither the person nor the health care system can guarantee these commitments. Natural disasters as well as human-caused situations such as wars and political conflicts, cause interruptions in drug supply that could be detrimental to PLHIV, and give rise to drug resistance [221]. Yet, even in the absence of such catastrophic events, drug stock-outs are far from rare in many settings [222–224]. Decentralization of ART puts further stress on supply-chains, yet continuous provision of drugs and other essential medical equipment must be secured.

Attrition from care due to patients becoming lost to follow-up (LTFU) is problematic for many HIV programs [225,226]. Reasons for becoming LTFU varies between individuals, and in different geographical and cultural contexts [227]. Through decentralization and integration of ART into primary health care, HIV care has become more accessible to many PLHIV. As hoped, several studies indicate that this has improved retention in care. MSF reported lower LTFU rates from ART programs receiving their support, when integrated care was provided compared with vertical programs [228]. In a case study from Mozambique, Pfeiffer et al. reported increased ART uptake and reduced LTFU when integrating ART services into rural primary health facilities [229]. Similarly, Topp et al. reported increased entry into HIV care at integrated facilities in Lusaka, Zambia, but with the drawback of increased waiting times for all patients accessing the facilities [230]. From Ethiopia, Balcha et al. reported a 65% reduction in risk of LTFU for patients receiving health center-based ART, compared with hospital-based care [231]. This is encouraging since retention on ART in LMIC has been estimated as low as 65%-70% after 36 months of treatment [232], and the obvious increased risk of death for subjects no longer in care [233]

In a systematic review and meta-analysis, Boender et al. reported on long-term virological outcomes of 1st line ART in LMIC [234]. The analysis included 163

studies, 137 of which were from SSA. Virological suppression remained high with suppression rates above 80% during up to 48 months of follow-up. However, when considering subjects no longer in care as non-suppressed, the suppression rates decreased to around 60%. Additionally, the authors emphasize that these findings reflect patients at clinics with access to virological monitoring, which likely represent well-resourced ART sites not representative for most health center-based care [234]. Furthermore, in this large review covering publications from 2006 until 2013, none of the included studies was performed in Ethiopia.

Decentralization of ART means that care is provided at a lower level within the health system. The effect of such decentralization on mortality was therefore of concern as decentralization began. Reassuringly, several studies have shown either similar rates of death [202], or even improved survival in those receiving decentralized compared with centralized HIV care [235,236]. Contrastingly, others have reported high early mortality and difficulties in managing those with pronounced immunosuppression, in particular those with concomitant TB [203,237].

Many countries severely affected by HIV are also highly burdened by TB. These two epidemics overlap to a great extent – especially in SSA. It is therefore essential to determine that subjects with concomitant TB have similar outcome of ART as those without TB. In a systematic review and meta-analysis, Soeters et al. investigated the impact of TB treatment at start of ART on subsequent virological and immunological response to ART [238]. In total, 25 studies were identified, 17 of which examined the impact of TB treatment on ART outcome as main exposure of interest. The authors conclude that TB treatment at ART initiation does not impair virologic suppression or CD4 count gain. They do note, however, that none of the investigated studies were limited to bacteriologically confirmed TB cases, and only one study described this subset. In another analysis by Soeters et al. the effect of TB treatment at ART initiation on subsequent mortality was investigated [239]. Again, it was noted that few (2/22) studies included subsets of bacteriologically confirmed TB cases and most studies were based in specialized HIV clinics. Intriguingly, TB treatment was associated with increased risk of death after about a year of ART, but not before [239].

When investigating the impact of concomitant TB on outcome of ART, the diagnostic methods and screening strategy used to diagnose TB can greatly affect the results and even give misleading information. Especially if TB diagnosis is only based on routine diagnostics generally available (sputum smear microscopy and clinical assessment), and initiated through passive case-finding. In such settings, cases of TB presenting with atypical symptoms or asymptotically would be misclassified as non-TB cases [156,157]. In turn, this can result in a diminished difference regarding impact of TB on outcome of ART. This could

explain why some studies, in contrast to the review by Soeters et al., found a clearly negative impact of prevalent and incident TB on ART outcome, both regarding VF and mortality [240,241]. These studies were performed in Cape Town, South Africa, where access to sophisticated TB diagnostic methods ensured that the risk of misclassification of subjects regarding concomitant TB was low.

Few studies with adequate diagnostic methods for TB have been performed at health centers providing ART. It is possible that the additional complexity of both TB treatment and ART, with increased risks of drug-drug interactions, overlapping side effects, and an increased pill burden, in addition to complications such as TB-IRIS, could be overwhelming at such settings with limited both human and technical resources.

In 2017, worrying data regarding increasing levels of resistance to antiretroviral drugs commonly used in RLS was presented by WHO [11]. The prevalence of both pretreatment and acquired drug resistance are reaching levels where the effectiveness of current 1st line ART regimens is seriously threatened. In particular, resistance against efavirenz has already become so common in several countries that its place as a 1st line antiretroviral drug should be questioned. Efavirenz is one of the most affordable and widely used antiretroviral drugs, with the important benefit of minimal interaction with TB drugs. The implications for PLHIV both with and without concomitant TB could prove to be deleterious in such settings, in particular for those receiving decentralized care without access to alternative drugs.

HIV care in RLS is faced with a true dilemma. Continued expansion of ART is crucial in order to reach all PLHIV. Yet, resources for such expansion are not unlimited. Through decentralization and task-shifting, continued increment in both cost-effectiveness and treatment coverage could be achieved. It is, nonetheless, essential that such expansion does not compromise quality of care. Additionally, it is likely that not all PLHIV are suitable for such care. In particular, those with most advanced disease manifestations might benefit from continued referral to higher tiers of care.

Furthermore, monitoring of subjects on ART is both difficult at decentralized facilities and incur high costs. Yet, it is vital in order to identify individuals with failing ART before resistance mutations have accumulated and their health deteriorated. It is possible that alternative strategies for monitoring the effectiveness of ART could utilize existing monitoring resources more efficiently, without compromising quality.

Aims of the present investigation

The general objective of the studies presented in this thesis was to evaluate the outcome of ART provided at decentralized facilities managed by non-physician clinicians in a RLS with high burden of concomitant TB.

The specific aims of the four studies included were:

- Comparison of short-term outcome of ART in subjects with and without concomitant TB, in particular rates of virological suppression 6 months after ART initiation.
- Determine risk of mortality in the early (6 months) period after clinic registration in patients seeking HIV care at health centers before and after starting ART, and analyze factors associated with such mortality that could be used to identify individuals at high risk of mortality.
- Investigate the long-term outcome of ART with focus on the impact of concomitant TB at start of ART on subsequent rates of mortality, LTFU, lack of virologic suppression, and immunological recovery during up to four years of follow-up.
- Construct an algorithm that through categorization of subjects regarding risk of virological failure 12 months after ART initiation could be used at decentralized facilities for targeted VL testing.

Materials and Methods

Setting

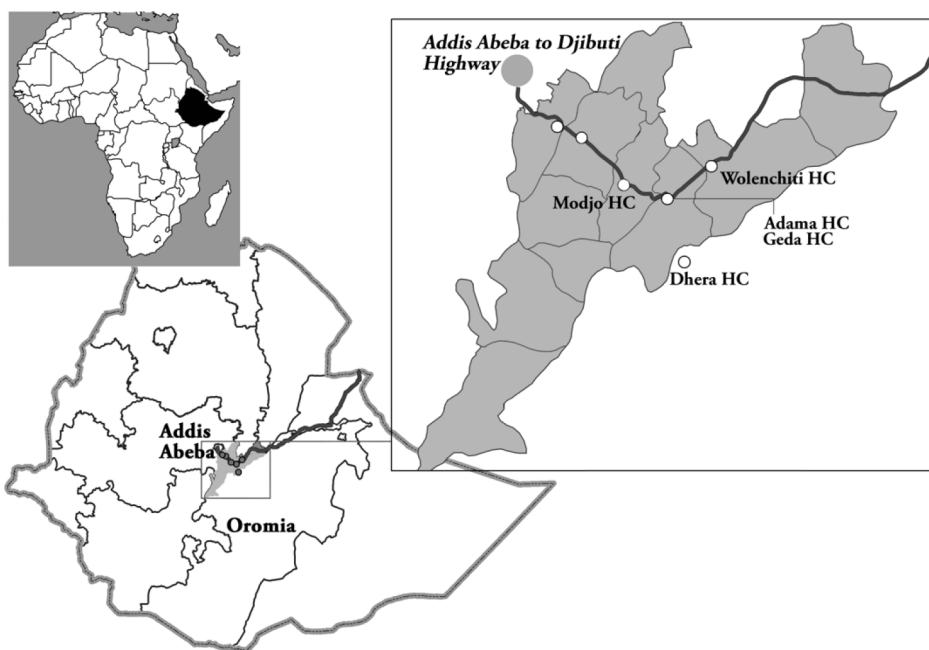


Figure 6:
Map of Ethiopia, Oromia, and East Shoa with the Addis Ababa – Djibouti highway and participating health centers.

The studies included in this thesis were conducted at all five public health centers (Adama HC, Geda HC, Dhera HC, Modjo HC, and Wolenchiti HC) providing ART at the time of the study in the city of Adama and adjacent rural and suburban districts. These health centers covered an uptake area with approximately 600,000 inhabitants. Adama is situated on the heavily trafficked Addis Ababa – Djibouti highway, that functions as the major transport route of goods being imported and exported from Ethiopia. This route is considered a high-risk corridor for HIV infection in Ethiopia.



Figure 7.
Addis Ababa – Djibouti high-way passing through Wolenchiti nearby one of the participating health centers.

Non-physician clinicians with 3-4 years of academic training provide care at these health centers. Apart from HIV services, the health centers consist of several different sections such as outpatient department, antenatal clinic, delivery service, and TB clinic. Health center clinicians attend the different sections according to rotating schedules. When assigned to the ART clinic, they are fully responsible for all aspects of HIV care, including treatment initiation and follow-up.

Participants

Inclusion criteria for participation in the study cohort were: age 18 years or greater, eligibility to start ART (defined as CD4 count below 350 cells/mm³ and/or WHO stage 4 disease, in line with Ethiopian guidelines in use at the time), and residence within the catchment area of any of the study sites. All patients who fulfilled the inclusion criteria were eligible for inclusion. The inclusion period lasted from October 2011 until March 2013.

Subjects with current or previous ART (except for short-course treatment for PMTCT), or TB treatment for more than 2 weeks before inclusion were excluded from the study cohort. These exclusion criteria were used since we aimed to study ART naïve subjects and to avoid misclassification of subjects with active TB but

negative diagnostic investigations due to ongoing TB treatment. Furthermore, individuals that did not provide samples for TB investigations were also excluded.

Study procedures

Health center staff interviewed and physically examined all patients as they were included in the study cohort. Detailed demographic and clinical data were recorded using structured questionnaires. At the baseline visit, blood for CD4 cell and complete blood counts were obtained, and plasma aliquots were stored at -80°C for later VL determination. All participants also underwent bacteriological investigations for active TB. Laboratory results, including TB diagnostics, were communicated to the responsible clinicians who decided if and when to start ART and/or TB treatment.

Participants were then followed for up to four years until study closure on December 31st, 2015. Follow-up visits were scheduled at months 1, 2, 3, 6, 9, and 12, with biannual visits thereafter. At each follow-up visit, health center staff interviewed and physically examined the patient following structured questionnaires. Blood sampling was repeated on visits at months 1, 3, 6, 12, and subsequent visits. The clinicians were also instructed to repeat all TB diagnostic investigations in case of clinically suspected active TB at any time during the study follow-up.

Subjects more than one day late for a follow-up visit were contacted by telephone or traced by health extension workers in order to re-engage them in care or to determine their outcome of care.

Adherence to the study protocol was ensured by weekly visits to each study site by the study investigators or members of the research team. Data registered at the health centers were continuously entered into an electronic database by trained data clerks. All data were thereafter cross-checked to minimize the risk of erroneously entered parameters.

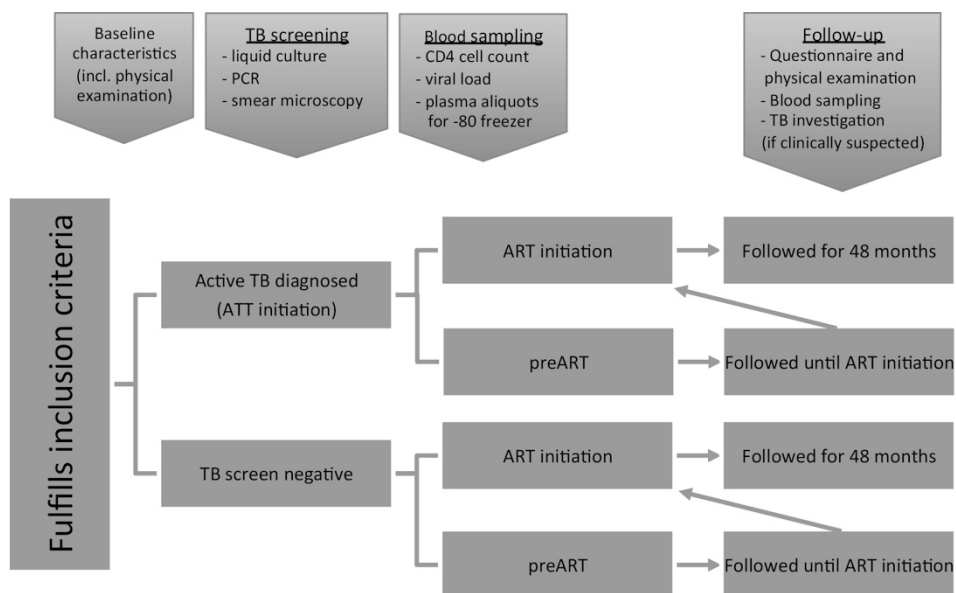


Figure 8:
Flow chart over study follow-up procedure.

Laboratory procedures

HIV was diagnosed based on results from routine care that use serial rapid diagnostic tests. Complete blood counts were performed using Sysmex KX-21 (Sysmex Corp, Kobe, Japan), and CD4 cell counts using the BD FACSCalibur cytometer (Becton Dickinson, San Jose, CA) at the regional laboratory in Adama.

Investigations for TB were performed on sputum samples and, in case of peripheral lymphadenopathy, on fine-needle aspirates using Ziehl-Neelsen staining, Xpert MTB/RIF (Cepheid, Sunnyvale, CA), and liquid culture on a BACTEC MGIT 960 (BD Diagnostics, Franklin Lakes, NJ).

VL determinations were performed in batches during the study period on plasma samples stored at -80°C at the regional laboratory in Adama. The Abbott Real-Time HIV-1 assay (Abbott Molecular Inc., Des Plaines, IL) with a detection limit of 40 copies/mL was used for most study samples. At the end of the study, the regional laboratory switched to the Abbott m2000 RealTime System automated molecular platform (Abbott Molecular Inc., Des Plaines, IL) with a detection limit of 150 copies/mL. This new assay was used for the final samples of the study.

The regional laboratory in Adama participates regularly in external quality assurance performed by CDC (Atlanta, GA).

Study definitions

Concomitant TB was considered bacteriologically confirmed if any of the three diagnostic methods (smear microscopy, Xpert MTB/RIF, or culture) was positive for *Mtb*. Subjects with clinical TB fulfilled criteria according to Ethiopian National guidelines (symptoms and/or signs compatible with active TB supported by radiological results and lack of response to antibiotic therapy) but without bacteriological confirmation [219]. All TB diagnoses within 3 months of study inclusion were defined as prevalent TB, with diagnoses made later during follow-up defined as incident TB. For papers I, II, and IV, all participants with prevalent TB were considered as TB cases. For paper III, subjects with incident TB later during study follow-up, yet before starting ART, were also considered as TB cases.

Subjects more than 3 months late for a scheduled visit and who did not later return during follow-up were considered as LTFU. Tracing campaigns were performed during the study, and if a subject considered as LTFU was found to have died or moved to another location, the study outcome of that individual was changed accordingly.

In paper I, virological suppression was defined as either VL <40 copies/mL or <400 copies/mL after 6 months ART. In paper III, virological outcome was divided in three categories: virological suppression (<150 copies/mL), low-level viremia (150-1000 copies/mL) and high-level viremia (>1000 copies/mL). Any event of high-level viremia during follow-up after at least 6 months ART was defined as lack of virological suppression. In paper IV, virological failure was defined as VL \geq 1000 copies/mL after 12 months ART.

Statistical analysis

Comparisons of patient characteristics between groups were performed using Mann-Whitney's U test for continuous variables and chi-square test for categorical variables. Non-parametric tests were used since several continuous variables were not normally distributed. For ease of comparison, these tests were also used for normally distributed variables.

For survival analyses (papers I, II, and III) Kaplan-Meier curves were used to assess the temporal distribution of events. These curves could also give an indication on whether the risk attributed by the investigated variable was proportional over time.

Cox proportional hazards models were used to enable multivariate adjustments of the survival analyses. Variables considered for the multivariate analyses were assessed for the proportional hazards assumption using log-minus-log plots and by analysis of Schoenfeld residuals.

In paper I, multivariate logistic regression was used to analyze patient-related variables' association with virological suppression.

In paper IV, multivariate logistic regression was used to construct the Viral Load Testing Criteria. Variables with univariate association ($p < 0.3$) with virological failure (defined as a VL ≥ 1000 copies/mL) were entered into a multivariate model. Using stepwise removal of the least significant variable until only variables with $p < 0.05$ remained generated a model with variables independently associated with virological failure. These variables then constituted the Viral Load Testing Criteria. The performance of these criteria to correctly identify subjects with virological failure was evaluated using sensitivity, specificity, positive predictive value, and negative predictive value with 95% confidence intervals (CI). To further describe the discriminative potential, numbers needed to test to identify one subject with virological failure was calculated.

Ethical considerations

Ethical approval for all studies included in this thesis was obtained from the National Research Ethics Committee at the Ministry of Science and Technology of Ethiopia and the Regional Ethical Review Board of Lund University, Sweden. All study participants provided written informed consent. An impartial witness confirmed consent received from illiterate participants.

Results

Study cohort

Study inclusion period lasted for 17 months. During this time, all HIV-positive adults presenting at the ART clinics of the study health centers were assessed for their eligibility to be included. In total, 886 subjects were further interviewed and 812 of them were included in the study cohort. A majority were women (59%) and the median age at time of inclusion was 32 years (interquartile range [IQR], 28-40). The median CD4 cell count at study inclusion was 208 cells/mm³ (IQR, 116-320).

In all, 158 (19%) had concomitant TB of which 137 (87%) were bacteriologically confirmed. Subjects with TB were more likely to be men, and had lower body mass index (BMI), mid-upper arm circumference (MUAC), CD4 counts, and hemoglobin compared with subjects without TB. They also had signs of more advanced disease, as measured by lower Karnofsky performance status (KPS), and higher WHO clinical stages.

The 74 subjects that were further interviewed but excluded from the study cohort, were excluded since they did not have a CD4 count below 350 cells/mm³ or WHO stage 4 disease (n=13), and for not submitting samples for TB investigations (n=61). The 61 subjects excluded due to lack of samples did not differ regarding their baseline characteristics, compared with subjects included into the study cohort (Table 2).

Table 2. Comparison of characteristics of included subjects (n=812) and subjects excluded for not submitting samples for TB investigations (n=61).

Variable	Included	Excluded	P value*
Age, years	32 (28-40)	32 (28-40)	0.92
Gender, female	476 (59)	40 (66)	0.29
CD4 count, cells/mm ³	208 (116-320)	210 (135-292)	0.66
MUAC, cm	23 (21-24)	23 (20-25)	0.53
WHO clinical stage			0.37
Stage 1	141 (17)	11 (18)	
Stage 2	246 (30)	16 (27)	
Stage 3	322 (40)	21 (35)	
Stage 4	100 (12)	12 (20)	

Data presented as median (IQR) or n (%).

*P value derived using Mann-Whitney's U test for continuous variables and chi-square test for categorical variables.

During follow-up, 729 participants started ART, in median 1.2 months after being included into the study. The median CD4 cell count at ART initiation was 187 (IQR, 116-265). All 729 subjects initiated NNRTI-based 1st line ART, with efavirenz as the most common NNRTI component, started in 84% compared with nevirapine 16%. All regimens included lamivudine, and as third drug the NRTI tenofovir in 88%, zidovudine in 10%, and stavudine in 2%.

Among the 83 subjects that did not start ART, 22 (27%) died before ART initiation, 31 (37%) were LTFU, 23 (28%) transferred their care to other facilities, and 4 (5%) declined further study participation.

Whereas 158 participants had TB at or within 3 months of study inclusion, an additional 14 subjects were diagnosed with TB later during study follow-up (incident TB). The median time from inclusion until incident TB was 17 months (IQR, 12-25) during 1935 person-years of follow-up. This equals a TB incidence of 7.2/1000 person-years. Four of these incident TB cases had completed TB treatment previously during this study. An additional 4 subjects had not started ART by the time of incident TB diagnosis.

TB-IRIS was seldom reported by the health center clinicians, with suspected TB-IRIS in 7/158 (4%) subjects with TB.

At study closure on Dec 31st, 2015, 528 (65%) remained in active care, 82 (10%) had died, 89 (11%) were LTFU, 103 (13%) had reported transfer of care, and 10 (1%) had declined further participation in the study. Among the 103 subjects with reported transfer of care, 30 (29%) self-reported this transfer whereas the remaining 73 (71%) had formal transfers performed.

The four papers included in this thesis analyze the study cohort at different times during follow-up, sometimes partly overlapping (Figure 9). In addition, some

subjects included in the analyses of one paper could be excluded from other analyses. In the following sections, this will be clarified in more detail and the most important results from each paper will be presented.

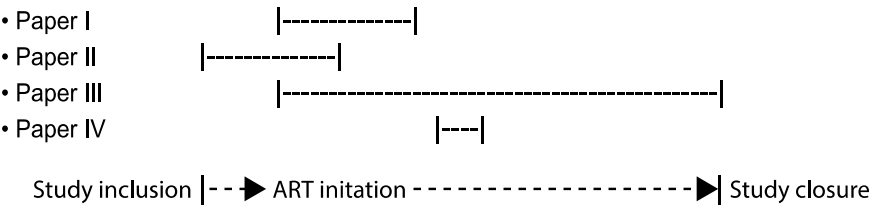


Figure 9.
The follow-up period studied in each paper included in the thesis.

Paper I

In the first paper, subjects started on ART before Dec 31st, 2013 were included (n=678). The primary study outcome was the rate of virological suppression after 6 months of ART, comparing subjects with and without concomitant TB. Two definitions of virological suppression were used in two separate analyses: VL <400 copies/mL and VL <40 copies/mL. Among the 678 started on ART, 561 (83%) remained in care after 6 months and were eligible for the virological outcome analysis. Data on 6-month VL was not available for 49 (9%), leaving 512 subjects with complete data for the main outcome analysis.

Virological suppression at <400 copies/mL was achieved for 89% and at <40 copies/mL for 72%. There was no difference in proportion of subjects achieving virological suppression with or without concomitant TB, $p=0.10$ for VL<400 and $p=0.74$ for VL<40 (Figure 10). In multivariate analysis, male gender was the only variable found to be associated with lower odds of achieving virological suppression: odd ratio 0.4 (95% CI, 0.2-0.7) for VL<400, and odds ratio 0.4 (95% CI, 0.3-0.6) for VL<40.

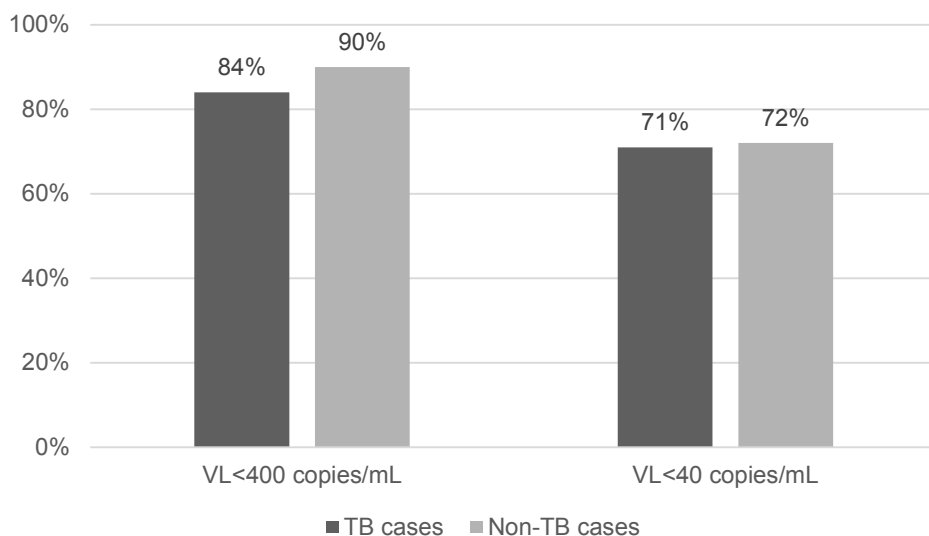


Figure 10. Proportions with virological suppression 6 months after starting ART, comparing subjects with and without concomitant TB.

Paper II

For paper II, all subjects in the study cohort were included in the analyses (n=812). The primary study outcome was all-cause mortality within 6 months of study inclusion. This study also aimed to investigate factors associated with such mortality, and whether these factors were similar for men and women, and for TB cases and non-TB cases, respectively.

At 6 months after inclusion, 37 out of 812 (5%) were confirmed dead, whereas 42 (5%) were LTFU, 34 (4%) had registered transfer of care, 20 (2%) had declined further follow-up (several of whom subsequently rejoined the study), and 679 (84%) remained in care. Deaths occurred in median 54 days (IQR, 30-87) after the participant was included in the study cohort. Half of the deceased participants had started ART prior to time of death.

Mortality was associated with reduced KPS and shorter MUAC. These associations were different for men and women. For men, MUAC remained associated with mortality along with CD4 counts below 100 cells/mm³. For women, KPS remained associated with mortality, along with reported cough. Comparing TB cases and non-TB cases also resulted in different variables being associated with mortality (Table 3).

Table 3. Adjusted hazard ratios (aHR) for mortality within 6 months of study inclusion.

