

From Department of Laboratory Medicine
Karolinska Institutet, Stockholm, Sweden

OPTIMIZATION OF PEDIATRICS ANTIRETROVIRAL TREATMENT OUTCOMES AMONG HIV INFECTED CHILDREN IN ETHIOPIA

Birkneh Tilahun Tadesse, MD



**Karolinska
Institutet**

Stockholm 2020

All previously published papers were reproduced with permission from the publisher.
Published by Karolinska Institutet.
Printed by Arkitektkopia AB, 2019
© Birkneh Tadesse, 2020
ISBN 978-91-7831-640-3

Optimization of Pediatrics Antiretroviral Treatment Outcomes among HIV infected Children in Ethiopia

THESIS FOR DOCTORAL DEGREE (Ph.D.)

The public defence for the degree of Doctor of Philosophy at Karolinska Institutet will be held at Karolinska Institutet, Huddinge/C1-huset/C1.87/För, Karolinska University Hospital, Huddinge.

Friday, January 17, 2020, at 09:30

By

Birkneh Tilahun Tadesse

Principal Supervisor:

Associate Professor Eleni Aklillu
Karolinska Institutet
Department of Laboratory Medicine
Division of Clinical Pharmacology

Co-supervisor(s):

Professor Eyasu Makonnen
Addis Ababa University
Department of Pharmacology

Doctor Degu Jerene
Management Sciences for Health
Department of Management
Sciences for Health

Opponent:

Professor Stefano Vella
Catholic University of Rome
Department of Global Health

Examination Board:

Professor Anna Mia Ekström
Karolinska Institutet
Department of Public Health
Sciences (PHS)
Division of Global Health

Professor Mats Målqvist
Uppsala University
Department of Department of
Women's and Children's Health
Division of International Child
Health and Nutrition

Associate Professor Gaetano Marrone
Karolinska Institutet
Department of Public Health Sciences
Division of Global Health

To my family!

ABSTRACT

Ending the HIV/AIDS epidemic by 2030 is a global agenda. To meet this global goal, having safe and effective antiretroviral therapy is a key requirement. In Ethiopia, the safety and efficacy of combination antiretroviral therapy in HIV infected pediatric population is poorly studied. In this thesis, I aimed at understanding the short- and long-term safety and efficacy of antiretroviral therapy among HIV infected children in Ethiopia. Clinical and laboratory data were recorded for a total of 870 HIV infected children in two parallel cohorts – EPDOS and EPHIC projects.

We first investigated the burden and correlates of pretreatment drug resistance (PDR) in **Paper I**. We observed that the overall rate of PDR was 14%. All cases with PDR had resistance to NNRTIs while ~9% harbored resistance solely to NNRTIs and ~5% harbored resistance to both NNRTIs and NRTIs. No resistance was observed to protease inhibitors.

In **Paper II**, among children who were followed for 48 weeks following initiation of treatment, we assessed virologic outcome of children at one year of cART initiation using Cox Proportional Hazards Model. In total, 94/110 (85.5%) achieved virological suppression to undetectable levels during the first year of treatment. Thirty-six (31.9%) experienced virologic rebound. Tenofovir-containing cART regimen and absence of PDR were associated with higher virologic suppression.

In **Paper III**, we explored burden and correlates of HIV drug resistance among children who failed treatment. Overall, 81% (73/90) of successfully genotyped participants had resistant mutations. From these, 69% (62/90) harbored dual drug class resistance. Strikingly, 42% of the participants harbored resistance to all four NRTIs recommended for second line use in the setting. Longer duration of cART and any regimen changes were associated with occurrence of drug resistance mutations.

In **Paper IV**, we investigated the long term renal and hepatic toxicities associated with antiretroviral therapy among cART experienced children. At study enrolment, 177(25.1%) and 83(11.8%) had high aspartate aminotransferase and alanine aminotransferase (ALT), respectively. Zidovudine or nevirapine containing regimens and viral load >1000 copies/mL were associated with elevated ALT. Twenty-four (3.4%) and 84(12.1%) of the children had elevated creatinine and blood urea nitrogen (BUN), respectively. A progressive increment in BUN and decrement in GFR were observed during the follow up period. Both AST and ALT exhibited a decreasing trend.

In **Paper V**, we compared the prevalence and correlates of dyslipidemia between cART naïve and experienced HIV infected children. Dyslipidemia was more common among cART experienced (70.2%) than naïve (58.1%) HIV infected children ($p=0.03$). Higher proportion of low HDLc (40.2% versus 23.4%, $p=0.006$) and hypertriglyceridemia (47.2% versus 35.8%, $p=0.02$) was observed among cART experienced HIV infected children as compared to naïve. No difference was observed in the proportion of total hypercholesterolemia and high LDLc levels between the groups. Undernutrition was associated with more dyslipidemia in the cART naïve group ($p=0.01$).

In **conclusion**, we showed that a high proportion of children with HIV infection in resource-limited settings do achieve virologic suppression during the first year of treatment initiation. We also confirmed that HIV drug resistance is a major cause of virologic treatment failure in children with limited virologic monitoring. On the other hand, the burden of PDR is low but predicts virologic outcome. Adverse events associated with cART are the major challenges for meeting the global UNAIDS targets in 2020 and 2030. The findings call for targeted monitoring and treatment of children in resource-limited settings.