Subgroup and variables	aHR (95% CI)
All	
Karnofsky status <80%	4.3 (1.8-10.1)
MUAC – per cm decrease	1.2 (1.1-1.4)
Men	
MUAC – per cm decrease	1.4 (1.1-1.8)
CD4 cell count, cells/mm ³	
>300	1.0
201-300	1.8 (0.3-13.2)
100-200	2.0 (0.3-11.2)
<100	6.8 (1.4-33.8)
Women	
Karnofsky status <80%	11.0 (2.3-51.5)
Reported cough	4.0 (1.1-14.4)
TB cases	
Reported cough	8.3 (1.1-65.1)
Non-TB cases	
Gender – men vs. women	2.5 (1.1-6.1)
Karnofsky status <80%	4.9 (1.8-13.2)
MUAC – per cm decrease	1.3 (1.1-1.6)

All five models adjusted for age as a continuous variable, CD4 counts as a categorical variable and antiretroviral treatment as a time-varying variable.

Paper III

In paper III, all subjects starting ART during study follow-up were included (n=729). The primary study outcomes were rates of adverse ART outcomes (all-cause mortality, LTFU, and lack of virological suppression) during long-term follow-up of the study cohort. The secondary outcome was immunological recovery during ART.

In addition to subjects with TB within 3 months of inclusion, four subjects had incident TB before starting ART and were therefore also considered as TB cases in paper III.

During a median of 3.0 years of follow-up, death was confirmed for 12/141 (9%) TB cases and 48/588 (8%) non-TB cases. Deaths occurred a median 8.6 months (IQR, 2.2-17.4) after ART initiation. Subjects with TB were not more likely to die, compared with non-TB cases, log rank $p=0.85$. In multivariate Cox analysis, low MUAC was associated with an increased likelihood of death.

In total, 11/141 (8%) TB cases, and 47/588 (8%) non-TB cases were LTFU after starting ART. Subjects were lost in median 6.6 months (IQR, 2.0-15.5) after starting ART. Subjects with TB did not have an increased likelihood of becoming LTFU, log rank $p=0.93$. In multivariate analysis, men were at an increased risk.

Adverse virological outcome of ART was defined as lack of virological suppression below 1000 copies/mL after at least six months ART. A total of 131/630 (21%) participants included in this analysis had lack of virological suppression during follow-up. In univariate analysis, TB was not associated with an increased likelihood of lack of virological suppression, log rank $p=0.15$. The median time from ART initiation until lack of virological suppression was 11.8 months (IQR, 6.0-17.5). In multivariate analysis, men, subjects with CD4 counts below 100 cells/mm³, and those with low MUAC, were at increased risk.

Table 4. Variables associated with mortality, LTFU, and lack of virological suppression during long term follow-up.

Outcome and variables	aHR (95% CI)
Mortality	
MUAC, dichotomized*	3.1 (1.7-5.9)
LTFU	
Gender – men vs. women	2.0 (1.2-3.5)
Lack of virological suppression	
Gender – men vs. women	2.0 (1.4-2.9)
MUAC, dichotomized*	2.1 (1.4-3.1)
CD4 count - <100 vs. >350 cells/mm ³	2.3 (1.2-4.5)

Models adjusted for TB status, gender, age, CD4 count, and MUAC, except for the LTFU model. MUAC not included in multivariate model for LTFU due to violation of proportional hazards assumption.

* MUAC dichotomized at <23 cm for women and <24 cm for men.

Immunological recovery was similar for TB cases and non-TB cases during follow-up. Nonetheless, even after three years of ART, two-thirds of the participants had CD4 counts below 500 cells/mm³ (Figure 11).

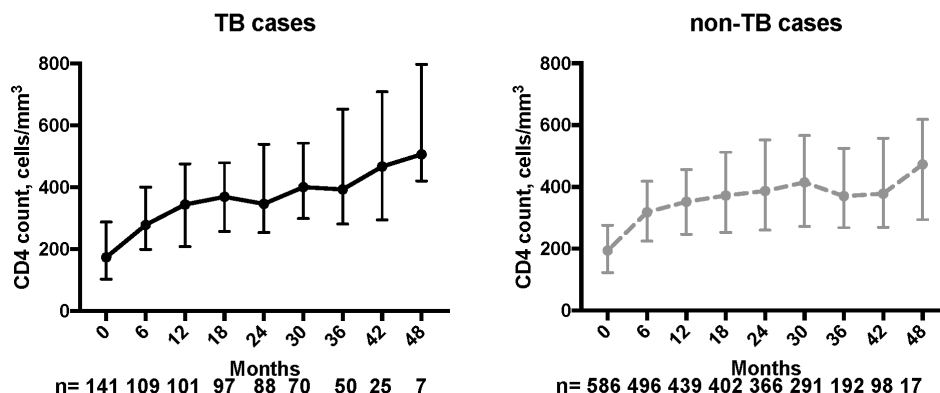


Figure 11.
Immunological recovery during ART.

Paper IV

Paper IV was a cross-sectional analysis of data 12 months after ART initiation. Subjects with a study visit 9-15 months after ART start, with an accompanying VL result, were included in this study (n=494). The primary aim was to develop an algorithm that could identify subjects with virological failure, defined as a VL ≥ 1000 copies/mL.

In all, 57/494 (12%) participants met the definition of virological failure 12 months after starting ART. In univariate analysis, gender, age, KPS, BMI, gender-specific MUAC, previous ART interruption, CD4 counts below 350 cells/mm³, hemoglobin, and lymphocyte count were associated with VF ($p < 0.3$). Tuberculosis did not show any association with virological failure (Table 5).

Table 5. Univariate odds ratios (OR) for virological failure (VF).

Variable	non-VF (n=437)	VF (n=57)	OR (95% CI)	P
Male gender	152 (31)	29 (51)	1.9 (1.1-3.4)	0.02
Age	32 (28-40)	36 (30-44)	1.0 (1.0-1.1)	0.09
Karnofsky status <90%	54 (12)	18 (32)	2.4 (1.3-4.4)	<0.01
Body mass index <18,5 kg/m ²	52 (12)	12 (21)	1.9 (1.0-3.9)	0.07
MUAC <23 cm ♀ / <24 cm ♂	95 (22)	24 (43)	2.6 (1.5-4.7)	<0.01
Baseline tuberculosis	78 (18)	10 (18)	1.0 (0.5-2.0)	0.96
Adherence <95%	67 (15)	9 (16)	1.0 (0.5-2.2)	0.94
Previous ART interruption	8 (2)	5 (9)	5.2 (1.6-16.3)	0.01
CD4 cell count <350 cells/ mm ³	199 (46)	46 (82)	5.4 (2.7-11.0)	<0.01
Lymphocyte count <1100 cells/mm ³	61 (14)	13 (24)	1.9 (1.0-3.7)	0.07
Haemoglobin <11.0 g/dL	52 (12)	11 (20)	1.8 (0.9-3.7)	0.11
Skin rash	17 (4)	2 (4)	0.9 (0.2-4.0)	0.89

Data presented as n (%) or median (IQR), if not stated otherwise.

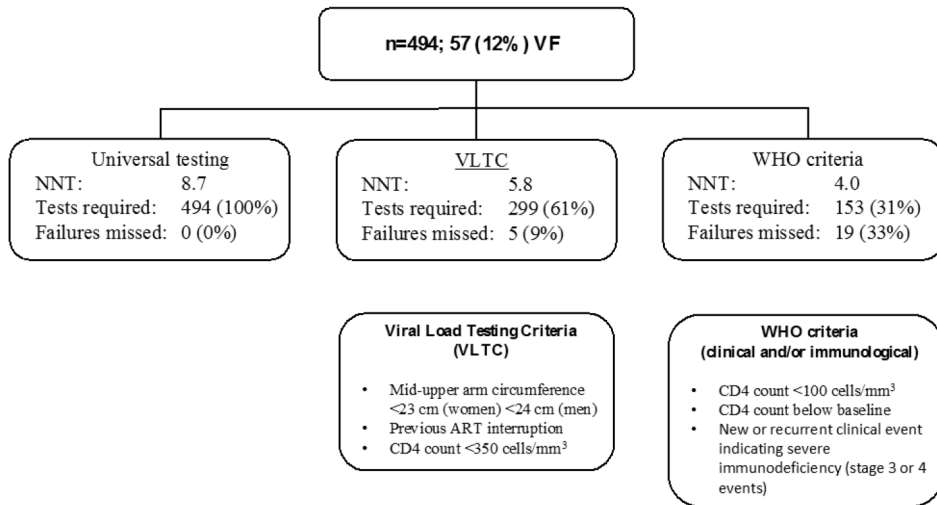
P value derived from the univariate logistic regression model.

In multivariate analysis, after stepwise removal of variables with $p > 0.05$, gender-specific MUAC, CD4 count below 350 cells/mm³, and previous ART interruption remained in the model. These variables constituted the Viral Load Testing Criteria (VLTC).

In derivation, the VLTC had 91% sensitivity (95% CI, 91-97) with 43% specificity (95% CI, 39-48). In comparison, the combined WHO immunological and clinical criteria had 67% sensitivity (95% CI, 53-79) with 74% specificity (95% CI, 69-78).

Compared with universal testing, the VLTC could reduce the proportion of subjects requiring VL testing by 39% with misclassification of 5/57 (9%) of subjects with virological failure.

Algorithms for viral load testing



[NNT, numbers needed to test to identify one individual with virological failure (VF)]

Figure 12.
Performance of algorithms for VL testing.

In summary, the VLTC could be used to rule out virological failure in 4/10, reducing the numbers needed to test from 8.7 (universal testing) to 5.8 (Figure 12). However, the performance of the VLTC needs to be confirmed in external validation before implementation in routine care can be considered.

Discussion

This thesis explores aspects of decentralized ART in RLS. In particular, the impact of concomitant TB is investigated. For this purpose, ART-naïve adults receiving care at Ethiopian public health centers were prospectively recruited and followed for up to four years after starting ART. In a study preceding this thesis, we could demonstrate that coinfection with active TB was common in this cohort [157]. TB is considered to be a leading cause of death in PLHIV in low-income countries (LIC) [242], and has been associated with increased mortality both before and after starting ART [241,243]. Furthermore, concomitant treatment of both HIV and TB is complicated due to potential drug-drug interactions, TB-IRIS, overlapping side-effects, and a large pill burden. In HIC, such dually infected patients would receive care by physicians specialized in infectious disease medicine. The situation in RLS is vastly different. With a limited diagnostic and therapeutic arsenal, these patients are cared for at health centers managed by non-physician clinicians. Suboptimal response to TB treatment and ART can be detrimental, with increased risk of mortality and morbidity, and of drug resistance accumulation and transmission of drug resistant viruses and mycobacteria.

We therefore compared clinical, virological, and immunological outcome of ART for subjects with and without concomitant TB. In papers I-III, different aspects of ART outcome are investigated.

Impact of concomitant TB on outcome of care

In paper I, virological suppression 6 months after ART initiation was analyzed with regard to concomitant TB. The 6-month time period was chosen since this interval constitutes the most critical part where ART and TB treatment often is given simultaneously. The duration of such contemporaneous treatment depends on when TB is diagnosed, and on timing of ART in relation to TB treatment. Although this study was not designed to elucidate the effect of this timing, it was clear that contemporaneous ART and TB treatment is common. Among TB cases, 72% had overlapping ART and TB treatment. Furthermore, despite being subjected to active TB case-finding at study inclusion, 20% initiated ART before

starting their TB treatment. Considering these data, it was encouraging that TB coinfection did not affect rates of virological suppression at 6 months of ART. Furthermore, subjects with TB did not have higher rates of mortality and had similar retention in care, compared with non-TB cases. These results were in agreement with a systematic review from 2014 comparing the effect of TB treatment on virologic response to ART [238]. Most studies included in that meta-analysis did not show a negative impact of concomitant ART and treatment for TB, except for two papers from the Khayelitsha HIV treatment program in Cape Town. In those reports, Boulle et al. reported an inferior virological outcome, both in short- and long-term, for those with concomitant TB and ART including nevirapine as NNRTI, but not for those with efavirenz [244,245]. In our study, 94% of TB cases starting ART had efavirenz-based treatment. Additionally, although most of the studies included in that review were conducted in LMIC, none were from a similar setting of decentralized, health center-based care. Therefore, by demonstrating the feasibility of combined TB treatment and ART at primary health care level, our study adds important evidence to this field.

However, we noted that a larger proportion of HIV/TB coinfecting subjects died before starting ART, compared with those without TB. Since we only included subjects that had initiated ART in the analysis in paper I, such early TB deaths possibly created a left-censoring. This could have inadvertently led to an underestimation of the impact of TB on outcome of ART, as recognized by others [239].

In the second paper, instead of using time of ART initiation as baseline, we used time of inclusion in the study cohort. Thereafter, all-cause mortality within 6 months of study inclusion was analyzed. We did find that TB cases had higher mortality compared with non-TB cases. In Kaplan-Meier analysis, we could see that this difference in mortality was most pronounced during the first month after study inclusion. In multivariate analysis, adjusted for disease severity and other characteristics, TB coinfection was not independently associated with mortality within 6 months of study inclusion. Although concomitant TB was not independently associated with such mortality, it is important to consider that many subjects with TB die before starting ART. Studies reporting outcome of ART often do not include events occurring before ART initiation. If such mortality is higher for a certain group (for example patients with TB), excluding that information can give too optimistic results when outcome of ART is reported [246].

In the first two papers, impact of concomitant TB on short-term outcome of care was investigated. It is possible, however, that TB could adversely impact long-term outcome of ART, despite limited adverse short-term effects [247]. In paper III, we therefore investigated outcome of ART during up to four years of follow-up (median 2.5 years). In this analysis, concomitant TB did not influence the long-

term risks of death, LTFU, or lack of virological suppression. Immunological recovery during ART was also similar for subjects with and without TB.

In summary, the results from papers I-III show that concomitant TB does not have a negative impact on decentralized ART at Ethiopian public health centers. The setting in which this study was conducted is similar to that where many PLHIV in RLS receive care, and it is therefore reassuring that we could demonstrate the feasibility of concomitant ART and TB treatment managed by non-physicians.

Although we did not observe an adverse impact of concomitant TB on outcome of ART, not all subjects had satisfactory outcome of care (Figure 13). Overall, 5% of study participants were confirmed dead within 6 months of inclusion with an additional 5% being LTFU. Virological suppression below 40 copies/mL by 6 months of ART was not attained for nearly one third. Finally, in the long-term analysis, one third of patients initiating ART had unsatisfactory long-term outcomes. Could this be considered as an overall satisfactory outcome of decentralized ART? If those with concomitant TB are not at highest risk of poor outcome of care, who is?

In order to improve management and outcome of care, identification of individuals at high risk of adverse outcome is important. Therefore, we also investigated factors, besides concomitant TB, with potential association with such adverse outcomes. Interestingly, some of these factors recurred in several different analyses. Whereas male gender was associated with both short- and long-term lack of virological suppression and with an increased risk of becoming LTFU, low MUAC was associated with mortality, both early and long-term, and with long-term lack of virological suppression.

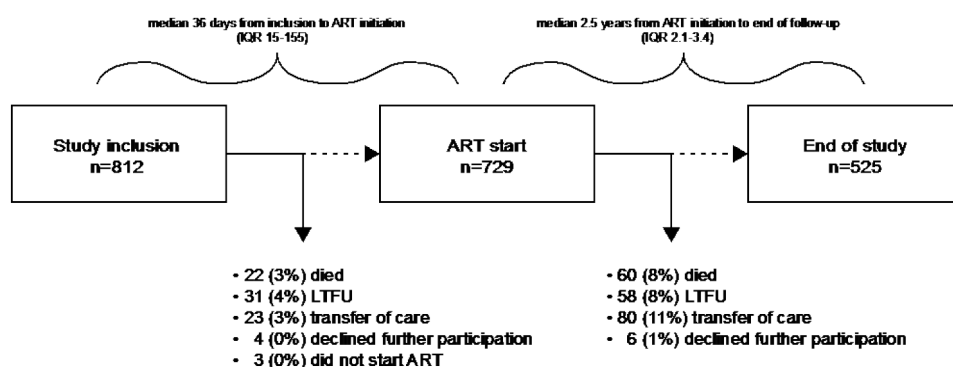


Figure 13. Flow of study participants with regard to outcomes before and after ART initiation, until end of follow-up.

Factors associated with adverse outcome of care

Male gender

Men have been recognized to have worse outcome of HIV care in several studies. In a large meta-analysis, Beckham et al. concluded that HIV-positive men are greater risk of all-cause mortality compared with women while on ART in LMIC [248]. The observed nearly 50% increase in risk persisted over time and was significant in all geographic regions studied, but slightly lower in studies from East Africa (19% increased risk). Similar increased risk of mortality for men both before and after starting ART have been reported elsewhere [243,249].

Why did we not observe a similar increased risk of death for our male participants? In both paper II and paper III, we did in fact observe a trend towards increased risk of death in univariate analysis (lower bound of 95% CI was 0.9 in both analyses). It is therefore likely that we would have reached statistical significance, in univariate analysis, if we had studied a larger population. Yet, these associations diminished after adjustments for other variables, in particular factors associated with more advanced disease. It is therefore unlikely that we missed any increment in risk of death for our male participants related to gender per se. A limitation to this conclusion is the fact that men were at increased risk of becoming LTFU.

It is not possible to ascertain the true outcome of subjects LTFU. At least in our study, they would not have been defined as LTFU if the true outcome could have been determined. It is clear, however, that a proportion of LTFU from ART programs is due to mortality. Two studies from Ethiopia estimated the proportion of deaths among subjects LTFU. In Gondar, Wubshet et al. estimated this proportion to 48%, while Mekuria et al. found 23% of subjects LTFU to have died in study conducted in Addis Ababa [250,251]. Although these studies give an indication that a large proportion LTFU from Ethiopian ART programs dies, this information should be interpreted with caution when speculating on the outcome of subjects LTFU in our study. It is cautioned against assuming the outcome of subjects LTFU if not recent data from the same setting exists [233]. Furthermore, it is clear that a proportion LTFU seek care elsewhere, so called “silent transfers” [252]. Indeed, in a meta-analysis of 23 studies, 5-50% traced LTFU patients were such “silent transfers”. From Ethiopia, it has also been reported that several subjects LTFU instead seek help from traditional healers and religious treatments (holy water) [253]. This was also observed in our study, when subjects coming back after periods being LTFU reported such reasons for their period of absence from the health center ART program. Considering these difficulties in estimating

the outcome for subjects LTFU, we chose to handle this outcome separate, except for a sensitivity analysis in paper I where death and LTFU were analyzed together.

Men did not have an increased risk of death in our study, but the virological outcome of ART was worse for men than women. In particular, men were 60% less likely to be virally suppressed by 6 months of ART (paper I) and were nearly twice as likely to experience lack of virological suppression during the long-term follow-up (paper III). Several reasons for the inferior outcome of ART for men compared with women has been suggested, such as worse adherence (with higher LTFU as found in our study) and treatment initiation at more advanced disease due to late presentation to care [254].

Malnutrition

We used BMI and MUAC to measure malnutrition in our study. These measurements are obviously highly correlated since both are measurements of malnutrition, even though slightly different aspects of it. Interestingly, MUAC had a stronger association compared with BMI in all multivariate analyses performed (as indicated by loss of statistical significance when both variables were entered into a model). Furthermore, we consider MUAC to be a simpler measurement than BMI since it only requires a measuring tape and takes seconds to perform by a layperson with minimal instructions required. We found MUAC to be associated with early and long-term mortality, and with lack of virological suppression both at 12 months after ART initiation (paper IV), and during long-term follow-up (paper III).

That malnutrition was associated with an increased risk of mortality in this study was not surprising, as it has been shown in numerous previous studies [225,245,255]. Yet, the level of increased risk was worryingly high (22% increased risk of early mortality per cm shorter MUAC and three times increased hazard of long-term mortality). What is the cause of malnutrition in our study participants and why does it lead to an increased risk of death?

Advanced HIV infection is associated with weight loss and severe wasting. HIV wasting syndrome (defined as weight loss of more than 10%) is a recognized WHO stage 4 clinical condition. Yet, malnutrition in PLHIV most likely depends on several overlapping conditions. Infections, such as malaria, TB, and diarrheal diseases, are common comorbidities in many settings where HIV is common. Furthermore, food insecurity is a recurring problem in many RLS, affecting socio-economically vulnerable individuals in particular. HIV-related malignancies, and OIs such as *Mycobacterium avium* complex (MAC), visceral leishmaniasis, and disseminated fungal infections can also contribute to malnutrition [134,256].

The association between TB and malnutrition is of particular interest. It is generally accepted that patients suffering from active TB commonly show signs of cachexia. At the same time, malnutrition is a risk factor for developing TB disease [146]. In line with this, Balcha et al. found low MUAC to be a predictor for active TB in our cohort participants [257]. Additionally, malnutrition has been associated with worse outcome of TB treatment [258,259]. It is clear that establishing cause-and-effect regarding malnutrition and aspects of TB disease and its treatment is far from straightforward. Similarly, it is difficult to speculate on the causal link between MUAC and the elevated risks of mortality and poor virological outcome that we found in our cohort. It is possible that some non-TB cases in our cohort with severe malnutrition actually had unrecognized TB. Our TB investigations were focused on pulmonary and lymph node TB. Consequently, even though 21 subjects with negative bacteriological investigations for TB still were considered as clinical TB cases, some cases of active TB could have been missed by our protocol. In addition, other unrecognized OIs could also have played a detrimental role in malnourished individuals that died. These speculations are supported by findings from several autopsy studies performed in Africa, that show high levels of unrecognized pathogens, and TB in particular, in deceased HIV-positive individuals [124,159,160,260].

Malnourishment could also reflect socio-economical vulnerability. Preliminary data from our cohort indicate that illiterate subjects and those residing in rural communities were more likely to have low MUAC. We also found subjects without permanent accommodation and those unemployed to be at an increased risk of death (unpublished data). One could speculate that such vulnerable individuals are overwhelmed by issues related to their day-to-day survival, such as acquiring something to eat and finding a safe place to spend the night, and that this in turn could have a negative effect on their medical treatment [261].

What are the implications of these findings?

At study closure, the majority of participants remained in care – 528/699 (76%), subjects transferring out or declining participation excluded. In addition, most of these 528 participants were virologically suppressed at their last clinical visit (86%). These findings are reassuring regarding outcome of ART for the majority of PLHIV receiving care at Ethiopian health centers. However, 82 subjects (12%) died, and 89 (13%) were LTFU during a median of three years follow-up. Nine percent of subjects in care at end of follow-up had high-level viremia (VL >1000 copies/mL), in addition to those previously transferred out due to suspected, or confirmed, virological failure. This shows, that even though many PLHIV have

good treatment outcome, there are several individuals at high risk of adverse outcome. If such high-risk individuals could be identified early, ideally at first visit the health centers, targeted interventions could potentially improve their outcome.

According to our data, men and subjects with malnutrition are two such groups at higher risk for adverse outcome of care. Then what should be done?

Men are recognized to be at an increased risk of presenting to care at more advanced stages of HIV than women [262]. This could be caused both by gender-related differences in health seeking behavior, but also due to health facilities being less available for men. Through improvements in maternal health care in many RLS, women living with HIV can be identified and linked to care early. Similar screening programs have not been widely implemented for men and could be considered, especially if targeted towards high-risk groups [263]. Similarly, once in care, it could be beneficial to be extra vigilant of men being late for their visits, initiating tracing attempts to re-engage them in care without much delay.

Interventions targeting malnourished individuals is also difficult. Nutrition assessment and support are already included in most HIV programs [264,265]. Yet, the fact that we still found malnutrition to be such a strong indicator of adverse outcome of care shows that current interventions are insufficient. This indicates that malnourishment in these individuals does not simply reflect too little food, but rather a more complex picture [134]. Investigations for concomitant TB or other OIs should be performed, the socio-economic situation and its potential impact on subsequent care should be assessed, and ART should be initiated without unnecessary delay.

As more countries adopt the WHO endorsed test-and-treat strategy, more people with less advanced HIV will initiate ART. Whereas evidence indicate that this will improve outcome of care for these individuals, it could potentially lead to additional crowding of ART clinics. In such a scenario, it will be increasingly important to be able to identify those in immediate need of above-mentioned investigations and treatment. Differentiated care packages with fast-track investigation and treatment for high-risk individuals is an attractive option for such a scenario.

Targeted viral load testing

It is generally agreed, that VL monitoring of individuals on ART provide valuable information that help clinicians evaluate the effectiveness of ART. It can also serve as an important tool in adherence counselling. Unfortunately,

implementation of VL monitoring in RLS has been problematic. In many settings, access to VL is severely restricted, and expansion of VL capacities is hampered by high cost and technical requirements. The situation is similar in Ethiopia. Although annual VL monitoring has been part of routine care according to National guidelines since 2015, the actual number of patients being tested remains low. During this study, we experienced the difficulties of performing VL testing. Essential reagents with short shelf-life were often unavailable, the machines require sophisticated maintenance, and safety cabinets failed to work.

An alternative to annual VL testing of all subjects on ART, is testing targeted towards individuals at high risk of virological failure. Such strategy requires fewer tests to be performed, and can thereby be an attractive option for settings with limited VL capacities. However, the algorithm behind such targeted testing must be highly sensitive to not misclassify subjects with virological failure since these would not be offered a test.

In table 6, previously developed algorithms for targeted VL testing are compiled. Since none of these had been validated in Ethiopia, nor in a decentralized setting, we first attempted to perform such a validation. We chose the Clinical Predictor Score (CPS) developed by Lynen et al. in Cambodia [103]. The CPS was chosen for its relative simplicity and since this was the only algorithm that had been validated in a setting different from where it was developed. Unfortunately, there were some essential components included in the CPS that we did not record in our study questionnaires: we did not record papular pruritic eruption (PPE), and we did not estimate adherence using a visual analogue scale (VAS). Finally, since all our participants were ART naïve at study inclusion, the first variable ‘ART exposure’ did not apply. Nevertheless, using the more general term ‘skin rash’ instead of PPE, and a three-question panel instead of VAS for adherence [266], we tested the performance of this modified CPS on our dataset 12 months after ART initiation. The modified CPS could categorize individuals at high risk of virological failure with 79% sensitivity, similar to the first validation of the CPS in Cambodia [104]. Yet, the specificity was lower at 45%. This reduced performance could possibly be explained by our modification of the original CPS. This was not a correct validation of the CPS for two main reasons; first, we modified the original CPS, second, we applied the algorithm on our registered data retrospectively. Consequently, we instead pursued to develop a new algorithm optimized for use at facilities providing decentralized ART in settings with limited VL capacity.

Table 6. Previously developed algorithms for targeted viral load testing.

Setting	Year	Failure (VF) definition	Subjects (% VF)	Method	Variables	Se Sp
Hospital Cambodia [103]	2005- 2007	VL>1000x1	764 (8%)*	Likelihood ratios	ART exposure CD4 < baseline 25% or 50% drop in CD4 from peak PPE Hemoglobin drop ≥1 g/dL <95% adherence (VAS)	63.2 66.0
Hospital Cambodia [104]	2010- 2011	VL>1000x1	1490 (3%)	N/A	As above	77.8 60.4
Hospital Cambodia [105]	2010- 2011	VL>1000x1	1490 (3%)**	Likelihood ratios	CPS 3: ART exposure CD4<250 PPE <95% adherence (VAS) WHO stage 3 or 4	78.2 41.3
Hospital Uganda [100]	2006	VL>1000x1***	496 (10%>400 8%>1000)	Logistic regression	30% fall in CD4 Ever missed ART >2 days	67 82
Hospital Uganda [98]	2004- 2009	VL>1000x1	443 (21%****)	Likelihood ratios	CD4 ≤200 MCV ≤95 fl <90% adherence (VAS) Shingles Severe pneumonia Prurigo Thrush EPTB	71.8 47.3
HIV clinic South Africa [99]	2004- 2010	VL>400x2*****	3657 (25%)	Cox model	<u>Baseline:</u> Gender Age >40 years CD4 <100 WHO stage 3 or 4 Albumin <25 g/L <u>Current:</u> Hemoglobin drop >20% MCV <100 fl Missed ARV pickup >7 days CD4 <200 WHO stage 3 or 4 "New condition" Regimen change	57.1 50.5

* Analysis based on visits, 764 subjects contributed 1803 visits (5% VF)

** Algorithm derived using cohort from 2010-2011 and performance tested on cohort from 2005-2007

*** VL>400 copies/mL was used for derivation, and VL>1000 copies/mL for validation

**** Analysis based on visits, 443 subjects contributed 2668 visits (5% VF), performance is tested on Ugandan cohort from 2006.

***** All subjects had to achieve VL<400 copies/mL prior to VF in order to be included.

There are several ways to develop a clinical algorithm. We aimed to identify variables that could easily be recorded by health center staff with minimal training and without the requirements of complicated calculations, or sophisticated laboratory facilities. We chose logistic regression to identify such variables. Through multivariate analysis, few variables with strong independent association with virological failure remained in the final model thereby making the algorithm easier to use. Lynen et al. used a different statistical method to develop their algorithm, based on likelihood ratios instead of odds ratios [267,268]. For comparison, we applied that method to our material. The resulting model was similar, but we chose the logistic regression method mainly since that method has been used by our group previously. Furthermore, we consider logistic regression to be more widely used in our research field compared with likelihood ratio calculations, and this could improve the readers understanding of how our model was derived.

The algorithm we developed, the Viral Load Testing Criteria (VLTC), consists of three variables: MUAC with gender-specific thresholds (<23 cm for women and <24 cm for men), CD4 count below 350 cells/mm³, and ART interruption of more than one day. In derivation, the VLTC did have high sensitivity (91%) and acceptable specificity (43%), in categorizing our study participant regarding risk of virological failure (defined as a VL \geq 1000 copies/mL). Before implementation of any algorithm could be considered, performance must be assessed in a population different from the derivation population. There are several ways to perform so called ‘internal validations’ [269], but we chose not to perform such validation for several reasons. First, although results from an internal validation consistent with those of the derivation indicate that the performance estimated in derivation is true, external validation must still be performed. Second, our study cohort data is in part limited since our inclusion criteria includes ‘CD4 count below 350 cells/mm³’. Many countries have raised the CD4 threshold for ART initiation, or removed it altogether. Hence, validation of the VLTC in a population not selected on basis of CD4 count before ART initiation would be more generalizable for use in current ART programs. Third, the main objective of a validation would be to evaluate the performance 12 months after ART initiation, since this was the time point it was derived for. It would, nevertheless, be of interest to validate the performance of the VLTC to identify subjects with high risk of VF later during their ART as well.

We did plan for a validation study during February-April 2017. Unfortunately, mainly due to continued issues related with the VL testing facility, the study could not be performed at that time. Instead, we plan to validate the VLTC February-April 2018.

Limitations

The principal aim of this thesis was to evaluate outcome of ART at Ethiopian health centers. The study protocol should hence interfere as little as possible with routine care. Yet, some interference was required in order to collect necessary data. While we wanted as much information as possible on our study participants, such as TB status, virological outcome of ART, and more, we did not want our additional investigations to influence routine care. While it was mandatory from an ethical perspective that all additional data should be communicated without delay to the responsible clinician to benefit the patients, this was also a scientific dilemma since that may have affected the outcome of care for a proportion of our participants.

First, through the intensified TB case-finding at study inclusion we could categorize participants regarding concomitant TB. This was crucial to study the impact of TB on ART outcome. Concurrently, this is an important intervention since several subjects that were identified as TB cases through this approach would not have been diagnosed as timely, or not at all, without this case-finding strategy (23% of TB cases were positive in smear microscopy). Early identification and prompt treatment for TB, in particular among PLHIV, are essential for satisfactory outcome TB treatment. We therefore report our findings in light of this intervention, i.e. concomitant TB did not have a negative impact on outcome of ART, at least when it is identified timely and accurately.

Second, our study protocol included biannual VL during ART, which was not part of routine care. Since it would be unethical to withhold such crucial information from the care providers, this could have been another important interference with routine care. However, through factors out of our control, VL testing during this study was not performed continuously. Instead, study samples were tested in batches. As soon as VL results were available, they were communicated to the health centers. Nonetheless, the delay between blood sampling and VL result delivery was often prolonged up to several months. As a result, the study intervention through biannual VL testing proved to be less than we expected.

Third, since outcome of subjects LTFU is unknown, we aimed to minimize this proportion as much as possible. We noted, that even though tracing of patients late for their visits to ART clinics are mandated in Ethiopian National ART guidelines, this was not routinely performed at all sites. We therefore provided assistance by engaging health extension workers to perform this task. While performing weekly monitoring, we also assisted by calling subjects late for their visits, a task that usually is performed by tracers at the health center. Finally, to ascertain the outcome of subjects LTFU for a prolonged period, our research team conducted annual tracing campaign with home visits. Hereby, several subjects LTFU were

found to have died or had their care transferred to another facility. Yet, some individuals were found alive and could be motivated to return to care, thereby reducing the numbers LTFU from routine care. Important with regard to determining the outcome of subjects LTFU, this intervention should not have altered outcome of ART at a large extent.

At study inclusion, active TB was found in 158 (19%) participants with an additional 14 cases of incident TB during study follow-up. This shows that our TB case-finding protocol was effective. It is, nonetheless, likely that some cases of active TB were misclassified. Autopsy studies indicate that TB may be missed, even in settings where active case-finding is performed. We estimated the proportion of such missed TB cases to be small in our study. Yet, if these subjects had worse outcome of care this would have reduced the actual difference between TB subjects and non-TB subjects that we found in our study. Additionally, the burden of other severe OIs among study participants could not be determined with certainty due to the limited diagnostic tools available at the health centers. In a separate cohort, we investigated the prevalence of cryptococcal antigenemia in ART-naïve patients receiving care in the HIV clinic at Adama Regional Hospital [270]. Among 129 subjects investigated (median CD4 count 210 cells/mm³; 23% <100 cells/mm³), prevalence of cryptococcal antigenemia was low (1.6%). This indicates that the burden of cryptococcosis is low in the geographic area where our study was performed.

In line with this, causes of death were not determined with certainty for deceased participants. We could ascertain that the deaths in our study were due to illness and not attributable to accidents or other unnatural causes. The addition of autopsies would have provided important information, but this was not possible to perform for several reasons.

Finally, 103 (13%) of included participants had their care transferred to other facilities during the study. Although these participants contributed important information as long as they remained in the study, the final outcome is unknown. In survival analyses, these subjects were censored at last study visit. This approach is commonly used in similar studies. However, in a South African study, subjects transferring out had an elevated risk of death, in particular within three months of transfer [271]. Tracing of subjects transferring out could therefore have added important information to our study.

Elimination of AIDS by 2030

In this thesis, some aspects of providing concomitant ART and treatment for TB at Ethiopian health centers has been investigated. Through papers I-III, we could show that concomitant TB did not have a negative impact on outcome of ART. These data are important since they show that continued provision of care under these circumstances can be considered.

In 2014, UNAIDS launched the 90-90-90 campaign [272]. It stipulated what UNAIDS consider that all countries affected by the HIV epidemic should strive for: by 2020, 90% of all PLHIV should know their HIV status, 90% of them should receive sustained ART, and 90% of those on ART should be virally suppressed. This could in theory bring an end the global AIDS epidemic by 2030. This ambitious strategy would, except for a great impact on number of lives saved and new HIV infections averted, incur high costs to implement. Nonetheless, in 2017, for the first time, more than half of all PLHIV have access to ART [11]. AIDS-related deaths have been reduced by almost 50% since 2005. Encouragingly, in Eastern and Southern Africa, the world region most affected by HIV, the number of new HIV infections has declined by nearly one third since 2010. However, there are two major reasons for concern regarding the continued success in the fight against HIV/AIDS – lack of sufficient funding and increased prevalence of drug resistant HIV.

In order to meet the ambitious 90-90-90 goal, great financial investments are required. Compared with 2014, investment in HIV in LMIC needs to increase by 91% by 2020 [273]. In Ethiopia, more than 86% of funding for HIV/AIDS has been provided by PEPFAR and the Global fund [210]. As elsewhere great increase in funding is required to reach 90-90-90 by 2020. Contrastingly, the support from PEPFAR is declining, from US\$200 million in 2015 to a projected US\$104 million in 2020 [210]. Therefore, great increase in domestic funding is required even to sustain current level of care. In a country with several competing priorities, such as health care besides HIV/AIDS, investments in education, and improved infrastructure, to name a few, allocation of additional funds for HIV can be problematic.

It is clear that improved cost-effectiveness will be essential. Hopefully, costs associated with antiretroviral agents will continue to decline, but costs associated with monitoring of ART must be given attention. Currently, annual VL monitoring is not performed on all patients on ART mainly due to limited capacity. As we report, nearly 80% of our study participants achieved sustained virological suppression. Annual VL on subjects with persistent viral suppression could therefore be considered as resources misspent, and if such individuals could be identified with simple algorithms this might help to use existing resources more

efficiently. It is nonetheless crucial, that subjects with failing ART are identified in time, before resistance mutations have accumulated with potential detrimental effects on the individual's health and onward spread of drug resistant HIV in the community.

The increase in drug resistance that has become increasingly evident in recent years is truly a cause for great concern. In 2011, Hamers et al. could show that the level of drug resistance in six African countries was associated with the time since start of ART roll-out [61]. In 2016, high levels of tenofovir resistance was found in subjects with virological failure, with highest levels in SSA (57%) [62]. Tenofovir resistance is of particular concern, given that it is a main component in current pre-exposure prophylaxis (PrEP) regimens [274–276]. In 2017, WHO reported that levels of pretreatment resistance to the main NNRTI components efavirenz or nevirapine reached 10% or above in 6/11 countries contributing survey data [11]. These NNRTIs are the most affordable and widely used drugs in current 1st line ART globally. Yearly increment in such NNRTI resistance was greatest in studies from Eastern Africa, where three out of four participating countries had >10% NNRTI resistance.

In our cohort, we are conducting drug resistance testing on subjects failing to suppress VL below 500 copies/mL by 12 months ART. Until now, resistance data is available for 45/72 of these subjects. NNRTI resistance was detected in two thirds, and NRTI resistance in 40% with dual NNRTI/NRTI resistance in nearly 40%. These preliminary data emphasize the need to identify the minority of subjects that do not respond satisfactorily to ART. If left unnoticed, these individuals are at high risk of treatment failure with subsequent AIDS and related high risk of death. Furthermore, they can serve as sources for transmission of resistant viruses in their communities.

Through continued, and expanded, provision of decentralized ART, with targeted VL testing and identification of individuals at high risk of virological failure, the cost-effectiveness of HIV care in RLS could potentially be improved – without reduced effectiveness. It is clear, however, that this will not be sufficient. Funding must also increase, both for drugs and for laboratory monitoring (including drug resistance testing for selected cases), most likely through increased allocation of domestic resources in affected countries. In light of current levels of pretreatment drug resistance in many RLS, with even higher levels among treatment experienced and in those failing 1st line ART, current 1st line ART regimens will also need to be revised.

Conclusions

- Combined ART and TB treatment at decentralized facilities in RLS is feasible, with similar rates of virological suppression both at 6 months and during continued follow-up.
- Rates of mortality are high among recently registered HIV/TB coinfecting subjects with advanced disease characteristics, and often occur before ART has been initiated.
- Men have higher risk of poor virological outcome of ART, and are at increased risk of becoming LTFU.
- Subjects with malnutrition are at high risk of both short- and long-term mortality, and have worse long-term virological outcome of ART.
- In derivation, the Viral Load Testing Criteria based on MUAC, current CD4 count, and previous interruption of ART, identified subjects with virological failure with high sensitivity. If external validation confirms this performance, it could be used for targeting VL testing in RLS.

Future perspectives

We found that most patients receiving decentralized ART at Ethiopian health centers had a good and sustained response to treatment. Recently, Ethiopia adopted test-and-treat offering ART to all PLHIV regardless of CD4 count. This move forward will enable more PLHIV to have access to ART which can further reduce HIV-associated morbidity and mortality, and reduce the number of new HIV-infections. However, it will also put a great strain on the health care system [277]. Although PLHIV need close and regular monitoring before they start ART, this monitoring is often more intense after ART has been initiated. It is essential to establish and maintain virological suppression, and to keep the patients motivated for lifelong adherence to ART. Already at insufficient capacity, VL monitoring of all patients on ART will be problematic and expensive.

We constructed an algorithm, the VLTC, that could reduce the number of VL tests required, but with a specificity of 43% in derivation, a great number of VL test will still need to be performed. Additionally, the VLTC includes CD4 count below 350 cells/mm³ as an indicator of virological failure. We did try to develop an algorithm without the use of CD4 counts, but the performance loss was too great. Far from all health centers in Ethiopia are equipped with point-of-care CD4 count analyzers, and although CD4 counts are usually available within a few days at most health centers, test result delivery days after the actual patient visit complicates subsequent care. Return visits are difficult to organize and incur extra costs for the patient in form of transportation and potentially another day's loss of income. If CD4 counts could be omitted altogether from the VLTC, or similar algorithms, the usability would be greatly improved.

Until recently, no such algorithm existed. However, Pastor et al. have presented data on interferon-inducible protein-10 (IP-10) and its performance regarding identification of subjects on ART with detectable viremia [278]. In their model, IP-10 had 92% sensitivity and 60% specificity to detect subjects with VL >150 copies/mL. The predictive capacity did not improve when CD4 count was included in their model. If these results are confirmed, IP-10, either alone or in combination with clinical algorithms such as the VLTC, could be an important tool in monitoring of ART. In particular, such biomarker has the potential to be developed into a simple point-of-care test.

Ideally, implementation of highly sensitive and specific algorithms regarding risk of virological failure would not only reduce the number of VL tests required, but altogether replace VL as monitoring tool. It can be argued, that the usefulness of VL is limited in many HIV clinics in RLS today. Even at sites where VL is available, result delivery is often delayed and rarely available while the patient still is at the clinic. In addition to the limited usefulness of a VL result weeks or months after the actual patient visit, it is still impossible to know whether drug resistance mutations are present or not. If expansion of VL testing facilities can be bypassed, resources could instead be used to implement drug resistance testing on selected patient samples.

Beside the technical issues concerning virological monitoring of an increasing number of patients on ART, human resources at health facilities providing ART are at risk of becoming overwhelmed. One potential solution to this could be further decentralization of ART for patients on stable treatment and considered to be at low risk of adverse ART outcome. In this thesis, factors associated with adverse outcomes have been presented. Yet, these results could also be interpreted in reverse, for example that women without malnutrition as a group are more likely to respond well to ART. If such data could be refined, it could be used to identify low-risk individuals suitable for down referral to more peripheral health facilities. In Ethiopia, health posts constitute the most peripheral health facility. Health extension workers at such sites often have good knowledge of the surrounding community and its people, and could be a valuable resource to alleviate overburdened health center clinicians as more PLHIV need monitoring and support. Through the use of simple clinical algorithms with or without point-of-care tests, patients with failing treatment could be identified and referred back to health centers or hospitals.

Correspondingly, some patients are at high risk of death soon after being engaged in care at health centers. Late presentation to care at advanced stages of HIV infection is an enduring challenge to HIV programs [279]. Individuals presenting with advanced disease manifestations to a health center-based HIV clinic should probably be considered for referral to higher tiers of care. Yet, referral has also been shown to be associated with high mortality [271]. Hakim et al., suggested an alternative approach to tackle this problem in a recent trial. In this trial, HIV-positive adults and children with CD4 counts below 100 cells/mm³ were randomized to receive standard care or ‘enhanced prophylaxis’ [280]. The enhanced prophylaxis consisted of isoniazide, fluconazole, azithromycin, and albendazole, in addition to standard co-trimoxazole prophylaxis. This enhanced antimicrobial prophylaxis combined with ART, resulted in reduced rates of death without a negative impact on virological suppression. Although risking the development of antimicrobial resistance through increased prescription of

antimicrobial drugs, this approach could be considered for at-risk individuals at sites with limited diagnostic capacities and poor referral systems.

Finally, given that prevalence of drug resistance to antiretroviral drugs included in current 1st line ART regimens already have reached high levels in many countries [11], alternative 1st line regimens need to be evaluated for its use in decentralized ART. This is of particular importance in settings where HIV/TB coinfection is common. Many put their hope to INSTIs, and especially dolutegravir, for their high genetic barrier to resistance [281]. As these relatively new drugs are introduced in settings where concomitant TB is common, the effectiveness of dose-adjusted dolutegravir in combination with rifampicin-containing TB treatment must be carefully monitored. Should resistance emerge even to INSTIs, completely new classes of antiretroviral drugs will be needed before long.

After reading this thesis, it should be clear that the fight against HIV/AIDS in RLS have come a long way compared with the desolate situation 15 years ago. Yet, it should be equally obvious that several obstacles and threats remain that could jeopardize the achievements made. It is crucial, that the grip on HIV remains tight and that efforts to reach all PLHIV with effective and durable treatment are maintained.

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Paper I



High Rates of Virological Suppression in a Cohort of Human Immunodeficiency Virus-Positive Adults Receiving Antiretroviral Therapy in Ethiopian Health Centers Irrespective of Concomitant Tuberculosis

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Background. Antiretroviral therapy (ART) initiation during treatment for tuberculosis (TB) improves survival in human immunodeficiency virus (HIV)/TB-coinfected patients. We compared virological suppression (VS) rates, mortality, and retention in care in HIV-positive adults receiving care in 5 Ethiopian health centers with regard to TB coinfection.

Methods. Human immunodeficiency virus-positive ART-naïve adults eligible for ART initiation were prospectively recruited. At inclusion, all patients underwent microbiological investigations for TB (sputum smear, liquid culture, and polymerase chain reaction). Virological suppression rates after 6 months of ART (VS; viral load <40 and <400 copies/mL) with regard to TB status was the primary outcome. The impact of HIV/TB coinfection on VS rates was determined by multivariate regression analysis. Mortality and retention in care were analyzed by proportional hazard models.

Results. Among 812 participants (TB, 158; non-TB, 654), 678 started ART during the follow-up period (TB, 135; non-TB, 543). No difference in retention in care between TB and non-TB patients was observed during follow-up; 25 (3.7%) patients died, and 17 (2.5%) were lost to follow-up ($P = .30$ and $P = .83$, respectively). Overall rates of VS at 6 months were 72.1% (<40 copies/mL) and 88.7% (<400 copies/mL), with similar results for subjects with and without TB coinfection (<40 copies/mL: 65 of 92 [70.7%] vs 304 of 420 [72.4%], $P = .74$; <400 copies/mL: 77 of 92 [83.7%] vs 377 of 420 [89.8%], $P = .10$, respectively).

Conclusions. High rates of VS can be achieved in adults receiving ART at health centers, with no significant difference with regard to TB coinfection. These findings demonstrate the feasibility of combined ART and anti-TB treatment in primary healthcare in low-income countries.

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Keywords. ART; Ethiopia; health centers; outcome; tuberculosis.

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Human immunodeficiency virus (HIV) infection is the strongest risk factor known for developing active tuberculosis (TB), and TB remains the most common opportunistic infection globally [1]. Rates of HIV coinfection among TB patients are high in sub-Saharan Africa, exceeding 50% in some countries [1–3]. In many cases, HIV infection is first detected at presentation with TB, and most such persons have advanced immunosuppression at the time of HIV diagnosis. The risk of death among HIV/TB-coinfected people is greatly increased

compared with HIV-infected subjects without TB [4–7]. Survival for coinfecting persons is significantly improved if antiretroviral therapy (ART) is started during the course of anti-TB treatment (ATT) [8–10]. Consequently, the World Health Organization (WHO) recommends starting ART during ATT for all HIV/TB-coinfecting patients, preferably during the first 2 months of ATT [11].

However, ART initiation during ATT may be associated with complications. The risk of immune reconstitution inflammatory syndrome (IRIS) is elevated for patients starting ART during the initial months of ATT; although IRIS is rarely fatal, this condition may be difficult to recognize and manage in low-resource settings [12]. Furthermore, concomitant ATT and ART might compromise adherence and increase the risk of side effects and drug-drug interactions, all of which could adversely impact treatment outcome [12, 13]. More importantly, inadequate suppression of viral replication during suboptimal ART promotes selection of HIV variants with mutations conferring antiretroviral drug resistance [14].

Although most HIV/TB-coinfecting subjects live in resource-limited settings, initial studies on the efficacy of concomitant ART and ATT were performed in high- and middle-income regions [15–17]. Recently, Soeters et al [18] presented a systematic review on the effect of ATT on ART outcome that showed virologic suppression rates in patients receiving concomitant ART/ATT comparable to those in subjects only receiving ART. This review included 8 studies conducted in sub-Saharan Africa (5 of 8 from South Africa), which were mainly based in hospitals or specialized ART clinics. To increase treatment coverage, continued decentralization of ART services to primary health centers is needed. This process requires simplification of management due to less qualified staff and limited access to laboratory monitoring; in particular, viral load testing is rarely available. Although comparable outcomes of ART in clinics managed by nurses compared with physician-run facilities have been reported [19, 20], the outcome of ART provided at public health centers for patients with HIV/TB coinfection is not well documented.

In Ethiopia, an increasing number of patients receive ART at public health centers throughout the country since decentralization of ART began in 2006. We have recently reported a high prevalence of TB detected through intensified case-finding in a cohort of adults receiving care in 5 health centers in an uptake area of Central Ethiopia [3]. The current study compares the outcome of ART after 6 months with regard to the presence of concomitant TB among participants in this cohort, with particular focus on virologic suppression (VS) rates.

MATERIALS AND METHODS

Setting

This study was performed in all public health centers (Adama, Dhera, Geda, Mojo, and Wolenchiti) providing ART in the city

of Adama, Oromia Regional State, Ethiopia, and adjacent rural and suburban districts, covering an uptake area with approximately 600 000 inhabitants. Adama is situated on the highway connecting Addis Ababa and Djibouti, which is considered a high-risk corridor for HIV infection in Ethiopia. In 2005, the estimated HIV prevalence in Adama was 9%, compared with the average national prevalence of 3.5% [21].

Non-physician clinicians with 3–4 years of academic training provide care at Ethiopian public health centers. Apart from HIV services, health centers consist of several different sections, such as outpatient department, antenatal clinic, delivery service, and TB clinic. Clinicians attend the different sections according to rotating schedules; although assigned to ART clinics, they are fully responsible for care, treatment initiation, and follow-up of HIV-positive patients. In the 2008 Ethiopian National ART guidelines, ART initiation was recommended for all patients with CD4 cell counts <200 cells/ μ L, patients with WHO clinical stage 3 and CD4 cell counts <350 cells/ μ L, and patients with WHO clinical stage 4 irrespective of CD4 cell counts [22]. Since 2012, the Ethiopian National ART guidelines recommend ART initiation for all patients with CD4 cell counts <350 cells/ μ L or WHO clinical stage 4 [23]. The first-line treatment regimen for TB consists of an intensive phase with rifampicin, isoniazid, pyrazinamide, and ethambutol for 8 weeks, followed by a 4-month continuation phase with isoniazid and rifampin [23].

Study Participants

Human immunodeficiency virus-positive patients presenting to the study health centers between October 3, 2011 and March 1, 2013 were eligible for inclusion if the following criteria were met: age 18 years or greater, eligibility to start ART (defined as a documented CD4 cell count <350 cells/ μ L and/or WHO stage 4 disease, in accordance with the 2012 Ethiopian National ART guidelines [23]), and residency in the catchment area of any of the study sites. Patients with current or previous ART, as well as patients on ATT for more than 2 weeks before inclusion, were excluded. This cohort has since been continuously followed. Patients who have started ART since inclusion constitute the study population for the current study, with follow-up data collected until data abstraction on December 31, 2013.

Methods

At inclusion, detailed demographic and clinical data, including physical examination details, were collected using structured questionnaires. All patient-related study procedures were performed by health center staff who had been trained and certified to provide ART according to the national Ethiopian guidelines [22]. Blood for CD4 cell and complete blood counts was obtained, with storage of plasma for viral load measurements. In association with inclusion, all patients underwent bacteriological investigations for intensified TB case-finding. For this purpose, participants were asked to provide 2 paired morning

sputa, expectorated on consecutive days. In case of peripheral lymphadenopathy, fine-needle aspirates (FNA) were obtained. Blood and bacteriological results were communicated to the responsible clinicians who decided if and when to start ART or ATT. In accordance with Ethiopian national TB guidelines, patients could also be diagnosed and treated for TB based on clinical and radiological criteria [23].

Participants were observed until ART initiation, and at months 1, 2, 3, and 6 thereafter. At each visit, structured investigations were repeated, covering disease symptoms, adherence, and medications used, as well as physical examination. Blood tests were repeated until ART initiation (at months 3, 6, 12, and 18) and at months 1, 3, and 6 after ART initiation. After 6 months of ART, viral load testing was performed. Upon clinical suspicion of TB at any time during follow-up, the responsible clinician was encouraged to repeat TB diagnostics according to the study protocol. Tracing was recommended for patients more than 1 day late for scheduled visits.

To ensure adherence to the study protocol, the investigators (A. R., T. T. B., P. B.) or members of the research team conducted weekly visits to each health center and monitored all study data. Trained data clerks continuously entered study data into databases, with cross-checking of all entries after digitalization.

Laboratory Analyses

All laboratory analyses, except TB cultures, were performed at Adama Regional Laboratory. CD4 cell counts were analyzed using BD FACSCalibur cytometer (Becton Dickinson, San Jose, CA). Sputum and FNA samples were analyzed with direct smear microscopy using Ziehl-Neelsen staining and Xpert MTB/RIF (Cepheid, Sunnyvale, CA) for polymerase chain reaction. Liquid cultures for TB were performed at International Clinical Laboratories, Addis Ababa, using a BACTEC MGIT 960 (BD Diagnostics, Franklin Lakes, NJ).

Plasma HIV-RNA levels were determined using Abbott Real-Time HIV-1 assay (Abbott Molecular Inc., Des Plaines, IL) with a detection limit of 40 copies/mL. External quality assurance of the regional laboratory is regularly performed by the Center for Disease Control and Prevention (Atlanta, GA).

Statistical Analysis

The primary study outcome was the rate of VS after 6 months of ART in patients with prevalent TB at inclusion, compared with those without TB. Retention in care and mortality during the first 6 months after starting ART and CD4 cell count evolution during this period were secondary outcomes. Human immunodeficiency virus/TB cases were defined as subjects with either bacteriologically confirmed or clinically diagnosed TB at inclusion, or patients who started ATT within 3 months after inclusion (based on either bacteriological or clinical criteria). The 3-month follow-up period for the definition of prevalent TB was used to reduce the risk of misclassification of patients

with unrecognized active TB at baseline; subjects with TB presenting during the first 3 months of follow-up were hence considered to represent cases of prevalent TB. All other subjects were defined as HIV-only cases. Participants who received a diagnosis of TB after 3 months of follow-up were considered to represent cases of incident TB, and they were excluded from analysis.

Patients more than 3 months late for a scheduled follow-up visit were considered as lost to follow-up; in case such individuals returned to care, their follow-up was resumed. For blood test results at ART initiation, the results closest in time were chosen, allowing samples collected 6 months before and 1 week after ART initiation; similar criteria were applied for 6-month blood results, allowing samples collected between 3 and 9 months after ART initiation. The time distribution of patient outcomes (mortality and lost to follow-up) was analyzed using Kaplan-Meier curves. Time of follow-up started at ART initiation and ended at 6 months of ART, last study visit before reaching an outcome (death, lost to follow-up, transferred out, or declined further follow-up), or December 31, 2013, whichever came first. Cox proportional hazard models were used to compare HIV/TB with HIV-only, adjusting for gender, age, and CD4 cell counts at ART initiation.

Virological suppression was defined as a viral load <40 copies/mL; a separate analysis for viral load <400 copies/mL was also performed. For sensitivity analysis, patients with missing 6-month viral loads, or not in active care at the 6-month follow-up, were defined as nonvirologically suppressed.

Between groups comparisons of patient characteristics were performed using Mann-Whitney *U* test for continuous variables and the χ^2 test for categorical variables. Variables associated with VS were analyzed by logistic regression. Variables with a *P* value < .20 were entered in the multivariate analysis. Two-sided hypotheses and tests were used for all statistical inferences. *P* values < .05 were considered statistically significant. All analyses were performed using SPSS, version 21 (IBM Corp, Armonk, NY).

Ethical Approval

Ethical approval was obtained from the national Research Ethics Review Committee at the Ministry of Science and Technology of Ethiopia and the Regional Ethical Review Board of Lund University, Sweden. All study participants provided written informed consent. An impartial witness confirmed consent received from illiterate study participants.

RESULTS

Patient Characteristics

During the inclusion period, 886 patients were screened for eligibility; 812 participants (59% female) completed TB investigations (Figure 1). Characteristics of TB among these patients

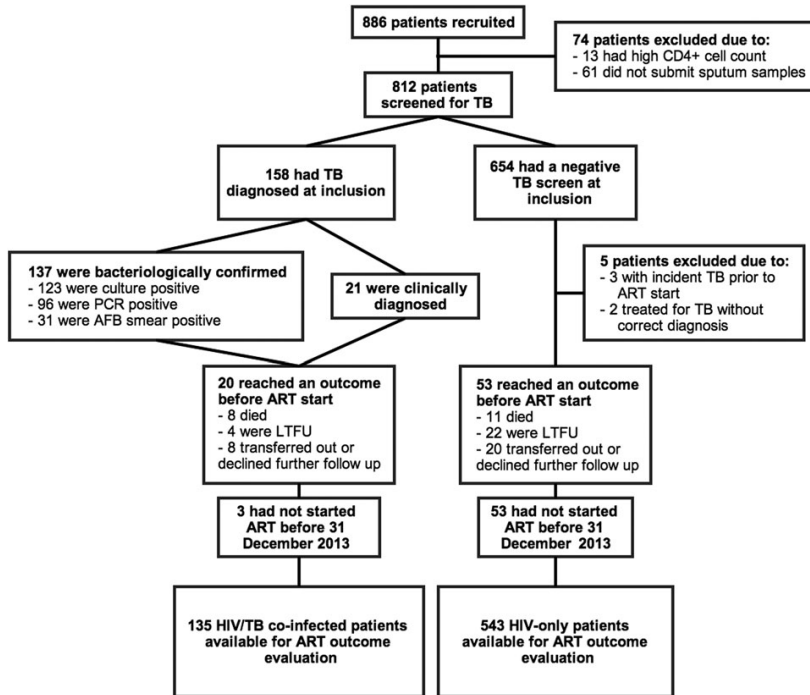


Figure 1. Flowchart of the study participants.

have been described in detail [3]. Tuberculosis was diagnosed in 158 of these subjects (137 bacteriologically confirmed, 8 clinically diagnosed at inclusion, 13 clinically diagnosed within 3 months of baseline).

Seventy-three participants discontinued study follow-up after completion of baseline investigations and before having initiated ART (Figure 1). At the time of data abstraction, the proportion of HIV/TB cases that died before starting ART was higher than that among HIV-only (8 of 158 [5.1%] vs 11 of 649 [1.7%]; $P = .04$). The median time from study inclusion until ART initiation was 14 days (interquartile range [IQR], 6–41). Three HIV/TB cases who initiated ART did not start ART during follow-up; 1 died, 1 was transferred out, and 1 started ART >6 months after ART initiation (due to long delay for TB culture result delivery).

Table 1 shows the characteristics of 678 participants who were included for analysis of ART outcomes. Five participants starting ART were excluded from this analysis (2 subjects initiated ART due to erroneously reported positive TB results; 3 patients were diagnosed with incident TB before starting ART [Figure 1]). Antiretroviral therapy was initiated at a median of

33 days (IQR, 15–116) after study inclusion, and blood samples were obtained at a median of 25 days (IQR, 8–43) before ART initiation. Most HIV/TB patients were male, in contrast to HIV-only cases. Persons with HIV/TB had lower (1) body mass index (BMI), (2) mid-upper arm circumference (MUAC), and (3) hemoglobin levels. CD4 cell counts showed a trend of being lower among HIV/TB cases, but the distribution of CD4 cell count strata was similar between the groups. Efavirenz was the most commonly used nonnucleoside reverse-transcriptase inhibitor irrespective of TB status. Among HIV/TB patients, 6 of 135 (4%) started ART within 2 weeks, 51 of 135 (38%) started ART between 2 and 8 weeks, and 43 of 135 (32%) started ART after 8 weeks of ART initiation; ART was initiated before ART in 35 of 135 (26%).

ART Outcome in Participants With and Without Concomitant TB Survival and Retention in Care

During the 6-month follow-up after ART initiation, 25 (3.7%) died, 17 (2.5%) were lost to follow-up, and 75 (11.1%) transferred out or declined further follow-up, leaving 561 (82.7%) patients in active care (Table 2). Kaplan-Meier estimates showed

Table 1. Characteristics of 678 Participants Initiating ART*

	HIV/TB (n = 135)	HIV Only (n = 543)	<i>P</i> Value ^a
Male sex, n (%)	74 (54.8)	209 (38.5)	<.01
Age, years	34 (28–42)	32 (28–40)	.25
BMI, kg/m ²	19 (17–20)	19 (18–21)	<.01
MUAC, cm	21.5 (20.0–23.0)	23.0 (21.0–25.0)	<.01
Karnofsky performance score, n (%)			.14
<70	6 (4.5)	24 (4.5)	
70 or 80	72 (54.5)	242 (45.2)	
90 or 100	54 (40.9)	269 (50.3)	
On co-trimoxazole at study inclusion, n (%)	98 (73.1)	420 (77.6)	.27
Newly enrolled in HIV care, n (%)	51 (37.8)	166 (30.7)	.12
CD4 cell count, cells/μL ^b	161 (98–243)	184 (118–256)	.05
CD4 cell strata, n (%), cells/μL			.11
<100	34 (25.8)	103 (19.3)	
100–200	53 (40.2)	203 (37.9)	
201–350	31 (23.5)	180 (33.6)	
>350	14 (10.6)	49 (9.2)	
CD4 cell percentage, %	11.0 (7.8–15.0)	11.0 (7.0–15.0)	.91
Hemoglobin, g/dL ^c	10.4 (9.2–12.0)	11.6 (10.5–12.7)	<.01
White blood cell count, ×10 ⁹ cells/L	4.7 (3.7–6.2)	4.2 (3.4–5.4)	<.01
Total lymphocyte count, ×10 ⁹ cells/L	1.3 (1.0–1.8)	1.4 (1.1–1.8)	.30
ART regimen – n (%) ^d			<.01
Nevirapine-based	10 (7.4)	113 (20.8)	
Efavirenz-based	125 (92.6)	430 (79.2)	

Abbreviations: ART, antiretroviral therapy; BMI, body mass index; HIV, human immunodeficiency virus; IQR, interquartile range; MUAC, mid-upper arm circumference; TB, tuberculosis.

* Variables presented as median (IQR), if not stated otherwise. *P* values of < .05 have been indicated in bold.

^a The *P* values were calculated using Mann-Whitney *U* or χ^2 test, as appropriate.

^b CD4 cell counts were available for 667 patients.

^c Blood cell counts were available for 639 patients.

^d Nucleoside/nucleotide components: lamivudine 100%; stavudine 2%; zidovudine 12%; tenofovir 86%.

no differences in the time distribution of events (death, lost to follow-up, or both) between HIV/TB cases and HIV-only during ART (Supplementary Figure 1). Unadjusted hazard ratios (HRs) were nonsignificant for death (HR, 1.57; 95% confidence interval [CI], .66–3.77), loss to follow-up (HR, 0.87; 95% CI, .25–3.04), and death and loss to follow-up combined (HR, 1.27; 95% CI, .62–2.58). The HRs remained nonsignificant after adjustment for age, gender, and CD4 cell count at ART initiation (Table 3).

Table 2. Patient Survival and Retention in Care During 6 Months Follow-up After ART Initiation*

	HIV/TB (n = 135)	HIV Only (n = 543)	<i>P</i> Value ^a
Patient Outcome			.38
Active in care	108 (80.0)	453 (83.4)	
Died	7 (5.2)	18 (3.3)	
Lost to follow-up	3 (2.2)	14 (2.6)	
Transferred out	7 (5.2)	11 (2.0)	
Declined further follow-up	2 (1.5)	12 (2.2)	
Data abstraction before 6-month follow-up	8 (5.9)	35 (6.4)	

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; IQR, interquartile range; TB, tuberculosis.

* Variables presented as no. (%).

^a The *P* value was calculated using χ^2 test.

Virologic Suppression

Six-month follow-up blood samples were provided a median of 25 weeks (IQR, 24–27) after ART initiation, with available viral load results for 512 of 561 (91.3%) patients. The presence of TB coinfection showed no association with the rate of VS in uni- or multivariate analysis (Supplementary Table 1). In total, 369 of 512 (72.1%) achieved a viral load <40 copies/mL; 65 of 92 (70.7%) and 304 of 420 (72.4%) for HIV/TB cases and HIV-only, respectively (*P* = .74; Table 4). Using the higher threshold (<400 copies/mL), 454 of 512 (88.7%) achieved VS; 77 of 92 (83.7%) and 377 of 420 (89.8%) for HIV/TB cases and HIV-only, respectively (*P* = .10).

In univariate analysis, using either <40 copies/mL or <400 copies/mL as definition for VS, female gender, higher BMI, and MUAC, as well as higher CD4 cell count and percentage at ART initiation were associated with higher odds of viral suppression. In multivariate analysis, female gender retained significant association with VS (*P* < .01) for viral load <40 copies/mL,

Table 3. Hazard Ratios for Mortality, Lost to Follow-up, and Combined Outcome Mortality or Lost to Follow-up Among Patients Initiating ART

	Hazard Ratios HIV/TB Coinfected versus HIV-Only			
	Unadjusted (95% CI)	<i>P</i> Value	Adjusted ^a (95% CI)	<i>P</i> Value
Died	1.57 (.66–3.77)	.31	1.35 (.53–3.48)	.53
Lost to follow-up	0.87 (.25–3.04)	.83	0.89 (.25–3.15)	.85
Died or lost to follow-up	1.27 (.62–2.58)	.51	1.15 (.54–2.45)	.71

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; HIV, human immunodeficiency virus; TB, tuberculosis.

^a Adjusted for age, sex, and CD4 cell count at ART initiation.

Table 4. Viral Suppression and CD4 Cell Distribution at 6 Months After ART Initiation*

	HIV/TB (n = 108)	HIV-Only (n = 453)	<i>P</i> Value ^a
Viral load, n (%), copies/mL ^b			
<40	65 (70.7)	304 (72.4)	.74
<400	77 (83.7)	377 (89.8)	.10
CD4 cell count, cells/μL ^c	247 (190–349)	300 (205–393)	.04
CD4 cell count, n (%), cells/μL			
<100	6 (6.5)	27 (6.8)	.29
100–200	20 (21.7)	67 (16.8)	
201–350	43 (46.7)	165 (41.5)	
>350	23 (25.0)	139 (34.9)	
CD4 cell percentage, % (IQR)	15 (12–22)	17 (12–22)	.27
CD4 cell increase, cells/μL ^d	87 (26–178)	103 (38–173)	.49

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; IQR, interquartile range; TB, tuberculosis.

* Variables presented as median (IQR), if not stated otherwise. *P* values of < .05 have been indicated in bold.

^a The *P* values were calculated using Mann-Whitney *U* or χ^2 test, as appropriate.

^b Viral loads were available for 512 patients.

^c CD4 cell counts were available for 490 patients.

^d CD4 cell increase data were available for 488 patients.

in addition to higher MUAC for viral load <400 copies/mL (Supplementary Table 1). In the sensitivity analysis, defining patients with missing 6-month viral loads or not in active care at the 6-month follow-up as nonvirologically suppressed, the proportion achieving VS (<40 copies/mL) was 369 of 641 (57.6%); 65 of 128 (50.8%) and 304 of 513 (59.3%) for HIV/TB cases and HIV-only, respectively (*P* = .08). In multivariate analysis, female gender remained significantly associated with VS (Supplementary Table 2).

CD4 Cell Evolution

At baseline, median CD4 cell counts were slightly lower in HIV/TB-coinfected subjects (Table 1). This difference remained and was statistically significant at 6 months: median CD4 cell counts 247 and 300 cells/μL (*P* = .04) for HIV/TB and HIV-only, respectively (Table 4). However, the median increase of CD4 cell counts during ART did not show significant difference with regard to TB status: median CD4 cell counts increase 87 and 103 cells/μL (*P* = .49) for HIV/TB and HIV-only, respectively.

DISCUSSION

In this prospective cohort study performed at Ethiopian health centers, no difference in the rates of VS at 6 months after ART

initiation was detected with regard to the presence of concomitant TB and ATT. Furthermore, TB coinfection did not affect rates of mortality and retention in care during ART. This finding provides support for current recommendations to initiate ART during the course of ATT for subjects with HIV/TB coinfection, and it demonstrates the feasibility of this strategy even at primary healthcare level in resource-limited settings.

Our results on ART outcome in HIV/TB-coinfected subjects are in agreement with a recent review [18]. Viral suppression (using the definition of viral load <400 copies/mL) was achieved by 89% of participants, with no difference with regard to TB coinfection. There was no difference between the 2 groups when using a stricter definition of VS (<40 copies/mL), although suppression was less pronounced (72%). In multivariate analysis, male gender was the only variable found to be associated with a lower likelihood of achieving VS.

The rates of VS found in this cohort are comparable with those reported elsewhere [13, 16, 24]. However, most previous studies on the outcome of ART in TB patients have been based in hospitals or specialized ART clinics. Our study provides confirmatory data from an Ethiopian primary healthcare setting, typical for low-income countries in sub-Saharan Africa where great proportions of people living with HIV and TB are managed.

For comparison of VS rates, we included subjects for whom a viral load result obtained at 6 months after ART initiation was available. In an attempt to assess whether retention in care or availability of samples for viral load testing could have affected our results, we performed a sensitivity analysis, defining patients with missing viral load results, or not in active care at the 6-month follow-up, as non-VS; yet, TB coinfection remained nonsignificantly associated with VS (*P* = .08).

Despite having a higher frequency of negative prognostic characteristics (such as lower BMI, MUAC, and hemoglobin levels), HIV/TB cases did not manifest increased mortality during ART. Furthermore, a greater proportion of these persons were of male gender, which has also been associated with higher mortality [25]. Although some studies have reported an increased risk of death in TB-coinfected subjects starting ART [5], a recent meta-analysis did not identify any such difference for short-term mortality [26]; however, the risk of death was elevated at 1 year after ART initiation in HIV/TB-coinfected patients. For the current analysis, we restricted follow-up to 6 months after ART, because we mainly wished to exclude a potential negative impact of concomitant ATT on ART outcome. The observation that long-term outcome may be compromised in TB-coinfected patients suggests that other factors are involved [27, 28]; such as delayed immune recovery among patients with HIV/TB coinfection [29, 30]. In our cohort, TB cases tended to have lower CD4 cell levels than subjects without TB at inclusion. Despite similar increases in CD4 cell counts, HIV/TB-coinfected persons had significantly lower median

CD4 cell counts after 6 months of ART, with >25% having levels <200 cells/ μ L.

Our study participants consisted of HIV-positive adults who fulfilled criteria for starting ART; at inclusion, all patients underwent microbiological investigation for TB. These investigations led to detection of active TB in 137 patients (16.9%), among whom only 13 had been diagnosed with TB previously [3]. It is likely that this active case-finding approach contributed to satisfactory ART outcomes. Incident TB has been associated with poor ART outcome [31], and by screening all patients several cases were probably detected at less advanced stages of TB disease. Yet, 5.1% of HIV/TB-coinfected patients died before ART initiation compared with 1.7% among those with negative TB screening. This result illustrates the need for early ART initiation during ATT, especially for individuals with advanced immunosuppression [8–10].

Our study has some limitations. It was conducted in a limited uptake area; however, we consider the participating health centers to be representative of primary healthcare in sub-Saharan Africa, and except for the intensified TB screening at study inclusion, no special interventions were introduced that might have improved treatment outcomes. We did not analyze adherence data or rates of adverse events (including IRIS) in the 2 groups. Furthermore, we did not analyze viral load at baseline. Tuberculosis coinfection is associated with significantly increased HIV replication [32], yet rates of viral suppression at 6 months of ART were similar in subjects with and without TB, suggesting that this factor has not obscured a potential disadvantage in coinfecting patients. Despite the use of intensified TB case-finding at baseline, it is possible that some cases of extrapulmonary TB were missed, which could have led to misclassification. To reduce this risk, we used a follow-up period of 3 months after inclusion for the definition of prevalent TB. However, the occurrence of TB after inclusion was low.

In conclusion, rates of VS were high; however, no significant difference was found with regard to the presence of concomitant TB in these HIV-positive adults receiving care at Ethiopian health centers managed by non-physician clinicians. These findings demonstrate the feasibility of combined ART and ATT at primary healthcare level in low-income countries.

Supplementary Material

Supplementary material is available online at *Open Forum Infectious Diseases* (<http://OpenForumInfectiousDiseases.oxfordjournals.org/>).

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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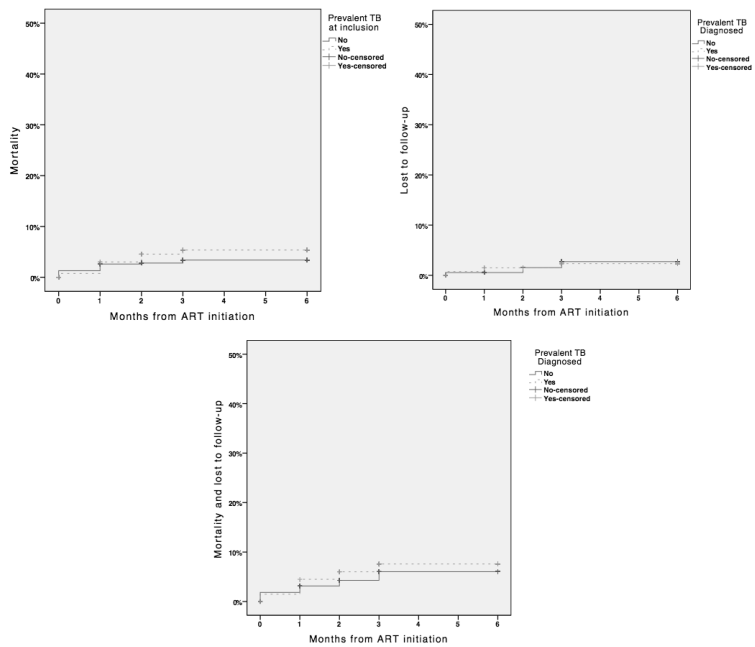
Supplementary Table 1. Univariate and multivariate odds ratios for viral suppression defined as viral load <40 copies/mL or <400 copies/mL at 6 months after ART initiation.

	Viral load <40 copies/mL ^a			Viral load <400 copies/mL ^a		
	OR (95% CI)	P	Multivariate OR (95% CI)	OR (95% CI)	P	Multivariate OR (95% CI)
Variables at study inclusion:						
Age	1.0 (1.0-1.0)	0.95		1.0 (1.0-1.1)	0.29	
Gender						
Male	1.0		1.0	1.0		1.0
Female	2.3 (1.5-3.4)	<0.01	2.6 (1.7-4.0)	2.5 (1.5-4.4)	<0.01	2.7 (1.5-5.1)
On co-trimoxazole prophylaxis						
No	1.0			1.0		1.0
Yes	0.9 (0.5-1.4)	0.49		0.5 (0.2-1.1)	0.10	0.5 (0.2-1.2)
Newly enrolled in HIV care						
No	1.0			1.0		
Yes	1.0 (0.7-1.6)	0.88		0.8 (0.5-1.5)	0.52	
HIV/TB co-infection						
No	1.0			1.0		1.0
Yes	0.9 (0.6-1.5)	0.73		0.6 (0.3-1.1)	0.10	0.7 (0.3-1.4)
Variables at ART initiation:						
BMI (per 1 kg/m ² higher)	1.1 (1.0-1.2)	<0.01	1.0 (0.9-1.1)	1.1 (1.0-1.3)	0.03	1.0 (0.8-1.1)
MUAC (per cm greater)	1.1 (1.1-1.2)	<0.01	1.1 (1.0-1.3)	1.2 (1.1-1.3)	<0.01	1.2 (1.0-1.5)
Kamofsky score		0.21			0.35	
<70	1.0			1.0		
70 or 80	0.9 (0.4-3.6)			1.8 (0.6-5.7)		
90 or 100	1.3 (0.5-3.6)			2.3 (0.7-7.4)		
CD4 cell count, cells/ μ L						
<100	1.0	0.03	1.0	1.0	<0.01	1.0
100-200	1.4 (0.8-2.3)		1.1 (0.6-2.0)	2.9 (1.5-5.8)		2.5 (1.2-5.4)
>200	2.0 (1.2-3.3)		1.4 (0.7-2.7)	3.0 (1.6-5.9)		1.9 (0.8-4.4)
CD4 cell percentage (per 1% higher)	1.0 (1.0-1.1)	0.01	1.0 (1.0-1.1)	1.1 (1.0-1.1)	0.02	1.0 (1.0-1.1)
Hemoglobin (per 1 g/dL higher)	1.1 (1.0-1.2)	0.07	1.1 (0.9-1.2)	1.1 (1.0-1.3)	0.11	1.0 (0.8-1.2)
Treatment-related variables:						
ART regimen						
Nevirapine-based	1.0			1.0		1.0
Efavirenz-based	1.2 (0.8-2.0)	0.41		1.6 (0.9-3.0)	0.14	2.0 (1.0-4.2)

P values of <0.05 have been indicated in bold.

Abbreviations: ART, antiretroviral therapy; OR, odds ratio; CI, confidence interval; BMI, body mass index; MUAC, mid-upper arm circumference.

^a Viral load results were available for 512/561 (91.2%) patients reaching 6-month follow-up.



Supplemental Figure 1. Kaplan-Meier plots depicting mortality, lost to follow-up, and mortality and lost to follow-up combined.

Supplementary Table 2. Uni- and multivariate odds ratios for viral suppression (<40 copies/mL) at 6 months after ART initiation, defining cases not in active care, or having missing viral load results, as non-virologically suppressed.

	Viral load <40 copies/mL			
	Univariate OR (95% CI)	P	Multivariate OR (95% CI)	P
Variables at study inclusion:				
Age	1.0 (1.0-1.0)	0.86		
Gender				
Male	1.0		1.0	
Female	2.1 (1.6-3.0)	<0.01	2.5 (1.7-3.5)	<0.01
On CoTrim prophylaxis				
No	1.0			
Yes	1.0 (0.7-1.5)	0.99		
Newly enrolled in HIV care				
No	1.0			
Yes	0.8 (0.6-1.1)	0.21		
HIV/TB co-infection				
No	1.0		1.0	
Yes	0.7 (0.5-1.1)	0.08	1.0 (0.7-1.6)	0.86
Variables at ART initiation:				
BMI (per 1 kg/m ² higher)	1.1 (1.1-1.2)	<0.01	1.0 (0.9-1.1)	0.58
MUAC (per cm greater)	1.1 (1.1-1.2)	<0.01	1.1 (1.0-1.2)	0.33
Karnofsky score		0.05		0.81
<70	1.0		1.0	
70 or 80	1.8 (0.8-3.9)		1.2 (0.5-2.8)	
90 or 100	1.2 (0.6-2.7)		1.1 (0.5-2.5)	
CD4 cell count, cells/ μ L		<0.01		0.28
<100	1.0		1.0	
100-200	1.3 (0.9-2.0)		1.0 (0.6-1.7)	
>200	2.0 (1.3-3.1)		1.4 (0.8-2.5)	
CD4 cell percentage (per 1% higher)	1.0 (1.0-1.1)	0.01	1.0 (1.0-1.0)	0.65
Hemoglobin (per 1 g/dL higher)	1.1 (1.0-1.2)	0.02	1.1 (1.0-1.2)	0.22
Treatment-related variables:				
ART regimen				
Nevirapine-based	1.0			
Efavirenz-based	1.1 (0.8-1.7)	0.53		

P values of <0.05 have been indicated in bold.

Abbreviations: ART, antiretroviral therapy; OR, odds ratio; CI, confidence interval; CoTrim, co-trimoxazole; BMI, body mass index; MUAC, mid-upper arm circumference.

Paper II



RESEARCH ARTICLE

Factors Associated with Early Mortality in HIV-Positive Men and Women Investigated for Tuberculosis at Ethiopian Health Centers

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Abstract

Introduction

Despite increasing access to antiretroviral treatment (ART) in low-income countries, HIV-related mortality is high, especially in the first months following ART initiation. We aimed to evaluate the impact of TB coinfection on early mortality and to assess gender-specific predictors of mortality in a cohort of Ethiopian adults subjected to intensified casefinding for active TB before starting ART.

Material and Methods

Prospectively recruited ART-eligible adults (n = 812, 58.6% female) at five Ethiopian health centers were followed for 6 months. At inclusion sputum culture, Xpert MTB/RIF, and smear microscopy were performed (158/812 [19.5%] had TB). Primary outcome was all-cause mortality. We used multivariate Cox models to identify predictors of mortality.

Results

In total, 37/812 (4.6%) participants died, 12 (32.4%) of whom had TB. Karnofsky performance score (KPS) and mid-upper arm circumference (MUAC) were associated with mortality in the whole population. However, the associations were different in men and women. In men, only MUAC remained associated with mortality (adjusted hazard ratio [aHR] 0.71 [95% CI 0.57–0.88]). In women, KPS <80% was associated with mortality (aHR 10.95 [95% CI 2.33–51.49]), as well as presence of cough (aHR 3.98 [95% CI 1.10–14.36]). Cough was also associated with mortality for TB cases (aHR 8.30 [95% CI 1.06–65.14]), but not for non-TB cases.

Competing Interests: The authors have declared that no competing interests exist.

Conclusions

In HIV-positive Ethiopian adults managed at health centers, mortality was associated with reduced performance score and malnutrition, with different distribution with regard to gender and TB coinfection. These robust variables could be used at clinic registration to identify persons at increased risk of early mortality.

Introduction

Although survival in people living with HIV (PLWH) has increased during the last decade as a result of expanding access to antiretroviral treatment (ART), HIV-related mortality remains high, especially in sub-Saharan Africa where 800,000 deaths were caused by HIV/AIDS in 2014 [1]. The risk of death among PLWH mainly depends on the presence of coinfections and the severity of immunosuppression at ART initiation [2–4]. Since clinical and immunological outcomes are better if ART is started before advanced immunosuppression has developed [5,6], current WHO guidelines recommend that ART should be initiated in everyone living with HIV irrespective of CD4 cell count [7].

Despite these revised criteria for ART, large proportions of PLWH still present with advanced immunosuppression at HIV diagnosis. According to a recent meta-analysis estimating temporal trends in disease status at presentation to care and ART initiation, mean CD4 cell counts did not increase significantly from 2002 to 2012 in sub-Saharan Africa [8]. Although all PLWH have an indication to start ART, subjects with advanced immunosuppression must be identified and prioritized for early ART initiation due to their elevated risk of death [7]. This is illustrated by studies from South Africa that show great accumulated mortality with delayed treatment initiation during the first month after registration for patients with CD4 cell counts <50 cells/ μ L and/or WHO stage IV disease [4,9].

Besides CD4 cell count at ART initiation, other factors may be involved in HIV-related mortality. For example, low body-mass index (BMI), anaemia, and increasing age have been associated with elevated risk of death in PLWH starting ART [2,10,11]. Risk factors for death in HIV infection may also vary with regard to gender. Male PLWH are often reported to have excess mortality after starting ART compared to female PLWH [10], but gender-specific risk factors for mortality have not been thoroughly studied [12].

Importantly, the underlying causes of death in PLWH show regional variations, and are incompletely understood. In sub-Saharan Africa, most fatalities in PLWH are considered to be caused by tuberculosis (TB) [13]. This is supported by autopsy studies [14–16], revealing high rates of TB, also in persons who had been investigated for TB before death [3]. Although correct diagnosis and treatment of TB is recognized as a critical component of HIV care [17], identification of TB in PLWH is difficult, and it is likely that active TB is missed in many persons starting ART in resource-limited settings.

We have previously presented data on TB coinfection and ART outcomes in a cohort of treatment-naïve HIV-positive adults who met criteria for ART recruited at Ethiopian health centers [18]. Despite intensified casefinding for TB and access to therapy for both TB and HIV around 5% of participants died during the first 6 months after inclusion. We hypothesized that robust clinical characteristics present at inclusion, might be used to identify individuals at high risk of 6-month mortality in routine health care. Since both TB coinfection and male gender have been associated with excess mortality in other studies, we determined predictors of early mortality in this cohort specifically for men and women as well as for participants with and

without TB coinfection. Furthermore, considering that most patients who present to HIV care do not initiate ART directly at presentation, we analyzed mortality from the time of first clinic visit instead of from the time of ART initiation.

Material

The cohort used for this study was recruited to determine the prevalence of active TB in ART eligible patients at Ethiopian health centers, to evaluate different diagnostic methods for TB and to determine the outcome of ART and TB treatment. The cohort and study setting have previously been described in detail [18,19]. In brief, PLWH presenting at all five public health centers providing HIV care in the city of Adama, Oromia region, central Ethiopia, and adjacent rural and suburban districts were assessed for eligibility. The health centers (2 urban, 2 semi-urban, 1 rural) are situated on the highway connecting Addis Ababa and Djibouti, which is considered to be a high-risk corridor for HIV infection in Ethiopia. Non-physician clinicians (nurses or health officers with 3–4 years of academic training) deliver all care at these health centers. Inclusion criteria for the cohort were: age 18 years or greater, indication for ART according to Ethiopian guidelines at the time of recruitment (CD4 cell count <350 cells/ μ L and/or WHO stage IV), and residency within the catchment area. Patients with previous or current ART and/or TB treatment for more than 2 weeks at the time of screening were excluded. Participants were recruited from October 2011 until March 2013. The cohort is continuously followed with regular study visits to evaluate the long-term outcome of ART.

Methods

At inclusion, trained non-physician clinicians collected detailed clinical data, including findings from physical examination, following a structured questionnaire. All participants (irrespective of symptoms) were requested to provide sputum samples for TB diagnostics by liquid culture, GeneXpert MTB/RIF, and smear microscopy. In case of peripheral lymphadenopathy, TB diagnostics were performed on fine-needle aspirates. Patients who did not submit sputum samples were excluded from the cohort since they could not be categorized for TB. Blood samples were obtained for complete blood and CD4 cell counts. Results from blood tests and TB investigations were available to the responsible clinicians. Detailed description of the TB diagnostic procedure has been published previously [19]. For this study a participant was defined as a TB case either by bacteriological confirmation (positive result by any of the three bacteriological methods) or clinical criteria. Cases of clinically diagnosed TB fulfilled criteria according to the Ethiopian National TB guidelines (symptoms and/or signs compatible with active TB supported by radiological results and lack of response to antibiotic therapy) [20]. All TB cases diagnosed within 3 months after inclusion were considered as prevalent TB.

Follow-up visits were scheduled at months 1, 2, 3, and 6 after study inclusion. In case of symptoms or signs suggestive of TB during follow-up study clinicians were encouraged to repeat TB diagnostics according to the baseline protocol. Health extension workers traced patients with missed appointments by telephone and home visits. The health center clinicians were responsible for starting ART and treating opportunistic infections (including TB) in accordance with national guidelines [20,21], without the involvement of the study investigators. Study data was continuously entered into an electronic database with regular crosschecking by trained data clerks.

Statistical analysis

The primary outcome for this study was all-cause mortality within 6 months of study inclusion. Analysis of predictors for this outcome was based on characteristics collected at study inclusion.

This analysis was first performed for the whole population, and then separately with regard to gender and TB status, respectively. Time-at-risk was defined as days from inclusion until time of death or 6 months after inclusion. Patients more than 3 months late for a scheduled visit were defined as lost to follow-up (LTFU) and their follow-up time was right censored at the last study visit. For patients who transferred their care to other facilities or who declined further follow-up similar right censoring was used.

Patient characteristics were summarized using frequencies and percentages for categorical variables and median and interquartile range (IQR) for continuous variables. For all continuous variables, plots of beta estimates for the primary outcome for each quintile of the variable were assessed to identify each variable's functional form in order to define appropriate cut-off values. Mid-upper arm circumference (MUAC) and BMI were kept as continuous variables. CD4 cell count was categorized by quartiles, and haemoglobin according to the WHO anaemia cut-offs, merging no anaemia (haemoglobin ≥ 13.0 and ≥ 12.0 g/dL for men and women, respectively) and mild anaemia (haemoglobin 11.0–12.9 g/dL and 11.0–11.9 g/dL for men and women, respectively). Karnofsky performance score (KPS) was dichotomized at $<80\%$ differentiating those unable to carry out normal activities or do active work. Patient characteristics for male and female participants, as well as for TB cases and non-TB cases, were compared using χ^2 -test for categorical, and Mann-Whitney's U-test for continuous variables.

Kaplan-Meier survival analyses were used to investigate overall survival from inclusion, using the log rank test to compare male and female participants as well as TB cases and non-TB cases, respectively. Cox proportional hazards models were used to investigate baseline variables' correlation with mortality. In order to further assess associations with mortality, four separate models were fitted for males, females, TB cases, and non-TB cases, respectively. All variables considered for the Cox models were assessed for the proportional hazards assumption using Kaplan-Meier and log minus log plots, and by analysing each variables' interaction with time. Only variables fulfilling the assumption were included in the final models.

Variables from each univariate model with a p value <0.3 were entered into multivariate models, whereby the least significant variables were removed using a backward stepwise procedure until only variables with $p < 0.05$ remained in the model. At each step, the beta estimates of variables remaining in the model were analysed for possible interaction with the variable removed. A change in beta estimate of $>20\%$ was considered to indicate a possible interaction, which then was further evaluated. The multivariate models were adjusted for age and CD4 cell count, as well as ART status, included as a time-varying 0/1 variable. Time-to-ART was defined as days from study inclusion until start of ART.

To account for missing data, secondary analyses were performed in which the multivariate models were refitted excluding variables with $<95\%$ available data from the start of the stepwise procedure. The final model generated was then compared with the original model to assess the possible bias derived from cases with missing data.

For all analyses, a p value <0.05 was considered to indicate statistical significance. All analyses were performed using SPSS, version 21 (IBM Corp, Armonk, NY).

Ethical approval

Ethical approval was obtained from the National Research Ethics Committee at the Ministry of Science and Technology of Ethiopia and the Regional Ethical Review Board of Lund University, Sweden. All study participants provided written informed consent.

The health center clinicians were responsible for all aspects of HIV and TB care in line with national guidelines. All laboratory results were communicated back to the responsible clinician, including all TB results. No treatments were withheld from the patients due to this study.

Results

Participants

A total of 886 PLWH were screened for eligibility; 74 were excluded and 812 participants (58.6% female) were included in the study cohort (Fig 1). Among these, 158 (19.5%) were diagnosed with TB (137 [86.7%] bacteriologically confirmed, 21 [13.3%] clinically diagnosed, Fig 1). Characteristics of study participants are shown in Table 1. The median age of men was higher than that of women ($p < 0.01$). Median CD4 cell counts were lower in men compared to women ($p = 0.01$), and in subjects with compared to those without TB ($p < 0.01$). No case of incident TB was diagnosed during the 6-month follow-up period.

Survival and retention in care

At 6 months after inclusion 679 participants (83.6%) remained in care, 37 (4.6%) were confirmed dead, 42 (5.2%) LTFU, 35 (4.3%) had registered transfer of care, and 20 (2.5%) declined further follow-up. ART was started in 564 (69.5%) subjects a median of 27 days (IQR 12–57) after inclusion. The median time to death for the 37 deceased participants was 54 days (IQR 30–87); 18 (48.6%) died before and 19 (51.4%) died after starting ART. In the latter group, death occurred a median of 51 days (IQR 33–72) after starting ART. In total, 12 (32.4%) of the deceased participants had been diagnosed with TB; 8 (66.7%) of these had not started ART and 4 (33.3%) died after having started ART (Table 2). Among the 25 deceased non-TB cases 10 (40.0%) had not started ART and 15 (60.0%) died after having started ART. The median age of deceased TB cases was 30 years (IQR 28–35; 5 male, 7 female), as compared to 35 years (IQR 28–42; 15 male, 10 female) for non-TB cases.

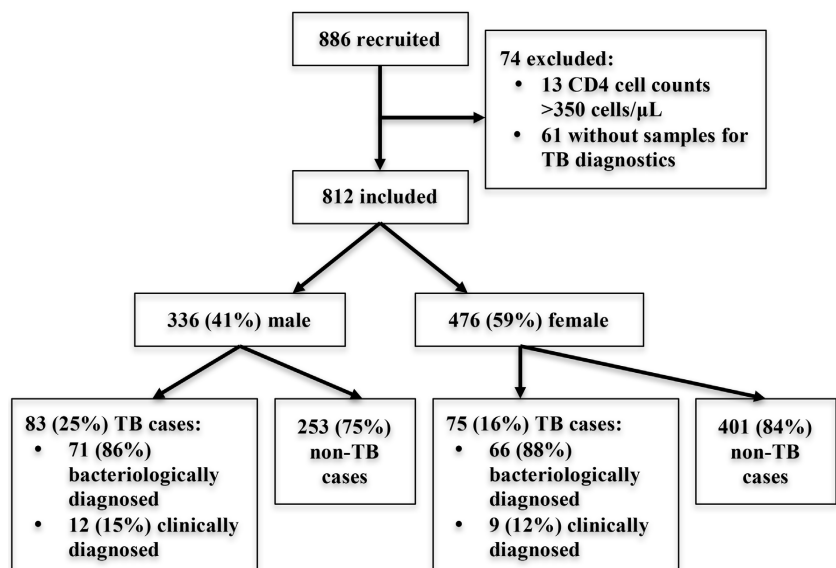


Fig 1. Flow chart of study participants.

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Table 1. Characteristics of study participants at inclusion.

	All n = 812	Male n = 336	Female n = 476	P ¹	TB cases n = 158	Non-TB cases n = 654	P ²
Age—years	32 (28–40)	35 (30–42)	30 (26–36)	<0.01	34 (28–40)	32 (28–39)	0.18
Gender—female	476 (59)	—	—		75 (47)	401 (61)	<0.01
BMI—kg/m ²	18.9 (17.3–21.0)	18.8 (17.2–20.3)	19.1 (17.4–21.6)	<0.01	17.7 (16.3–19.6)	19.2 (17.6–21.3)	<0.01
MUAC—cm	23 (21–24)	23 (21–24)	22 (20–24)	0.51	21 (19–23)	23 (21–25)	<0.01
CD4 cell count—cells/ μ L	208 (116–320)	191 (104–302)	219 (132–331)	0.01	170 (91–273)	220 (127–328)	<0.01
>300	229 (28)	85 (26)	144 (30)		33 (21)	196 (30)	
201–300	197 (24)	76 (23)	121 (26)		27 (17)	170 (26)	
100–200	223 (28)	93 (28)	130 (27)		53 (34)	170 (26)	
<100	158 (20)	78 (24)	80 (17)		45 (28)	113 (17)	
CD4 cell count—%age	12 (8–17)	11 (7–16)	13 (9–17)	<0.01	10 (9–12)	12 (8–17)	0.41
TB coinfection	158 (19)	83 (25)	75 (16)	<0.01	—	—	
Haemoglobin, g/dL				0.11			<0.01
>10.9	467 (61)	206 (66)	261 (58)		56 (38)	411 (67)	
8.0–10.9	268 (35)	97 (31)	171 (38)		79 (53)	189 (31)	
<8.0	27 (4)	11 (4)	16 (4)		14 (9)	13 (2)	
In care at study inclusion	557 (69)	218 (65)	339 (72)	0.05	93 (59)	464 (71)	<0.01
HIV test due to symptoms	442 (55)	205 (61)	237 (50)	<0.01	107 (68)	335 (51)	<0.01
Signs and symptoms							
Weight loss	514 (64)	238 (71)	276 (58)	<0.01	130 (82)	384 (59)	<0.01
Appetite loss	401 (50)	168 (50)	233 (49)	0.71	101 (64)	300 (46)	<0.01
Fatigue	553 (69)	233 (71)	320 (69)	0.56	131 (85)	422 (66)	<0.01
Fever	389 (48)	165 (49)	224 (47)	0.58	100 (64)	289 (44)	<0.01
Cough	326 (40)	149 (44)	177 (37)	0.04	98 (62)	228 (35)	<0.01
Night sweats	396 (49)	174 (52)	222 (47)	0.16	113 (72)	283 (43)	<0.01
Conjunctive pallor	126 (16)	58 (18)	68 (14)	0.22	41 (26)	85 (13)	<0.01
Karnofsky score <80%	275 (34)	120 (36)	155 (33)	0.35	89 (56)	186 (28)	<0.01
WHO clinical stage				0.01			<0.01
Stage 1	141 (17)	54 (16)	87 (18)		13 (8)	128 (20)	
Stage 2	246 (30)	84 (25)	162 (34)		31 (20)	215 (33)	
Stage 3	322 (40)	146 (44)	176 (37)		76 (48)	246 (38)	
Stage 4	100 (12)	50 (15)	50 (11)		28 (24)	62 (10)	

Data presented as n (%), or median (interquartile range). Data available for >97% for all variables, except haemoglobin with 762/812 (93.8%) available data.

Abbreviations: BMI, body-mass index; MUAC, mid-upper arm circumference; TB, tuberculosis.

¹P value comparing male and female using χ^2 test for categorical and Mann-Whitney's U-test for continuous variables.

²P value comparing TB cases and non-TB cases using χ^2 test for categorical and Mann-Whitney's U-test for continuous variables.

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No significant difference was found when comparing survival by gender ($p = 0.10$) using Kaplan-Meier plots. Non-TB cases had significantly better survival than TB cases ($p = 0.03$, Fig 2).

Factors associated with mortality

Complete univariate hazard ratios for all tested variables are presented in S1 Table.

In the multivariate analysis for the entire cohort, KPS < 80% and MUAC were independently associated with mortality (Table 3). The adjusted hazard ratio (aHR) for KPS < 80% was 4.30

Table 2. Mortality distribution with regard to ART, gender and TB status.

	Mortality		
	Total	Before ART start	After ART start
All	37/812 (4.6)	18 (48.6)	19 (51.4)
By gender:			
Male	20/336 (6.0)	9 (45.0)	11 (55.0)
Female	17/476 (3.6)	9 (52.9)	8 (47.1)
By TB status:			
TB-cases¹	12/158 (7.6)	8 (66.7)	4 (33.3)
Non-TB cases	25/654 (3.8)	10 (40.0)	15 (60.0)

Presented as n/N (%) and n (%). Abbreviations: ART, antiretroviral treatment.

¹6 (50.0%) of TB cases died before starting TB treatment.

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(95% CI 1.84–10.08). For each centimeter increase in MUAC the risk of mortality decreased with an aHR 0.82 (95% CI 0.71–0.94).

Among men MUAC had a stronger negative association with mortality, aHR 0.71 (95% CI 0.57–0.88); however, KPS<80 was not associated with mortality in men. Furthermore, male participants with CD4 cell counts <100 cells/ μ L had an aHR of 6.80 (95% CI 1.37–33.78) compared with CD4 cell counts >300 cells/ μ L.

For women KPS<80% had the overall strongest correlation with mortality with aHR 10.95 (95% CI 2.33–51.49), but the association between MUAC and mortality did not reach statistical

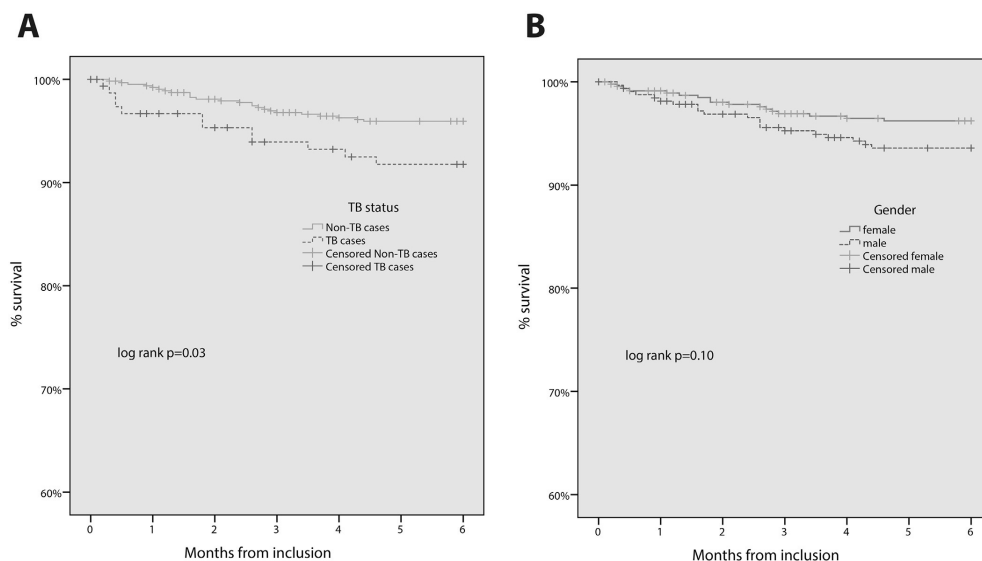


Fig 2. Kaplan-Meier plots comparing survival by gender (A) and tuberculosis coinfection (B).

doi:10.1371/journal.pone.0156602.g002

Table 3. Final multivariate Cox models for mortality after stepwise removal of non-significant variables; five separate models for all, males, females, TB cases and non-TB cases.

Subgroup and variables	aHR (95% CI)
All	
Karnofsky score <80%	4.30 (1.84–10.08)
MUAC—per cm increase	0.82 (0.71–0.94)
Male	
MUAC—per cm increase	0.71 (0.57–0.88)
CD4 cell count—cells/ μ L	
>300	1.00
201–300	1.82 (0.25–13.23)
100–200	1.95 (0.34–11.18)
<100	6.80 (1.37–33.78)
Female	
Karnofsky score <80%	10.95 (2.33–51.49)
Reported cough	3.98 (1.10–14.36)
TB cases	
Reported cough	8.30 (1.06–65.14)
Non-TB cases	
Gender, male vs. female	2.53 (1.05–6.12)
Karnofsky score <80%	4.89 (1.81–13.19)
MUAC—per cm increase	0.76 (0.63–0.91)

Abbreviations: aHR, adjusted hazard ratio; CI, confidence interval; MUAC, mid-upper arm circumference. Adjusted for age as a continuous variable, CD4 cell counts as a categorical variable and antiretroviral treatment as a time-varying variable.

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significance. Reported cough at study inclusion was independently associated with an increased risk of early mortality for female participants, aHR 3.98 (1.10–14.36).

In the multivariate model for TB cases, reported cough was the only variable that remained in the final model, aHR 8.30 (1.06–65.14).

Among participants not diagnosed with TB male gender was associated with mortality (aHR 2.53 [95% CI 1.05–6.12]), as well as KPS<80% aHR 4.89 (1.81–13.19) and MUAC aHR 0.76 (95% CI 0.63–0.91) per centimeter increase (Table 3).

Haemoglobin was missing for 50 (6.2%) participants. Therefore, all multivariate models were refitted excluding anaemia from the start. The final models did not change.

Discussion

In this cohort of HIV-positive Ethiopian adults, mortality during the first 6 months after inclusion was associated with reduced performance score and malnutrition (assessed by MUAC measurement). As part of the cohort study protocol, all participants had been investigated for active TB at inclusion. Although this resulted in detection of TB in 19.5% of subjects, persons with TB were at increased risk of death.

In contrast to other studies [2,10,11,22], we did not observe gender-related differences in the risk of death. It is possible that we did not observe a statistically significant survival difference for men versus women due to insufficient statistical power. However, we did find that factors associated with early mortality showed clear variations depending on gender. Thus, the significant association with lower MUAC was restricted to men, and low KPS to women.

Interestingly, CD4 cell count—considered the most important predictor of HIV progression—was independently associated with mortality only in the subset of men with levels <100 cells/ μL , as compared to men with >300 cells/ μL . Among reported symptoms, cough was associated with death in women. This symptom was also the only variable that showed independent association with death among TB-cases.

In this study, time-at-risk started at the time of study inclusion and we did not restrict the analysis of mortality to those who actually started ART. This design allowed us to estimate the proportion and risk factors for death also in ART-eligible persons who died before starting treatment. The median time between inclusion and death for deceased subjects was short (48 days), and they had advanced disease at presentation. In particular, a greater proportion had TB [18,19].

In the current WHO guidelines all PLWH should start ART [7]. However, HIV/TB coinfecting patients should start antituberculosis treatment before ART. Coinfecting patients with profound immunosuppression should receive ART immediately within the first 2 weeks of TB treatment. Otherwise ART should be started as soon as possible within the first 8 weeks of TB treatment [23]. These recommendations are based on three key clinical trials that showed a significantly improved survival for coinfecting patients if ART was started during the course of TB treatment [24–26]. Ethiopian guidelines for ART are concordant with the WHO recommendations [20]. It is possible that earlier consideration of ART for the study participants in our cohort that died could have improved survival, although our study was not designed to determine this.

In this study we aimed to include potential markers of mortality risk that would be possible to use at health center level in low-income countries. Based on previous findings from our own studies and on the scientific literature, we hypothesized that poor performance status and malnutrition would be associated with mortality. Karnofsky performance score was chosen as we wanted a general, standardized, and established measure of the performance status of participants that could be easily recorded by health care workers. Malnutrition can be measured using BMI and/or MUAC. Both our research group and others have shown independent association between MUAC and mortality in patients with TB [27,28]. Furthermore, we have also shown that low MUAC is associated with prevalent TB in HIV-positive adults [29]. A benefit of MUAC over BMI is the minimal need of equipment; all that is needed is a measuring tape. In line with our hypotheses, we found that low performance status and MUAC were independently associated with the risk of early death, whereas the level of CD4 cells did not have capacity to predict mortality. This suggests that these simple measurements might be used at HIV clinics to identify high-risk individuals. What interventions should then be undertaken to reduce the risk of death in patients manifesting these “warning signs”? Such persons should be prioritized for early ART initiation, which leads to improved survival [4,9]. Yet, half of patients who died had started ART. In agreement with another cohort study from a resource-limited setting most of these deaths occurred within the first 3 months after starting ART (in median 51 days after ART) [4].

Unfortunately, causes of death were not determined for our participants. This reflects the usual situation in low-income countries, and highlights the importance of detailed post-mortem investigation studies. We could ascertain, primarily by telephone calls to the family of the deceased, that the deaths in our study were due to illness and not attributable to accidents or other unnatural causes. It is possible that use of verbal autopsies could have elucidated causes of death with some greater accuracy [30]. In autopsy studies from sub-Saharan Africa TB has been reported as a leading cause of death; Wong et al found mycobacterial infections to be implicated in 69% of deaths in a cohort of HIV-positive adults in Johannesburg, South Africa

[15], and Bates et al reported TB in 66/101 (65%) HIV-positive patients autopsied in Lusaka, Zambia [16].

In our cohort, all participants had undergone active TB casefinding at inclusion, leading to diagnosis of TB in 158/812 persons, which in most cases (91.8%) had not been identified previously. Among participants not diagnosed with TB in our cohort, mortality was associated with male gender, low KPS and MUAC. Interestingly, these latter variables have also been found to be independently associated with prevalent TB in this cohort [29]. Self-reported cough was independently associated with death in women but not in men; in fact, among the non-TB cases that died 80.0% of women reported cough compared with 33.3% of the men. This phenomenon may be related to higher rates of poor quality sputum samples in women than men [31], and could indicate missed cases of pulmonary TB in women. Since our diagnostic protocol was focused on pulmonary TB it is possible that some cases of TB, in particular extrapulmonary disease, may have been missed, suggesting that the real burden of active TB in this population may be even higher than that found through sputum testing. Because of the difficulty in diagnosing TB in PLWH, and the severe consequences of missed TB in this population, some trials have evaluated the effect of empiric TB treatment to PLWH with advanced immunosuppression. Currently, the role of this treatment strategy is not obvious; in the REMEMBER trial empiric TB treatment did not lead to decreased mortality compared to standard care [32]. Using predictors for early mortality, such as those presented here might help to identify individuals who could benefit from empiric TB treatment [33].

This study was based on a large material of patients with structured and detailed categorization for active TB at inclusion. Furthermore, participants were recruited at Ethiopian public health centers, representing a typical health care setting for where most PLWH receive care in low-income countries.

Our study has certain limitations. For a considerable proportion of patients the outcome at 6 months was unknown (LTFU 42/812; 5.2%). LTFU is common in ART programs in sub-Saharan Africa, and studies on LTFU have identified different reasons for this phenomenon [34–36]. In a study from Ethiopia around 50% of subjects LTFU from an ART clinic were found to be dead when tracing was done [36]; other explanations may be “self-transfer” of care and resort to alternative therapies. In contrast to patients with confirmed death, characteristics of participants LTFU did not differ significantly from those remaining in care. However, their characteristics did differ from those that died. Median CD4 cell count was 99 cells/mm³ and 198 cells/mm³, median MUAC was 20 cm and 22 cm, and TB prevalence was 32% and 17%, for patients that died and were LTFU, respectively (S1 File). Our study did not aim to investigate reasons for LTFU. As part of our study protocol, we attempted to trace patients that were lost, but for a proportion this tracing was not successful. Although we could not determine the proportion of cases LTFU that have died, we do not consider early mortality to be a major outcome in this group. That 5.2% in our study were lost within 6 months is a major limitation. However, for the reasons mentioned above we do not consider the proportion of participants categorized as LTFU as having had any significant impact on the outcome of the study. Furthermore, we excluded persons who did not provide sputum samples for TB testing at inclusion, and TB investigations were mainly restricted to pulmonary disease. Before implementing the use of the predictors identified here in routine care it will be necessary to validate our findings in external cohorts.

Conclusions

In conclusion, we found that two simple clinical measurements have strong independent association with 6-month mortality in Ethiopian PLWH eligible to start ART: MUAC for men, and

KPS for women. These variables could be considered for use in routine care to identify subjects at high risk of early mortality. Such persons may particularly benefit from fast-track ART initiation, as well as intensified investigation for TB.

Supporting Information

S1 File. The raw data underlying all statistical analyses in the manuscript. (SAV)

S1 Table. Univariate Cox proportional hazards models for early mortality, separate models for all, male, female, TB cases, and non-TB cases, respectively. (DOCX)

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Author Contributions

Conceived and designed the experiments: AR TTB SS PB. Performed the experiments: AR TTB. Analyzed the data: AR NG. Contributed reagents/materials/analysis tools: ES PB. Wrote the paper: AR TTB SS PB.

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S1 Table. Univariate Cox proportional hazards models for early mortality, separate models for all, male, female, TB cases, and non-TB cases, respectively.

Variable	All			Male			Female			TB cases			Non-TB cases		
	HR (95% CI)	P	HR (95% CI)	HR (95% CI)	P	HR (95% CI)	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	P
Age – per year	1.00 (0.96-1.03)	0.902	1.00 (0.95-1.05)	0.907	0.98 (0.92-1.04)	0.470	0.94 (0.88-1.02)	0.125	1.02 (0.98-1.06)	0.403	2.48 (1.12-5.53)	0.026	1.02 (0.98-1.06)	0.403	0.026
Gender – female vs. male	1.71 (0.90-3.27)	0.102	–	–	<0.001	0.70 (0.58-0.84)	0.84 (0.70-1.01)	<0.001	0.57 (0.37-0.88)	0.057	0.61 (0.20-1.93)	<0.001	0.51 (0.19-1.18)	0.541	<0.001
BMI – per 1 kg/m ²	0.77 (0.67-0.88)	<0.001	0.68 (0.57-0.83)	<0.001	0.73 (0.60-0.87)	0.001	0.77 (0.60-0.99)	0.043	0.71 (0.61-0.83)	0.043	0.71 (0.61-0.83)	<0.001	0.71 (0.61-0.83)	0.043	<0.001
MUAC – per cm	0.72 (0.63-0.82)	<0.001	0.68 (0.57-0.83)	<0.001	0.73 (0.60-0.87)	0.001	0.77 (0.60-0.99)	0.043	0.71 (0.61-0.83)	0.043	0.71 (0.61-0.83)	<0.001	0.71 (0.61-0.83)	0.043	<0.001
CD4 cell count – cells/ μ L															
>300	1.0		1.0	1.0		1.0	1.0		1.0		1.0		1.0		
201-300	1.00 (0.31-3.28)	0.999	1.16 (0.16-8.24)	0.881	0.92 (0.21-4.10)	0.911	4.05 (0.42-38.92)	0.226	0.47 (0.09-2.43)	0.226	0.47 (0.09-2.43)	0.368	0.47 (0.09-2.43)	0.226	0.368
100-200	1.26 (0.42-3.75)	0.678	1.93 (0.35-10.53)	0.449	0.87 (0.20-3.90)	0.859	0.65 (0.04-10.32)	0.757	1.46 (0.45-4.77)	0.534	1.46 (0.45-4.77)	0.534	1.46 (0.45-4.77)	0.534	0.534
<100	4.82 (1.91-12.15)	0.001	6.68 (1.48-30.14)	0.014	3.47 (1.02-11.87)	0.047	6.14 (0.76-49.94)	0.009	4.10 (1.43-11.81)	0.009	4.10 (1.43-11.81)	0.009	4.10 (1.43-11.81)	0.009	0.009
CD4 cell %-age – per %	0.97 (0.92-1.02)	0.245	0.96 (0.89-1.03)	0.283	0.99 (0.92-1.07)	0.792	1.01 (0.94-1.09)	0.851	0.95 (0.89-1.01)	0.851	0.95 (0.89-1.01)	0.124	0.95 (0.89-1.01)	0.851	0.124
TB coinfection	2.09 (1.05-4.16)	0.036	1.01 (0.37-2.78)	0.985	4.14 (1.57-10.87)	0.004	–	–	–	–	–	–	–	–	–
Haemoglobin, g/dL															
>10.9	1.0		1.0	1.0		1.0	1.0		1.0		1.0		1.0		
8.0-10.9	1.97 (0.92-4.18)	0.079	2.52 (0.92-6.96)	0.074	1.61 (0.52-4.99)	0.409	0.77 (0.19-3.09)	0.716	2.49 (1.01-6.12)	0.716	2.49 (1.01-6.12)	0.047	2.49 (1.01-6.12)	0.716	0.047
<8.0	7.64 (2.72-21.44)	<0.001	6.00 (1.25-28.90)	0.025	9.48 (2.37-37.91)	0.001	3.47 (0.78-15.50)	0.104	8.05 (1.74-37.26)	0.104	8.05 (1.74-37.26)	0.008	8.05 (1.74-37.26)	0.104	0.008
In HIV care at inclusion	0.41 (0.22-0.79)	0.007	0.54 (0.22-1.29)	0.165	0.33 (0.13-0.87)	0.024	0.46 (0.15-1.46)	0.189	0.43 (0.20-0.94)	0.189	0.43 (0.20-0.94)	0.035	0.43 (0.20-0.94)	0.189	0.035
HIV test due to symptoms	1.83 (0.92-3.64)	0.085	1.56 (0.60-4.05)	0.364	1.94 (0.72-5.24)	0.193	0.96 (0.29-3.19)	0.945	2.13 (0.92-4.94)	0.945	2.13 (0.92-4.94)	0.078	2.13 (0.92-4.94)	0.945	0.078
Signs and symptoms															
Weight loss	6.73 (2.07-21.92)	0.002	8.30 (1.11-62.04)	0.039	5.42 (1.24-23.72)	0.025	2.47 (0.32-19.15)	0.386	8.21 (1.94-34.82)	0.386	8.21 (1.94-34.82)	0.004	8.21 (1.94-34.82)	0.386	0.004
Appetite loss	3.90 (1.78-8.54)	0.001	2.49 (0.96-6.48)	0.062	8.12 (1.86-35.49)	0.005	6.53 (0.84-50.56)	0.072	3.16 (1.32-7.58)	0.072	3.16 (1.32-7.58)	0.010	3.16 (1.32-7.58)	0.072	0.010
Fatigue	3.71 (1.31-10.49)	0.013	8.12 (1.08-60.84)	0.041	2.20 (0.63-7.67)	0.214	1.85 (0.24-14.46)	0.557	4.01 (1.20-13.41)	0.557	4.01 (1.20-13.41)	0.024	4.01 (1.20-13.41)	0.557	0.024
Fever	1.85 (0.95-3.59)	0.070	1.06 (0.44-2.55)	0.894	3.81 (1.24-11.67)	0.019	3.03 (0.66-13.81)	0.153	1.41 (0.64-3.08)	0.153	1.41 (0.64-3.08)	0.394	1.41 (0.64-3.08)	0.153	0.394
Cough	2.85 (1.45-5.59)	0.002	1.25 (0.52-3.01)	0.613	8.31 (2.39-28.92)	0.001	7.48 (0.97-57.95)	0.054	2.03 (0.93-4.45)	0.054	2.03 (0.93-4.45)	0.077	2.03 (0.93-4.45)	0.054	0.077
Night sweats	2.24 (0.13-4.47)	0.021	1.74 (0.69-4.26)	0.238	2.84 (1.00-8.05)	0.050	0.81 (0.24-2.68)	0.727	2.83 (1.22-6.56)	0.727	2.83 (1.22-6.56)	0.015	2.83 (1.22-6.56)	0.727	0.015
Conjunctive pallor	3.07 (1.56-6.03)	0.001	2.64 (1.05-6.62)	0.038	3.44 (1.27-9.29)	0.015	1.00 (0.27-3.69)	0.999	4.62 (2.07-10.28)	0.999	4.62 (2.07-10.28)	<0.001	4.62 (2.07-10.28)	0.999	<0.001
Karnofsky score <80%	7.86 (3.60-17.20)	<0.001	4.52 (1.74-11.75)	0.002	17.55 (4.01-76.76)	<0.001	4.24 (0.93-19.34)	0.062	8.86 (3.54-22.19)	0.062	8.86 (3.54-22.19)	<0.001	8.86 (3.54-22.19)	0.062	<0.001
WHO clinical stage															
Stage 1-2	1.0		1.0	1.0		1.0	1.0		1.0		1.0		1.0		
Stage 3	3.21 (1.34-7.69)	0.009	2.49 (0.78-7.94)	0.123	3.86 (1.02-14.53)	0.046	0.79 (0.18-3.52)	0.755	5.07 (1.67-15.40)	0.755	5.07 (1.67-15.40)	0.004	5.07 (1.67-15.40)	0.755	0.004
Stage 4	7.45 (2.93-18.92)	<0.001	4.74 (1.34-16.80)	0.016	10.90 (2.72-43.59)	0.001	2.17 (0.52-9.09)	0.289	10.68 (3.13-36.49)	0.289	10.68 (3.13-36.49)	<0.001	10.68 (3.13-36.49)	0.289	<0.001

Data presented as n (%), or median (interquartile range).

Abbreviations: HR, hazard ratio; CI, confidence interval; BMI, body mass index; MUAC, mid-upper arm circumference; TB, tuberculosis.

Bold p values are <0.3 indicating that the variable was included in the subsequent multivariable model.

Paper III



Long-term outcome of antiretroviral treatment in patients with and without concomitant tuberculosis receiving health center-based care – results from a prospective cohort study

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Keywords: HIV; antiretroviral treatment; tuberculosis; outcome; Ethiopia.

Main point: Adults initiating ART at decentralized facilities in Ethiopia had similar clinical, virological, and immunological outcome regardless of concomitant TB. However, one third of patients initiating ART had unsatisfactory long-term treatment outcomes, with higher risk for men and malnourished individuals.

Abstract

Background

In order to increase treatment coverage, antiretroviral treatment (ART) is provided through primary health care in low-income high-burden countries, where tuberculosis (TB) co-infection is common. We investigated the long-term outcome of health center-based ART, with regard to concomitant TB.

Methods

ART-naïve adults were included in a prospective cohort at Ethiopian health centers and followed for up to four years after starting ART. All participants were investigated for active TB at inclusion. The primary study outcomes were the impact of concomitant TB on all-cause mortality, loss to follow-up (LTFU), and lack of virological suppression (VS). Kaplan-Meier survival estimates and Cox proportional hazards models with multivariate adjustments were used.

Results

In total, 141/729 (19%) subjects had concomitant TB, 85% with bacteriological confirmation (median CD4 count TB: 169 cells/mm³ (IQR, 99-265), non-TB: 194 cells/mm³ (IQR, 122-275). During follow-up (median 2.5 years), 60 (8%) died and 58 (8%) were LTFU. After ≥6 months ART 131/630 (21%) had lack of VS. Concomitant TB did not influence the rates of death, LTFU or VS. Male gender and malnutrition were associated with higher risk of adverse outcomes. Regardless of TB co-infection status, even after 3 years of ART nearly two-thirds of participants had CD4 counts below 500 cells/mm³.

Conclusion

Concomitant TB did not impact treatment outcomes in adults investigated for active TB before starting ART at Ethiopian health centers. However, one third of patients had unsatisfactory long-term treatment outcomes and immunologic recovery was slow, illustrating the need for new interventions to optimize ART programs.

Introduction

Many reports on antiretroviral treatment (ART) outcomes in cohorts from low-income countries have shown results comparable to those in high-income settings, albeit with higher rates of early mortality, probably related to more advanced disease at ART initiation [1–3]. In order to increase ART coverage further in high-burden countries provision of ART is increasingly managed by nurses and integrated within existing primary health-care systems [4]. According to recent estimates >18 million people living with HIV (PLHIV) have started ART [5], but to reach the goal of 90% treatment coverage almost as many persons in addition need to start ART. Further attention is also required on achieving 90% rates of virological suppression in persons starting ART. Recent data show high rates of loss to follow-up (LTFU) in ART recipients [6,7], as well as rising occurrence of both acquired and transmitted antiretroviral drug resistance [8,9].

Although the median CD4 cell counts at ART initiation have increased during recent years [10], many patients in sub-Saharan Africa still have severe immunosuppression when HIV is diagnosed and ART can be started [11]. Furthermore, tuberculosis (TB) co-infection is common [12], and has been associated with worse ART outcomes [13], including persistently elevated rates of death [14]. Since the clinical manifestations of TB may be atypical and vague in PLHIV, TB is frequently missed [15]. Active TB case-finding has therefore been proposed as an intervention that could improve outcomes of patients with TB/HIV co-infection [16].

We have previously reported similar 6-month outcomes irrespective of concomitant TB in adults categorized for active TB before starting ART at Ethiopian health centers [17]. Here we aimed to compare long-term ART outcomes with regard to concomitant TB in these patients, and to identify factors associated with mortality, LTFU, and lack of virological suppression.

Methods

Setting

This study was performed in Ethiopian public health centers in and around Adama (population approximately 600,000). Non-physician clinicians provide all care at Ethiopian health centers, including management of TB and HIV. Since 2006, ART has been decentralized in Ethiopia by inclusion of an ART clinic in many health centers.

In 2012, the Ethiopian National ART guidelines recommended ART for all patients with CD4 counts <350 cells/mm³ and/or WHO stage 4 [18]. In 2015, these guidelines were revised with a higher CD4 count threshold for ART initiation at <500 cells/mm³ [19], with non-nucleoside reverse transcriptase inhibitor (NNRTI)-based 1st ART regimens in both versions. Second-line

ART is provided at hospital-based clinics to which patients with suspected treatment failure are referred.

At the time of this study, viral load (VL) determination was only performed in case of suspected treatment failure, based on immunological and clinical criteria [19].

Study participants

The study cohort has previously been described [17,20]. HIV-positive adults presenting to any of the five public health centers providing ART in the study uptake area from October 2011 until March 2013 were considered for inclusion. Inclusion criteria were: age ≥ 18 years, eligibility to start ART (defined as a documented CD4 count < 350 cells/mm³ and/or WHO stage 4 disease), and residence within the catchment area of the study sites. Patients reporting current or previous ART, or TB treatment for more than 2 weeks before inclusion, were excluded.

At inclusion, socio-demographic and medical information was collected following structured questionnaires. Blood samples were obtained for CD4 counts, with storage of plasma aliquots at -80°C for VL testing. Medical information was updated at subsequent visits along with repeated blood sampling. Patients were followed 3-monthly until ART initiation, whereby follow-up visits were scheduled for months 1, 2, 3, 6, 9, 12, and biannually thereafter until study closure on December 31, 2015.

At inclusion, all participants, regardless of symptoms suggestive of TB, were requested to submit two pairs of spontaneously expectorated morning samples for TB investigations (smear microscopy, GeneXpert MTB/RIF, and liquid culture) [20]. Subjects with peripheral lymphadenopathy were also referred for fine-needle aspiration for TB investigations. Participants who did not provide any sample for TB investigations were excluded.

Clinicians were instructed to repeat TB diagnostics according to the study protocol during follow-up in case of clinically suspected TB. All subjects diagnosed with TB within 3 months after starting ART were considered as TB cases with remaining subjects considered as non-TB cases. Patients diagnosed with TB ≥ 3 months after starting ART (incident TB) were considered as non-TB cases for the outcome analyses. TB cases were further categorized with regard to whether TB was bacteriologically confirmed or diagnosed on clinical grounds.

VL was performed in batches during the study period using the Abbott Real-Time HIV-1 assay (Abbott Molecular Inc., Des Plaines, IL; detection limit 40 copies/mL) or the Abbott m2000 RealTime System Automated molecular platforms (Abbott Molecular Inc., Des Plaines, IL; detection limit 150 copies/mL) with communication of results to care providers. In line with

national guidelines, subjects with VL ≥ 1000 copies/mL underwent adherence counselling followed by repeat VL testing before referral for second line ART [19].

During follow-up, tracing was recommended for patients more than 1 day late for a scheduled visit. Subjects more than 3 months late for a scheduled visit and who did not return during follow-up were considered as LTFU.

Statistical analysis

Comparison of patient characteristics between TB cases and non-TB cases was performed using Mann-Whitney U test for continuous variables and the chi-square test for categorical variables. All analyses were based on subjects with complete data regarding the variable(s) under study. The primary study outcomes were rates of adverse ART outcomes (all-cause mortality, LTFU, and lack of VS) with regard to TB category at ART start. The secondary outcome was immunologic recovery during ART.

In the mortality and LTFU analyses, time-at-risk started at ART initiation, whereas time-at-risk for virological outcome analysis started 6 months after ART initiation. For each outcome, subjects were followed until reaching the respective outcome, with censoring of remaining subjects at last registered visit. Subjects without subsequent follow-up visits after ART initiation were assigned 0.1 months' follow-up time so that they would contribute to the survival analyses. Virological outcomes were categorized as virological suppression (VS; VL < 150 copies/ml), low-level viremia (LLV; VL 150-1000 copies/ml) and high-level viremia (HLV; VL > 1000 copies/ml). Any event of HLV recorded during follow-up 6 months after ART initiation was defined as lack of VS. All subjects with at least one VL result during follow-up were included in this analysis.

Kaplan-Meier plots were used to graphically assess the temporal distribution of events, using the log rank test to compare subjects with and without TB. Cox proportional hazards models were used with adjustment for variables with previously reported associations with the outcomes. Whereas age was kept as a continuous variable, CD4 counts were categorized according to level of immunosuppression (<100, 100-200, 201-350, and >350 cells/mm³, respectively) and mid-upper arm circumference (MUAC) was dichotomized at gender-specific thresholds (<23 cm for women and <24 cm for men). All variables included in the models were assessed for the proportional hazards assumption using log-minus-log plots, and Schoenfeld residuals.

Two sensitivity analyses were performed. First, subjects with clinically diagnosed TB were excluded from the survival models to assess the impact of only bacteriologically confirmed TB.

Second, to assess whether intervals without available VL results during follow-up had any impact on the analysis of HLV, subjects with less than one VL per year had their follow-up censored at last VL before such an interval.

Immunological recovery during ART was described using medians and interquartile ranges (IQR) comparing TB cases and non-TB cases using Mann-Whitney U test at each follow-up. All analyses were performed using SPSS, version 21 (IBM Corp, Armonk, NY), and STATA, version 13.1 (StataCorp, College Station, TX). A p value <0.05 was considered to indicate statistical significance.

Ethical considerations

Ethical approval was obtained from the national Research Ethics Review Committee at the Ministry of Science and Technology of Ethiopia and the Regional Ethical Review Board of Lund University, Sweden. All study participants provided written informed consent.

Results

Participant characteristics

Out of 886 subjects identified to be eligible for inclusion 812 were included (Fig. 1). Characteristics of the 61 excluded subjects who did not submit samples for TB investigations did not differ significantly from those included (Table 1, Supplementary Material).

Active TB was diagnosed in connection with inclusion in 158/812 (19%) participants, with bacteriologic confirmation in 137/158 (87%). An additional 4 subjects were diagnosed during follow-up before starting ART (1/4 bacteriologically confirmed; Fig. 1).

During a median follow-up time of 3.0 years (IQR 2.1-3.4), 729 (90%) started ART. At study closure, 525 (72%) of subjects starting ART remained in care, whereas 60 (8%) had died, 58 (8%) were LTFU, 80 (11%) had reported transfer of care, and 6 (1%) had declined further participation in the study. Eighty-three subjects did not start ART during study follow-up (censoring event: death 22, LTFU 31, transfer of care 23, declined further study participation 4, end of study 3).

Characteristics of study participants at ART initiation are shown in table 1.

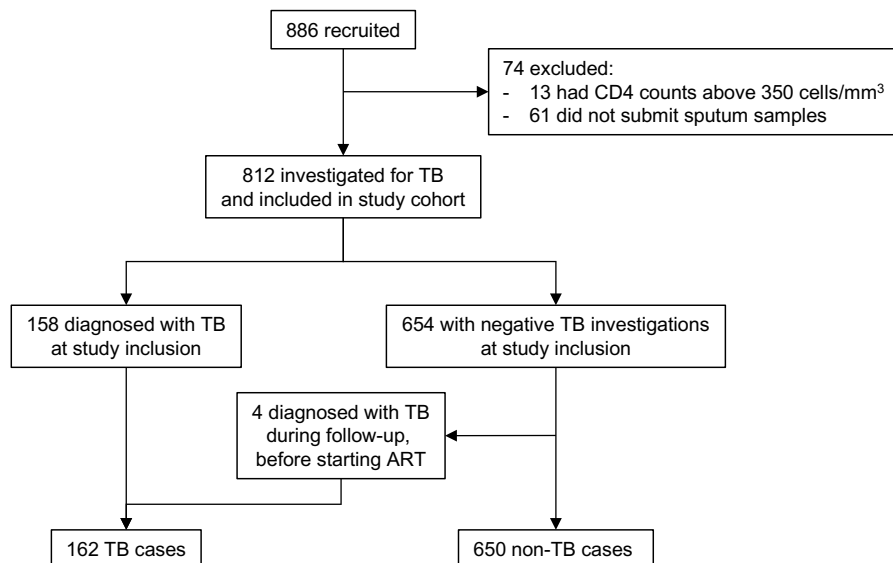


Figure 1. Study participant flow chart.

Table 1. Characteristics of cohort participants at start antiretroviral therapy (ART).

	Total n=729	TB cases n=141	Non-TB cases n=588	P
Women	431 (59)	65 (46)	366 (62)	<0.01
Age, years	32 (28-40)	34 (28-40)	32 (28-40)	0.49
MUAC, cm	23.0 (21.0-25.0)	21.5 (20.0-23.5)	23.0 (21.0-25.0)	<0.01
MUAC*, <23cm/<24cm	385 (53)	99 (70)	286 (49)	<0.01
CD4 count, median cells/mm³	187 (116-274)	169 (99-265)	194 (122-275)	0.03
<100	138 (19)	36 (26)	102 (17)	
100-200	264 (36)	52 (37)	212 (36)	
201-350	237 (33)	35 (25)	202 (35)	
>350	88 (12)	18 (13)	70 (12)	
Time to ART start, months	1.2 (0.5-5.1)	1.6 (0.9-3.4)	1.1 (0.5-5.5)	0.04
Initial ART regimen**				
NNRTI:				
efavirenz	612 (84)	132 (94)	480 (82)	<0.01
nevirapine	115 (16)	8 (6)	107 (18)	<0.01
NRTI:				
tenofovir	643 (88)	130 (93)	513 (87)	0.07
zidovudine	72 (10)	6 (4)	66 (11)	0.01
stavudine	12 (2)	4 (3)	8 (1)	0.26

Abbreviations: TB, tuberculosis; MUAC, mid-upper arm circumference; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleos(t)ide transcriptase inhibitor.

Data presented as n (%), or median (interquartile range).

P value derived using Mann Whitney U, chi-square, or Fisher's exact test, as appropriate.

*MUAC dichotomized at <23cm for women and <24cm for men.

**All regimens included lamivudine

Mortality

During 1643 person-years of follow-up after ART start, death was recorded in 12/141 (9%) TB cases and 48/588 (8%) non-TB cases. The median time from ART start to death was 8.6 months (IQR, 2.2-17.4), with 26 deaths (43%) occurring within 6 months of ART start. VL was available prior to time of death for 26/34 (76%; median duration from VL to death 4.9 months, IQR 3.4-7.4); 14 (54%) were virally suppressed, 11 (42%) had HLV and 1 (4%) had LLV.

TB was not associated with risk of death during ART, log rank $p=0.85$ (Fig. 2, panel A). In the unadjusted survival analysis, low MUAC was significantly associated with mortality. This association remained after multivariate adjustments (Table 2). Men had a trend towards higher risk of mortality in unadjusted analysis, $p=0.06$, but this trend did not remain after multivariate adjustments.

Loss to follow-up

Among 58 subjects lost to follow-up after ART initiation, 27/58 (47%) were lost within 6 months of ART start. In total, 11/141 (8%) TB cases and 47/588 (8%) non-TB cases were LTFU a median 6.6 months (IQR, 2.0-15.5) after starting ART, log rank $p=0.93$ (Fig. 2, panel B). VL was available prior to time of LTFU for 28 subjects (median duration from VL to last visit in study 1.2 months, IQR 0.0-4.8); 21 (75%) were virally suppressed, 6 (21%) had HLV, and 1 (4%) had LLV.

In unadjusted analysis, men had an increased risk of LTFU, and this association remained after multivariate adjustments, Table 1. MUAC was not included in the multivariate model as the proportional hazards assumption was not fulfilled due to a disproportionately higher risk of LTFU shortly after starting ART in subjects with lower MUAC, compared with later during follow-up.

Virological suppression

Six-hundred-thirty participants met criteria for inclusion in the virological outcome analysis. Among the 99 excluded subjects, 80 had less than 6 months of follow-up after ART start (death 26, LTFU 27, transfer-out 21, declined further participation 2, ART start within 6 months of study closure 4), and from 19 subjects no VL results were available during follow-up.

The 630 participants included in the virological outcome analysis had a median of 4 VL results after ≥ 6 months ART (IQR, 3-5). In total, 426 (68%) were virally suppressed on all occasions, 73 (12%) had LLV on at least one occasion, and 131 (21%) had HLV on at least one occasion. These proportions were similar when based on subjects with at least 2 VL ($n=591$) during

follow-up: 405 (69%) were virally suppressed on all occasions, 55 (9%) had LLV on at least one occasion, and 131 (22%) had HLV on at least one occasion.

A similar proportion of TB cases (26%) and non-TB cases (20%), $p=0.15$, had at least one occasion of HLV after ≥ 6 months of ART (lack of virological suppression). The median time from ART start to this event was 11.8 months (IQR, 6.0-17.5).

In survival analysis, TB was not significantly associated with HLV, log rank $p=0.14$ (Fig. 2, panel C). In unadjusted analysis, male gender, CD4 count <100 cells/mm³, and low MUAC were associated with HLV. The associations remained after multivariate adjustments (Table 2). To assess possible interaction between gender and the other variables, gender-stratified models were fitted. TB remained without association with HLV for both men ($p=0.35$) and women ($p=0.47$). For men, lower age was associated with an increased risk of HLV ($p=0.03$), whereas remaining variables had similar associations with HLV for both men and women compared with the unstratified analysis.

Subsequent VL data was available for 98/131 (75%) subjects with HLV. Of these, 43 had VS on at least one subsequent occasion (without recorded change of ART regimen in 34/43), 9 had LLV on at least one occasion, and 63 had HLV on at least one occasion.

Of the 522 participants remaining in care at end of follow-up, VL results were available for 516 (99%). The final virological status of these were 445 (86%) VS, 23 (5%) LLV, and 48 (9%) HLV, respectively.

Sensitivity analyses

Twenty-two TB cases without bacteriological confirmation were excluded in a sensitivity analysis with resulting multivariate models remaining largely unchanged (Table 2, Supplementary Material).

Eight subjects were excluded due to lack of VL data at 6 and 12 months after ART start, and an additional 26 subjects had their follow-up censored at last VL before a testing gap in the second sensitivity analysis. TB remained without significant association with HLV, log rank $p=0.15$. The multivariate model remained unchanged (Table 3, Supplementary Material).

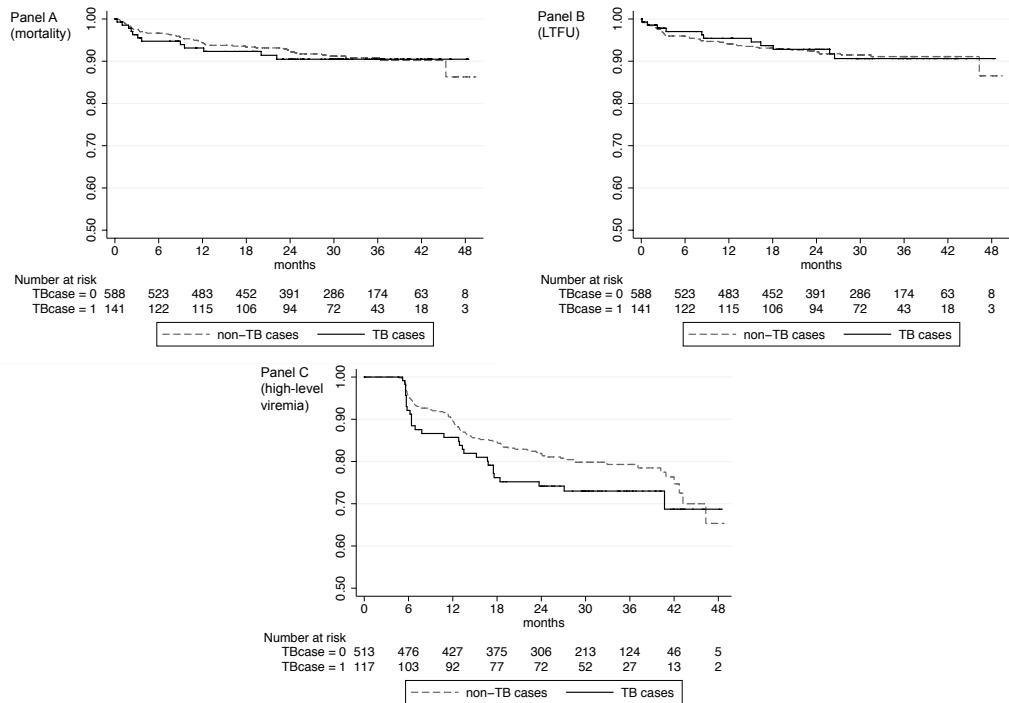


Figure 2. Kaplan-Meier plots for TB cases and non-TB cases regarding mortality (A), loss to follow-up (B), and high-level viremia (C).

Table 2. Adjusted (aHR) and unadjusted hazard ratios (HR) for mortality, LTFU and lack of virological suppression after ART initiation.

Variable	Mortality				LTFU				Lack of virological suppression			
	HR (95% CI)	P	aHR (95% CI)	P	HR (95% CI)	P	aHR (95% CI)	P	HR (95% CI)	P	aHR (95% CI)	P
TB case	1.1 (0.6-2.0)	0.85	0.8 (0.4-1.6)	0.62	1.0 (0.5-1.9)	0.99	0.9 (0.5-1.7)	0.76	1.4 (0.9-2.0)	0.15	1.0 (0.7-1.6)	0.88
Men	1.6 (1.0-2.7)	0.06	1.3 (0.8-2.3)	0.29	1.7 (1.0-2.9)	0.04	2.0 (1.2-3.5)	0.01	2.1 (1.5-3.0)	<0.01	2.0 (1.4-2.9)	<0.01
Age, per year	1.0 (1.0-1.0)	0.47	1.0 (1.0-1.0)	0.85	1.0 (1.0-1.0)	0.25	1.0 (0.9-1.0)	0.07	1.0 (1.0-1.0)	0.84	1.0 (1.0-1.0)	0.21
CD4 >350, cells/mm³	Reference		Reference		Reference		Reference		Reference		Reference	
CD4 201-350, cells/mm³	1.0 (0.4-2.4)	0.92	1.2 (0.5-3.1)	0.67	0.8 (0.3-2.1)	0.64	0.8 (0.3-2.1)	0.64	1.0 (0.5-1.9)	0.89	1.2 (0.6-2.4)	0.63
CD4 100-200, cells/mm³	0.8 (0.3-2.1)	0.71	0.9 (0.4-2.4)	0.86	1.5 (0.6-3.7)	0.34	1.6 (0.6-3.7)	0.33	1.2 (0.6-2.4)	0.52	1.4 (0.7-2.7)	0.33
CD4 <100, cells/mm³	2.1 (0.8-5.1)	0.12	1.8 (0.7-4.5)	0.20	0.9 (0.3-2.6)	0.90	0.9 (0.3-2.6)	0.87	2.5 (1.3-4.9)	0.01	2.3 (1.2-4.5)	0.01
MUAC*, <23cm/<24cm	3.5 (1.9-6.5)	<0.01	3.1 (1.7-5.9)	<0.01	1.4 (0.8-2.3)	0.25	**		2.5 (1.7-3.7)	<0.01	2.1 (1.4-3.1)	<0.01

Abbreviations: LTFU, lost to follow-up; MUAC, mid-upper arm circumference; CI, confidence interval

* MUAC dichotomized at <23cm for women and <24cm for men.

** MUAC did not fulfill the proportional hazards assumption for LTFU, therefore not included in the multivariate model.

Immunological recovery

Participants with TB had lower CD4 counts at ART start ($p=0.03$, Table 1). By 6 months, the median CD4 count remained lower in TB cases (267 cells/mm³, IQR 199-390) compared to non-TB cases (318 cells/mm³, IQR 225-414; $p=0.04$). At 12 months, however, median CD4

counts did not differ significantly for TB cases and non-TB cases; 343 cells/mm³ (IQR, 210-418) versus 352 cells/mm³ (IQR, 246-456), $p=0.58$. The similar CD4 count distribution among TB cases and non-TB cases remained during subsequent follow-up (Fig. 3).

Assessing whether early attrition influenced the apparent immunologic recovery, subjects with less than 6 months of follow-up after ART start ($n=80$) were excluded in an additional analysis. This did not affect the overall results (data not shown).

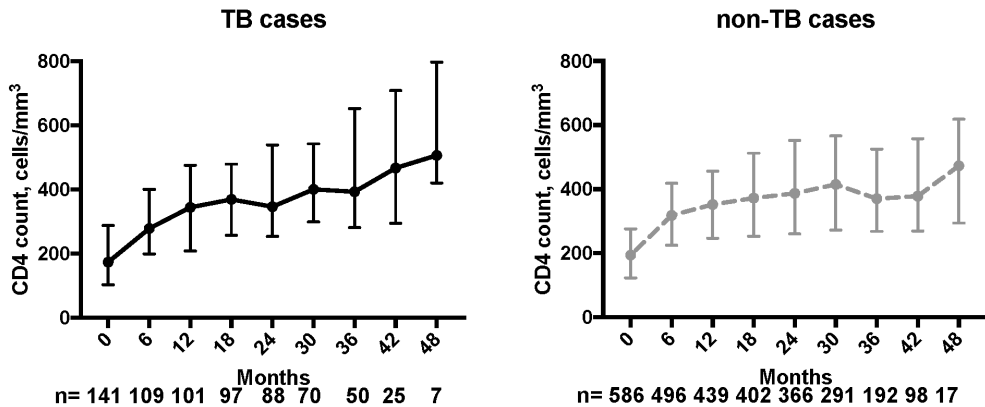


Figure 3. Immunologic recovery in ART recipients with regard to concomitant TB (plots with medians and interquartile ranges).

Discussion

In this cohort of adults starting ART at Ethiopian health centers a high proportion (86%) of subjects remaining in care had virological suppression at a median of 2.5 years after starting ART, irrespective of concomitant TB. These results are in line with a recent meta-analysis on 1st line ART outcome in Sub-Saharan Africa, reporting virological suppression in more than 80% for up to 60 months of ART [21].

TB is considered to be the leading cause of death in PLHIV in low-income countries [4], and TB co-infection has been associated with increased mortality both before and after starting ART [22,23]. In this cohort, however, concomitant TB did not influence the long-term risk of death, nor rates of LTFU or VS. All participants underwent intensified TB case-finding at inclusion, regardless of clinical presentation. It is likely that this strategy led to reduced mortality in co-infected patients.

Our results support that providing ART to PLHIV with TB is feasible and effective at health center level, with similar rates of virological suppression for both groups. These results are in agreement with a meta-analysis showing no influence of TB status at start of ART on virological suppression [24], but in contrast to our study most previous investigations of this issue have been performed at hospitals or specialized HIV clinics. Due to heterogeneity and inconclusiveness in available data, the risk of virological failure could not be assessed in that meta-analysis. However, in a previous study from South Africa (not included in the meta-analysis) with a high proportion of bacteriologically confirmed TB cases, TB at start of ART was associated with an increased risk of virological failure [13].

Nearly 80% of participants in our cohort had persistent viral suppression <1000 copies/mL. However, in an intention-to-treat analysis assuming subjects who were LTFU or died after starting ART to have lack of virological suppression, the treatment success rate declined to 67%. These results are in line with intention-to-treat estimates from Sub-Saharan Africa [21]. For our study, we chose to use the occurrence of VL >1000 copies/mL after at least 6 months ART to define lack of virological suppression. Among patients with available VL data, 21% of patients had at least one episode of HLTV. Although current guidelines require two VL measurements above this threshold to define virological failure, the majority of patients with recorded HLTV had persistent viremia in follow-up samples. Furthermore, since drug resistance mutations can accumulate rapidly during periods of HLTV in ART recipients [25,26], with subsequent risk of virological treatment failure [27] we think that even isolated episodes of HLTV should be regarded as unsatisfactory treatment outcomes. In addition, drug-resistant strains can be transmitted onwards [8].

The likelihood of HLTV was increased among men, subjects with CD4 counts <100 cells/mm³, and those with low MUAC. Low CD4 counts at ART initiation have previously been associated with lower rates of virological suppression during ART [13,28], and other studies have also observed higher risk of virological failure in men [29,30]. The underlying reason for the independent association between low MUAC and HLTV is not obvious, and may be related to factors not included in our analysis.

Although we did not find any gender-related difference regarding mortality, in contrast to other reports [31,32], malnutrition, measured by the gender-specific MUAC thresholds of <23 cm for women and <24 cm for men, was associated with a more than 3-fold increase in risk of death after starting ART. The association between low MUAC and mortality in PLHIV has been found by other researchers [33], and might be due to the presence of unrecognized opportunistic infections, including cases of TB not identified by our intensified case-finding protocol. This is

in accordance with autopsy studies from sub-Saharan Africa, demonstrating that TB is a common finding even among PLHIV who have undergone TB investigations before death [34,35].

Low MUAC was also associated with LTFU during the first 4 months after starting ART, which suggests that unreported mortality may explain LTFU in a subgroup of individuals during this period. This is in line with findings from South Africa which showed an elevated risk of death soon after becoming LTFU but not later [28]. Similarly, increased mortality was found in South African patients within 3 months after registered transfer of care [36]. In our cohort, transfer of care was noted in 11% of subjects, with similar proportions irrespective of concomitant TB. We were unable to assess the subsequent outcomes of these patients.

Previous studies have indicated that TB may negatively affect immunological recovery during ART [37–40], and it has been speculated that the increased risk of death after completion of TB treatment noted in some studies is related to persistent immunosuppression [14,37,38]. At 6 months after starting ART we observed lower median CD4 cell counts in co-infected patients [17], but during longer follow-up this difference disappeared among subjects who remained in care. Yet, regardless of TB co-infection status, even after 3 years of ART more than two-thirds of participants had CD4 counts below 500 cells/mm³, which is considered to represent the lower normal reference level. This phenomenon illustrates the need for earlier diagnosis and ART initiation during the course of HIV infection.

This study was based at public health centers providing nurse-based care, a representative setting for where many PLHIV receive ART globally. Through active case-finding for TB at inclusion into the study cohort, most subjects with concomitant TB were diagnosed with bacteriological confirmation. Furthermore, active case-finding allows for detection of active TB at earlier stages during the disease course. It should be noted, however, that our protocol was focused on pulmonary and peripheral lymph-node TB; thus, other types of TB might have been missed.

Some limitations should also be noted. Firstly, subjects included in this study were required to be ART-naïve, and to meet criteria for starting ART according to Ethiopian recommendations in use 2012-2015. Therefore, it is not certain that our findings apply to patients with previous ART exposure or to those with less advanced HIV disease. Secondly, we could not determine causes of death for deceased participants. Although a proportion of deaths could be due to TB [34,35], it has been suggested than conditions not directly associated with HIV account for an increasing proportion of deaths occurring later during ART [32]. Primarily by telephone calls to the family of the deceased, we could ascertain that most deaths in our study were due to

illness and not attributable to accidents or other unnatural causes. Finally, we did not have drug resistance data for subjects with HLV.

In conclusion, we found no impact of concomitant TB on treatment outcomes in adults initiating ART at decentralized facilities in Ethiopia. Overall, one third of patients initiating ART had unsatisfactory long-term treatment outcomes, with higher risk for men and malnourished individuals. Our findings support screening for TB and provision of ART to TB co-infected patients at health center level, but also illustrate the need for better treatment monitoring and interventions to optimize outcomes.

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Supplemental Table 1. Baseline characteristics of participants excluded due to lack of samples for tuberculosis investigations.

Characteristic	Excluded n=61	Included n=812
Age, years	32 (28-40)	32 (28-40)
Female	40 (66)	476 (59)
MUAC, cm	23 (20-25)	23 (21-24)
WHO stage		
1	11 (18)	141 (17)
2	16 (27)	246 (30)
3	21 (35)	322 (40)
4	12 (20)	100 (12)
CD4 count, median cells/mm ³	210 (132-295)	208 (116-320)

Abbreviations: MUAC, mid-upper arm circumference.

Data presented as n (%), or median (interquartile range).

Supplemental Table 2. Adjusted (aHR) and unadjusted hazard ratios (HR) for mortality, LTFU and lack of virological suppression after ART initiation, sensitivity analysis with clinical TB cases (n=22) excluded.

Variable	Mortality				LTFU				Lack of virological suppression			
	HR 95% CI	P	aHR 95% CI	P	HR 95% CI	P	aHR 95% CI	P	HR 95% CI	P	aHR 95% CI	P
TB case	0.7 0.3-1.6	0.41	0.6 0.3-1.3	0.20	1.1 0.5-2.1	0.88	0.9 0.5-1.8	0.82	1.3 0.8-2.0	0.24	1.0 0.6-2.6	0.99
Men	1.5 0.9-2.5	0.15	1.2 0.7-2.2	0.48	1.7 1.0-2.9	0.05	1.9 1.1-3.4	0.02	2.0 1.4-2.8	<0.01	1.9 1.3-2.8	<0.01
Age, per year	1.0 1.0-1.0	0.21	1.0 1.0-1.0	0.84	1.0 1.0-1.0	0.28	1.0 0.9-1.0	0.09	1.0 1.0-1.0	0.75	1.0 1.0-1.0	0.31
CD4 >350, cells/mm ³	Reference		Reference		Reference		Reference		Reference		Reference	
CD4 201-350, cells/mm ³	0.9 0.3-2.2	0.75	1.1 0.4-2.7	0.91	0.8 0.3-2.0	0.58	0.8 0.3-2.1	0.65	1.1 0.5-2.4	0.74	1.4 0.7-2.9	0.40
CD4 100-200, cells/mm ³	0.8 0.3-2.0	0.58	0.8 0.3-2.2	0.71	1.4 0.6-3.5	0.41	1.5 0.3-2.1	0.38	1.5 0.7-3.1	0.25	1.7 0.8-3.5	0.15
CD4 <100, cells/mm ³	1.7 0.7-4.3	0.27	1.5 0.6-3.8	0.41	0.9 0.3-2.5	0.85	0.9 0.3-2.5	0.81	2.8 1.4-5.8	0.01	2.6 1.2-5.3	0.01
MUAC*, <23cm/<24cm	3.2 1.7-6.0	<0.01	3.0 1.6-5.7	<0.01	1.4 0.8-2.3	0.24	**		2.5 1.7-3.6	<0.01	2.1 1.5-3.2	<0.01

Abbreviations: ART, antiretroviral treatment; LTFU, lost to follow-up; MUAC, mid-upper arm circumference; CI, confidence interval

*MUAC dichotomized at <23cm for women and <24cm for men.

** MUAC did not fulfill the proportional hazards assumption for LTFU, and was therefore not included in the multivariate model.

Supplemental Table 3. Adjusted (aHR) and unadjusted hazard ratios (HR) for lack of virological suppression after ART initiation, sensitivity analysis with time-at-risk adjusted for those with less than one viral load per year.

Variable	HR 95% CI	P	aHR 95% CI	P
TB case	1.4 0.9-2.1	0.15	1.0 0.7-1.6	0.84
Men	2.1 1.4-2.9	<0.01	2.0 1.3-2.9	<0.01
Age, per year	1.0 1.0-1.0	0.81	1.0 1.0-1.0	0.27
CD4 >350, cells/mm ³	Reference		Reference	
CD4 201-350, cells/mm ³	0.9 0.5-1.8	0.77	1.1 0.5-2.2	0.79
CD4 100-200, cells/mm ³	1.3 0.7-2.5	0.48	1.4 0.7-2.7	0.96
CD4 <100, cells/mm ³	2.4 1.2-4.7	0.01	2.1 1.1-4.2	0.03
MUAC*, <23cm/<24cm	2.5 1.7-3.6	<0.01	2.1 1.4-3.1	<0.01

Abbreviations: ART, antiretroviral treatment; LTFU, lost to follow-up; MUAC, mid-upper arm circumference; CI, confidence interval

*MUAC dichotomized at <23cm for women and <24cm for men.

Paper IV





ORIGINAL ARTICLE

OPEN ACCESS

Development of an algorithm for determination of the likelihood of virological failure in HIV-positive adults receiving antiretroviral therapy in decentralized care

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ABSTRACT

Background: Early identification of virological failure (VF) limits occurrence and spread of drug-resistant viruses in patients receiving antiretroviral treatment (ART). Viral load (VL) monitoring is therefore recommended, but capacities to comply with this are insufficient in many low-income countries. Clinical algorithms might identify persons at higher likelihood of VF to allocate VL resources.

Objectives: We aimed to construct a VF algorithm (the Viral Load Testing Criteria; VLTC) and compare its performance to the 2013 WHO treatment failure criteria.

Methods: Subjects with VL results available 1 year after ART start ($n = 494$) were identified from a cohort of ART-naïve adults ($n = 812$), prospectively recruited and followed 2011–2015 at Ethiopian health centres. VF was defined as $VL \geq 1000$ copies/mL. Variables recorded at the time of sampling, with potential association with VF, were used to construct the algorithm based on multivariate logistic regression.

Results: Fifty-seven individuals (12%) had VF, which was independently associated with CD4 count < 350 cells/mm³, previous ART interruption, and short mid-upper arm circumference (< 24 cm and < 23 cm, for men and women, respectively). These variables were included in the VLTC. In derivation, the VLTC identified 52/57 with VF; sensitivity 91%, specificity 43%, positive predictive value (PPV) 17%, negative predictive value (NPV) 97%. In comparison, the WHO criteria identified 38/57 with VF (sensitivity 67%, specificity 74%, PPV 25%, NPV 94%).

Conclusions: The VLTC identified subjects at greater likelihood of VF, with higher sensitivity and NPV than the WHO criteria. If external validation confirms this performance, these criteria could be used to allocate limited VL resources. Due to its limited specificity, it cannot be used to determine treatment failure in the absence of a confirmatory viral load.

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Background

Regular HIV-RNA quantification in plasma (viral load; VL) is the most accurate method to monitor antiretroviral treatment (ART), and has been routinely used in HIV care in high-income countries since ART became available [1,2]. VL monitoring allows for early detection of virological failure (VF) before clinical disease progression and accumulation of resistance mutations has occurred [3–5]. Viral load results can also be used for adherence counselling, and may save costs by preventing unnecessary switches to 2nd line ART [6,7]. For these reasons, the WHO recommends regular VL monitoring, at 6 and 12 months after ART start and annually thereafter, for all people receiving ART [8].

In 2015, 46% of the 36.9 million people living with HIV (PLHIV) in the world, of whom the majority reside in Sub-Saharan Africa, had started ART [9]. This

achievement has been made possible by decentralisation and integration of HIV care into primary health care. In these settings, access to VL monitoring is severely restricted [10], and expansion of viral load capacities is hampered by high cost and technical requirements [11].

In several fields of medicine algorithms are used to determine the likelihood of certain conditions being present, to target further investigations. This approach is also used in HIV care, especially for estimation of the risk of tuberculosis co-infection [12,13]. Some groups have also attempted to develop algorithms for determination of VF [14–17], but to our knowledge these algorithms are hitherto not in general use nor recommended in ART guidelines.

In areas where viral load monitoring is not available the WHO recommends using clinical and/or immunological criteria to identify patients failing on

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treatment [8]. However, these criteria are not evidence-based, and have poor performance [18]. Alternative strategies for detection of treatment failure are therefore required for ART programs in low-income countries until universal VL monitoring is established.

In Ethiopia, nearly 400,000 out of an estimated 781,000 PLHIV had started ART by 2016 [19,20], with most HIV care provided through health centres. Until 2015, viral load testing was only recommended for cases of clinically suspected treatment failure [21]. Although annual viral load testing is currently recommended for all patients receiving ART, the resources to comply with this are limited. The use of algorithms to prioritize patients for viral load testing should therefore be considered to optimize use of available laboratory resources.

For this purpose, we have constructed an algorithm intended for use in decentralized HIV care settings to identify subjects with increased likelihood of VF who need further evaluation with VL testing. The algorithm is based on robust variables independently associated with VF in cohort of adults receiving care at Ethiopian health centres. The performance of the algorithm is compared with the 2013 WHO failure criteria in our cohort participants.

Methods

Patient population

This study is based on a patient cohort prospectively recruited from October 2011 until March 2013 at all five public health centres providing ART in and around the city of Adama, Ethiopia (uptake area 600 000 inhabitants). The cohort was recruited to study methods to diagnose tuberculosis and virological failure in HIV positive adults. Detailed descriptions of the cohort has been published previously [22,23].

ART-naïve patients aged ≥ 18 years with recorded CD4 cell count < 350 cells/mm³ and/or WHO stage IV disease were eligible for enrolment in the cohort. Subjects with previous ART experience and/or tuberculosis treatment for > 2 weeks were excluded.

At inclusion, socio-demographic and medical information was collected, and at all subsequent visits symptoms and clinical findings were recorded following structured questionnaires. All patients enrolled in HIV care in Ethiopia receive adherence counselling at least twice before starting ART and adherence assessments are made at all clinical visits after ART initiation [21]. For study purposes, medication adherence was estimated using a three-question panel on: punctuality of daily tablet intake, number of missed doses weekly, and duration since last missed dose [24]. Treatment interruption of ART since last visit (for

any reason and at least one day's duration) was also recorded.

Follow-up visits after ART start were scheduled at months 1, 2, 3, 6, 9, 12, and biannually thereafter. Blood sampling for haematological parameters, CD4 cell counts and storage of plasma for later VL testing was performed in all participants at months 1, 3, 6, 12, and subsequent visits. Participants could, however, decline to give blood without being excluded from the study.

HIV-1 RNA quantification was performed on plasma aliquots stored at -80°C at the regional laboratory in Adama using Abbott Real-Time HIV-1 assay (Abbott Molecular Inc., Des Plaines, IL; detection limit 40 copies/mL) in batches during the study period. Results were communicated to care providers with recommendations to assess adherence and repeat viral load testing on subjects with VL ≥ 1000 copies/mL before referral for second line ART (according to national guidelines). Blood sampling was performed at the same visit as recording of symptoms and clinical findings, thereby blinding the clinicians performing the examinations with regard to VF. External quality assurance of the regional laboratory is regularly performed by the Center for Disease Control and Prevention (Atlanta, GA).

Subjects with a study visit 1 year (9–15 months) after ART start, with an accompanying viral load result, were included in this study.

The WHO criteria

The WHO 2013 clinical failure criterion is defined as a new or recurrent clinical event indicating severe immunodeficiency after 6 months of ART, whereas immunological failure is defined as a CD4 count below or at the value measured before starting ART or a CD4 count < 100 cells/mm³ [25]. In this study, all stage 3 and 4 events were considered to indicate severe immunodeficiency.

Statistical analysis

The aim of this study was to construct an algorithm with high sensitivity and acceptable specificity to identify subjects with VF 1 year after ART start. We used VL ≥ 1000 copies/mL as definition of VF.

To construct the algorithm, all variables registered at the 12-month visit were assessed for possible association with VF. Variables had to be considered robust with potential to be used in a decentralized care setting to be included. Since active case-finding for tuberculosis had been performed on the cohort at inclusion we included this parameter to evaluate its potential impact on VF.

We used routinely used threshold levels to dichotomize body-mass index (BMI; < 18.5 kg/m²), mid-upper

arm circumference (MUAC; <23 cm for women and <24 cm for men), haemoglobin (<11.0 g/dL), and lymphocyte count (<1100 cells/mm³) [26,27]. Karnofsky performance status (KPS), and CD4 count were analysed with ROC (receiver operating characteristics) curves and dichotomised at maximum sensitivity with acceptable specificity; KPS at <90% and CD4 count at <350 cells/mm³. Age was used as a continuous variable since no clinically useful threshold could be determined.

All variables were analysed with univariate logistic regression. Variables associated with VF in univariate analysis ($p < 0.3$) were entered into a multivariate regression model followed by stepwise backward removal of the least significant variable at each step until only variables independently associated with VF ($p < 0.05$) remained. The remaining variables constituted the VLTC.

The diagnostic accuracy of the VLTC and WHO criteria were evaluated using sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) with 95% CI. To further describe the discriminatory potential, numbers needed to test to identify one subject with VF (NNT) was calculated.

To assess possible effect modification (interaction) between the algorithm and gender, the performance of the algorithm and its individual components were also analysed separately for men and women.

We performed a sensitivity analysis including only subjects with complete data for all criteria included in the WHO criteria and VLTC, and assessed possible effects on the performance of the algorithms due to missing data. In this study, all participants underwent active case-finding for active tuberculosis at inclusion. To assess the impact of these investigations on the WHO clinical criteria, which includes incident tuberculosis during ART, subjects with tuberculosis were excluded in an additional sensitivity analysis. The statistical analyses were performed in SPSS version 21 (IBM Corp, Armonk, NY).

Results

Patient characteristics

Among the 812 individuals in the cohort, 729 (90%) started ART during the follow-up period, Figure 1. Baseline characteristics are shown in Table 1. Patients not remaining in the study until the 12-month visit could not be included in this study. A similar number of men and women did not remain in follow-up due to death (22 men and 21 women) or loss to follow-up (20 men and 19 women). Participants without viral load results and/or no registered study visit within the defined time frame 1 year after ART start, 116/610 (19%), were excluded. Characteristics of excluded participants were similar to those of included participants,

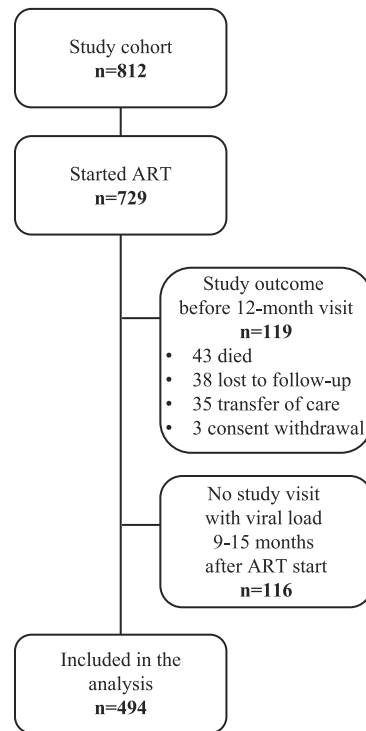


Figure 1. Flow chart of study participants.

except for a greater proportion of men being excluded due to unavailable data, Table 1. Seventy-five of the 82 participants without 12-month data remained in follow-up at 18 months of ART (2 were transferred out, 3 were lost to follow-up, and 2 declined further participation).

In total, 57 of the included 494 participants (12%) met our definition of VF 1 year after starting ART.

Derivation of the VLTC

In univariate analysis, the following variables were associated with VF ($p < 0.3$): gender, age, KPS, BMI, gender-specific MUAC, previous ART interruption, CD4 count <350 cells/mm³ haemoglobin and lymphocyte count, supplemental Table 1. Tuberculosis at inclusion, adherence <95%, and the clinical sign skin rash, did not show any association with VF.

After stepwise removal from the multivariate model the following variables remained: gender-specific MUAC, CD4 count <350 cells/mm³, and previous ART interruption, Table 2. Age was kept in the multivariate model for adjustments due to its univariate association with VF but without any clear threshold level.

Table 1. Baseline characteristics of study participants.

Characteristic	Started ART (n = 729)	12-month data available (n = 494)	12-month data unavailable (n = 116)	P
Age, years	32 (28–40)	33 (28–40)	30 (28–38)	0.13
Female	431 (59)	313 (63)	56 (48)	<0.01
BMI, kg/m ²	19 (18–21)	20 (18–22)	19 (18–21)	0.51
MUAC, cm	23 (21–25)	23 (21–25)	23 (21–25)	0.59
Karnofsky status, %	80 (80–90)	90 (80–90)	90 (80–90)	0.37
WHO clinical stage				0.68
Stage I	131 (18)	100 (20)	18 (16)	
Stage II	207 (28)	144 (29)	34 (29)	
Stage III	313 (43)	201 (41)	52 (45)	
Stage IV	77 (11)	48 (10)	12 (10)	
CD4 count, cells/mm ³	187 (116–274)	192 (127–274)	199 (115–302)	0.64
Lymphocyte count, cells/mm ³	1400 (1100–1800)	1400 (1100–1900)	1400 (1000–1800)	0.93
Haemoglobin, g/dL	11.5 (10.2–12.7)	11.6 (10.3–12.7)	11.6 (10.3–13.0)	0.93
Active tuberculosis at baseline	137 (19)	88 (18)	23 (21)	0.61

Data presented as n (%) of patients or median value (interquartile range).

P calculated with Chi-square and Mann-Whitney U test for categorical and continuous variables, respectively.

Abbreviations: ART, antiretroviral therapy; BMI, body-mass index; MUAC, mid-upper arm circumference.

Table 2. Variables associated with virological failure in multivariate analysis.

Variables	Unadjusted OR (95% CI)	Adjusted OR ^a (95% CI)
MUAC <23 cm ♀/ <24 cm ♂	2.6 (1.5–4.7)	2.7 (1.4–4.9)
CD4 count <350 cells/mm ³	5.2 (2.6–10.6)	5.5 (2.6–11.6)
Previous ART interruption	5.2 (1.6–16.6)	4.2 (1.2–14.1)

Abbreviations: OR, odds ratio; MUAC, mid-upper arm circumference.

^a Adjusted for age and the other variables remaining in the model.

Performance of the VLTC

A total of 299/494 (61%) had either a CD4 count <350 cells/mm³, a previous ART interruption, or MUAC below the gender-based threshold level; 52 of whom (17%) had VF. The NPV for determination of VF was 97% (95% CI, 94–99) with a corresponding sensitivity of 91% (95% CI, 91–97), Table 3. The specificity was moderate at 43% (95% CI, 39–48). Using the occurrence of any of the VLTC components to prompt a VL, the NNT decreased to 5.8 from 8.7 for universal testing. At higher thresholds, i.e. the occurrence of 2 or 3 VLTC components to prompt a VL, sensitivity markedly decreased (37% and 4%, respectively) and was therefore not considered in further analyses.

In comparison, the combined WHO criteria indicated VF in 153/494 (31%); 38 of whom (25%) had VF. The NPV was 94% (92–96) but the corresponding sensitivity was 67% (53–79), Table 3.

The VLTC had similar sensitivity for men and women, 90 and 93% respectively, but the specificity was higher among women resulting in a NPV of 94%

for men versus 99% for women, supplemental Table 2. In the multivariate age-adjusted model, including MUAC, CD4 < 350 cells/mm³, and previous ART interruption, the direction of associations remained the same for men and women.

Sensitivity analysis

Two sensitivity analyses were performed. First, a complete-case analysis including only those with full datasets regarding MUAC, CD4 count, treatment interruption, and WHO stage (n = 453/494; 55/57 with VF). The sensitivity increased from 91% to 95% for the VLTC, without any change for the remaining performance indicators, supplemental Table 2. The sensitivity for the combined WHO criteria decreased slightly from 67% to 64%. Second, subjects diagnosed with active tuberculosis at inclusion (n = 88) were excluded. The sensitivity of the AC increased slightly from 91% to 94% with no change of the remaining performance indicators, supplemental Table 2. The performance of the WHO criteria did not change.

Discussions

The discrepancy between the current recommendations for VL monitoring for all patients receiving ART and the insufficient capacity for VL testing constitutes a huge obstacle for ART programs in low-income countries, especially in view of the goal

Table 3. Performance of the Viral Load Testing Criteria (VLTC) and WHO criteria.

	n/N (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	NNT
VLTC:						
Any criteria	299/494 (61)	91 (81–97)	43 (39–48)	17 (16–19)	97 (94–99)	5.8
Two or more criteria	75/494 (15)	37 (24–51)	88 (84–91)	28 (20–37)	91 (90–93)	3.6
Three criteria	3/494 (1)	4 (0–12)	100 (99–100)	67 (16–96)	89 (88–91)	1.5
WHO criteria:						
Clinical	99/467 (21)	40 (28–54)	82 (77–85)	23 (17–31)	91 (89–92)	4.3
Immunological	79/490 (16)	45 (31–59)	88 (84–91)	32 (24–40)	92 (91–94)	3.2
Clinical and/or immunological	153/494 (31)	67 (53–79)	74 (69–78)	25 (21–30)	94 (92–96)	4.0

Abbreviations: CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; NNT, numbers needed to test.

of achieving universal ART coverage among PLHIV [8,10,28]. Although resources for VL monitoring are being scaled up in high-burden countries [10,19] it is important to consider alternative evidence-based strategies to monitor patients on ART. Algorithms that assess the likelihood of VF could help target resources for viral load testing in a cost-effective manner.

We have constructed such an algorithm using data recorded prospectively and blinded, in a cohort of patients starting ART at Ethiopian health centres; a representative setting for where most PLHIV globally receive ART. We decided to use only robust parameters that have low inter- and intra-observer variability. Furthermore, we did not consider data on trends in laboratory results since such information could be lacking at peripheral clinics and criteria requiring calculations can be error-prone [29].

The Viral Load Testing Criteria (VLTC) consists of three parameters all independently associated with an increased likelihood of VF: signs of malnutrition (measured by gender-specific MUAC thresholds), CD4 count <350 cells/mm³, and interruption of ART since last visit. These criteria are possible to use in most decentralized care settings in low-income countries.

Mid-upper arm circumference is a well-established marker of malnutrition, which also has been associated with mortality during ART [30,31]. Furthermore, we have shown its association with virological suppression (VL <400 copies/mL) at 6 months after ART start in this cohort [23]. Given the observational nature of this study, we cannot determine the mechanisms involved in this association. It is possible that reflects impoverishment in this population, but it could also be a consequence of continued HIV replication with HIV-related wasting.

Inadequate treatment adherence has been linked with the risk of VF [14,15], but such an association was not observed in our cohort. However, VF was more common in patients with treatment interruptions. We consider this parameter to be reliable and easy to measure compared with more complex assessments of adherence level. A similar criterion was part of clinical algorithm for VF developed in Uganda [17], and associated with VF in a study from South Africa [32].

Over the last decade availability of CD4 count testing has increased as part of ART roll-out [33]. Although CD4 count measurement may not be necessary for many patients with universal access to ART [34], this technology can still be useful in settings with limited treatment coverage or limited access to VL. We used a ROC curve to determine an appropriate threshold level for CD4 count. The threshold 350 cells/mm³ was chosen for its high sensitivity (82%) and acceptable specificity (54%) and

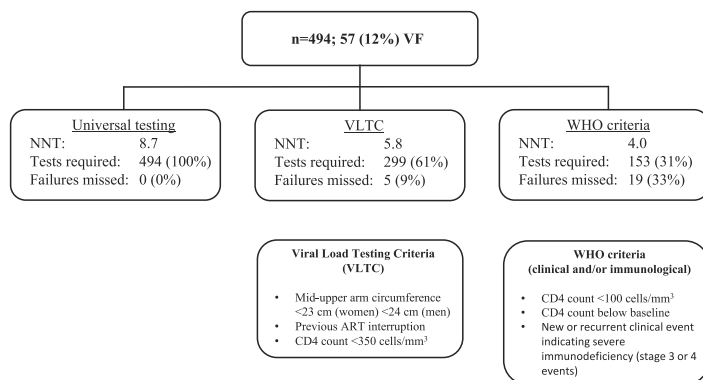
coincided with the median CD4 count at 12 months after ART start. Both CD4 count <100 cells/mm³ and CD4 count below baseline (WHO immunologic criteria) were associated with VF, but with low sensitivities at 23% and 32%, respectively. Different approaches in the use of CD4 counts to detect individuals with failing treatment has been suggested, such as risk charts [35] and CD4 gain percentile curves [36]. To keep the VLTC simple, and user-friendly we did not consider changes in CD4 count over time. However, the threshold indicating increased likelihood of VF is influenced by the CD4 count at treatment initiation. Indeed, our participants had a median CD4 count of 192 cells/mm³ at ART start, comparable with pre-ART counts in many African settings [37]. However, ART is now recommended for all PLHIV irrespective of CD4 counts [8], and it is likely that this will affect the performance of CD4 count data for identification of VF.

The use of algorithms will inevitably lead to some degree of misclassification. The VLTC had high sensitivity with acceptable specificity resulting in a NPV of 97%. Since the criteria should be regarded as a screening method to identify patients in need of VL testing to determine whether VF is present, sensitivity must be high. We considered construction of a scoring system based on sums of individual criteria, but since this compromised sensitivity markedly we decided to use the criteria separately.

There have been previous attempts to construct algorithms for targeted viral load testing based in Sub-Saharan Africa [15–17,35,36] and Cambodia [14]. Compared with these algorithms, the VLTC has few parameters, does not require any calculations, and only use information that can be available point-of-care. Despite this, it achieved high sensitivity in derivation. A clinical predictor score developed in Cambodia [14] achieved comparable sensitivity (78%) in a subsequent validation in Cambodia [38], but the sensitivity was low (51%) when validated in Uganda [15]. The clinical algorithms constructed in Sub-Saharan Africa (2 in Uganda and 1 in South Africa), had sensitivities ranging from 67% to 76% [15–17], external validations of these algorithms are yet to be published. The sensitivity of the 2013 WHO criteria was higher in this cohort (67%) compared with previously reported data [15,16,18]. For targeting viral load testing, however, misclassification of 33% of subjects with VF cannot be accepted.

The drawback of the high sensitivity for the VLTC is its limited specificity requiring VL testing of a large proportion (61%).

The development of point-of-care testing devices has great potential in improving access to VL with reduced turnaround time [39]. However, due to limited capacity of such devices, a combination with central, high-volume testing is still needed [40]. Algorithms such as the VLTC



[NNT, numbers needed to test to identify one individual with virological failure (VF)]

Figure 2. Algorithms for viral load testing.

could be considered for determination of subjects at highest risk of VF that should be for point-of-care testing, whereas remaining samples are sent to central laboratories. For such an approach, a combination of at least two criteria of the VLTC could be considered, increasing specificity to 88% for point-of-care testing.

This study was performed in health centres with nurse-based care, a setting in which most PLHIV receive their care. Data used for this study were prospectively collected from participants in a well-characterized cohort by nurse-clinicians blinded to the outcome of the study following a structured protocol. All participants were investigated for active tuberculosis at baseline. In line with a previous report from this cohort, VF was not associated with tuberculosis co-infection [23].

This study has some limitations. We defined VF as a single viral load ≥ 1000 copies/mL. Since some patients with a single elevated viral load level will have suppressed viremia on repeated testing [6], this definition could overestimate the rate of treatment failure. However, the VLTC is not intended to diagnose treatment failure (defined by the WHO as a VL above 1000 copies/mL at two consecutive measurements with adherence counselling in between [8]), but rather to identify patients at risk who need viral load testing. Data on drug resistance was not available for the participants of this study, information that unfortunately seldom is available in low-income settings. A proportion of subjects who started ART were excluded from analysis since a study visit with accompanying viral load was not available, which may have had some impact on the findings. In particular, a higher proportion of male participants were excluded for this reason, but few were lost to follow-up. Importantly, the VLTC has hitherto not been externally validated, which is necessary to assess its robustness before implementation in standard care. This also

concerns the performance of this algorithm among ART-experienced patients, who were not included in our cohort.

In conclusion, the VLTC consisting of three simple-to-measure criteria, was more sensitive than the 2013 WHO criteria in determining the likelihood of VF 1 year after starting ART. The VLTC could therefore be used to rule out VF in 4/10, reducing the numbers needed to test from 8.7 (universal testing) to 5.8 for each VF identified (Figure 2). VL resources could thereby be allocated more efficiently, with few cases of missed VF.

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Author contributions

Conceived and designed the study: AR TTB SS PM PB. Data collection: AR TTB PB. Analysed the data: AR PEI PM. Wrote the paper: AR PB. All authors read and approved the final manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

Ethics and consent

Ethical approval was obtained from the National Research Ethics Committee at the Ministry of Science and Technology of Ethiopia and the Regional Ethical Review

Board of Lund University, Sweden. All study participants provided written informed consent. Trial registration: NCT01433796. Registered 11 September 2011.

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Paper context

Virological monitoring is recommended for people receiving antiretroviral therapy, but monitoring capacities are insufficient in low-income countries. The World Health Organization's immunological and clinical failure criteria have poor performance for identification of virological failure. We present an algorithm (Viral Load Testing Criteria), that in derivation has high sensitivity and acceptable specificity to identify virological failure 12 months after treatment initiation. If externally validated, these criteria could be used to allocate viral load resources more efficiently.

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Supplemental Table 1. Univariate odds ratios for virological failure (VF).

Variable	Missing data	non-VF (n=437)	VF (n=57)	OR (95% CI)	P
Male gender	0	152 (31)	29 (51)	1.9 (1.1-3.4)	0.02
Age	0	32 (28-40)	36 (30-44)	1.0 (1.0-1.1)	0.09
Karnofsky status <90%	0	54 (12)	18 (32)	2.4 (1.3-4.4)	<0.01
Body-mass index <18,5 kg/m ²	9 (2)	52 (12)	12 (21)	1.9 (1.0-3.9)	0.07
MUAC <23 cm ♀ / <24 cm ♂	10 (2)	95 (22)	24 (43)	2.6 (1.5-4.7)	<0.01
Baseline tuberculosis	0	78 (18)	10 (18)	1.0 (0.5-2.0)	0.96
Adherence <95%	2 (0)	67 (15)	9 (16)	1.0 (0.5-2.2)	0.94
Previous ART interruption	0	8 (2)	5 (9)	5.2 (1.6-16.3)	0.01
CD4 cell count <350 cells/mm ³	4 (1)	199 (46)	46 (82)	5.4 (2.7-11.0)	<0.01
Lymphocyte count <1100 cells/mm ³	8 (2)	61 (14)	13 (24)	1.9 (1.0-3.7)	0.07
Haemoglobin <11.0 g/dL	3 (1)	52 (12)	11 (20)	1.8 (0.9-3.7)	0.11
Skin rash	0	17 (4)	2 (4)	0.9 (0.2-4.0)	0.89

Abbreviations: OR, odds ratio; CI, confidence interval; MUAC, mid-upper arm circumference; ART, antiretroviral treatment.

Data presented as n (%) or median (interquartile range) if not stated otherwise.

P value derived from the univariate logistic regression model.

Supplemental table 2. Performance of the Viral Load Testing Criteria (VLTC) and WHO criteria for men and women, excluding incomplete-cases, and excluding subject with baseline tuberculosis, respectively.

	n/N (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	NNT
VLTC*:						
Men only	129/181 (71)	90 (73-98)	32 (25-40)	20 (18-23)	94 (85-100)	5.0
Women only	170/313 (54)	93 (77-99)	49 (44-55)	15 (13-17)	99 (95-100)	6.5
Complete-cases	279/453 (62)	95 (85-99)	43 (38-48)	19 (17-20)	98 (95-99)	5.4
Subjects with tuberculosis excluded	242/406 (60)	94 (83-99)	45 (40-50)	18 (16-20)	98 (95-99)	5.5
WHO criteria*:						
Men only	68/181 (38)	62 (42-79)	67 (59-75)	26 (20-34)	90 (85-94)	3.8
Women only	85/313 (27)	71 (51-87)	77 (72-82)	24 (18-30)	96 (94-98)	4.3
Complete-cases	147/453 (32)	67 (53-79)	72 (68-77)	25 (21-30)	94 (92-96)	4.0
Subjects with tuberculosis excluded	119/453 (26)	64 (49-77)	75 (70-80)	25 (20-31)	94 (92-96)	4.0

Abbreviations: CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; NNT, numbers needed to test.

* VLTC, positive if any criterion is present; WHO criteria, combined clinical and/or immunological criteria.



Beyond research

The research station in Adama, Ethiopia, active since 2010, aims to through research improve aspects of health care for all people living there, and in other similar places. Yet, during these years, we have met many individuals suffering from extreme poverty and social exclusion. For many of them, especially single mothers and their children, improved health care will not suffice for them to survive. In 2017, we launched the non-governmental organization *End Vulnerability of Women* (EVOW) aiming to provide direct support to these women and their children through micro financing and income generating initiatives, health education and facilitated access to health services, and nutritional support.

If you would like to support our effort to improve the livelihood
for these women and children in Ethiopia please visit
www.evow-sweden.se