LIST OF SCIENTIFIC PAPERS

- I. **Tadesse BT**, Tsai O, Chala A, Chaka TE, Eromo T, Lapointe HR, Baraki B, Shahid A, Tadesse S, Makonnen E, Brumme ZL, Aklillu E, Brumme CJ. Prevalence and Correlates of Pre-Treatment HIV Drug Resistance among HIV-Infected Children in Ethiopia. *Viruses*. 2019 Sep 19;11(9). pii: E877. doi: 10.3390/v11090877..
- II. **Tadesse BT**, Chala A, Mukonzo J, Chaka TE, Tadesse S, Makonnen E, Brumme ZL, Brumme CJ, Aklillu E. Rates and correlates of short term virologic response among treatment-naïve HIV-infected children initiating antiretroviral therapy in Ethiopia: a multi-center prospective cohort study. *Pathogens*. 2019 Sep 24;8(4). pii: E161. doi: 10.3390/pathogens8040161..
- III. **Tadesse BT**, Kinloch NN, Baraki B, Lapointe HR, Cobarrubias KD, Brockman MA, Brumme CJ, Foster BA, Jerene D, Makonnen E, Aklillu E, Brumme ZL. High Levels of Dual-Class Drug Resistance in HIV-Infected Children Failing First-Line Antiretroviral Therapy in Southern Ethiopia. *Viruses* 2018, 10(2). pii: E60. doi: 10.3390/v10020060.
- IV. **Tadesse BT**, Foster BA, Kabeta A, Ayalew F, G HM, Jerene D, Makonnen E, Aklillu E. Hepatic and renal toxicity and associated factors among HIV-infected children on antiretroviral therapy: a prospective cohort study. *HIV Med* 2019, 20:147-156. doi: 10.1111/hiv.12693. Epub 2018 Nov 26.
- V. **Tadesse BT**, Foster BA, Chala A, Chaka TE, Bizuayehu T, Ayalew F, G HM, Tadesse S, Jerene D, Makonnen E, Aklillu E. HIV and cART-Associated Dyslipidemia Among HIV-Infected Children. *J Clin Med* 2019, 8 (4).pii: E430. doi: 10.3390/jcm8040430.

CONTENTS

1	Introduction	1
1.1	The HIV Epidemic in Children	3
1.1.1	Overview of Pediatric HIV infection	3
1.1.2	Pathogenesis of HIV in children	3
1.1.3	Burden of Pediatric HIV in Ethiopia	5
1.1.4	Diagnosis of HIV infection in children in Ethiopia	5
1.1.5	Pediatric HIV treatment in Ethiopia	6
1.2	Pediatric HIV Treatment Outcomes	7
1.2.1	Virological outcome following initiation of cART	8
1.2.2	First-line treatment failure and predictors in Ethiopia	8
1.2.3	Impact of HIV drug resistance on treatment success	9
1.2.4	Drug adverse events affecting treatment outcome	11
2	AIMS OF THE Research	13
2.1	Specific Aims	13
3	Materials and methods	14
3.1	Patient population	14
3.2	Diagnosis and treatment of HIV Infection	15
3.3	Patient recruitment and follow up	16
3.4	Study procedures: laboratory and clinical parameters	16
3.4.1	HIV Drug Resistance Testing	19
3.4.2	Blood Chemistry Tests	20
3.5	Statistical analysis and data management	21
3.6	Ethical considerations	23
4	Results and discussion	24
4.1	Pretreatment HIV Drug Resistance among HIV infected Children (paper I)	24
4.2	HIV treatment outcomes among HIV infected children (Paper II)	26
4.3	Prevalence and Correlates of Acquired HIV Drug Resistance (Paper III)	30
4.4	Liver and renal toxicities associated with long term cART use (paper IV)	32
4.5	Metabolic disturbances among HIV infected children on treatment (paper V)	34
5	Conclusions and recommendation	38
6	Acknowledgments	40
7	References	42

LIST OF ABBREVIATIONS/ACRONYMS

ABC	Abacavir
ADR	Acquired drug resistance
AIDS	Acquired immunodeficiency syndrome
ALT	Alanine aminotransferase
APRI	AST to Platelet Ratio Index
ART	Antiretroviral therapy
AST	Aspartate aminotransferase
AZT	Zidovudine
BAZ	Body mass index Z score
BUN	Blood urea nitrogen
cART	Combination antiretroviral therapy
CBC	Complete blood count
CDC	Centers for Disease Control
CPT	Cotrimoxazole preventive therapy
CVD	Cardiovascular diseases
DBS	Dried blood specimen
DILI	Drug induced liver injury
DNA	Deoxyribonucleic acid
DPS	Dried plasma specimen
DTG	Dolutegravir
DRM	Drug resistance mutations
ESA	East and Southern Africa
EFV	Efavirenz
FIB-4	Fibrosis score-4
GFR	Glomerular Filtration Rate
HAZ	Height-for-age Z score
HBSAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HDLc	High density lipoprotein cholesterol
HIV	Human immunodeficiency virus

HIVAN	HIV associated nephropathy
HIVdb	HIV drug resistance database
HIVDR	HIV drug resistance
HR	Hazard ratio
IAS	International AIDS Society
IQR	Interquartile range
IRB	Institutional Review Board
LDLc	Low density lipoprotein cholesterol
LMIC	Low- and middle-income countries
MTCT	Mother to child transmission
NAT	Nucleic acid tests
NNRTI	Non-nucleoside reverse transcriptase inhibitors
NRTI	Nucleoside reverse transcriptase inhibitors
NRERC	National Research and Ethics Review Committee
NVP	Nevirapine
PCR	Polymerase chain reaction
PDR	Pretreatment drug resistance
PI	Protease inhibitor
PMTCT	Prevention of mother to child transmission
PRD	Progressive renal disease
pVL	Plasma viral load
RNA	Ribonucleic acid
SNNPR	Southern Nations Nationalities and Peoples Region
TB	Tuberculosis
TDF	Tenofovir
TDR	Transmitted drug resistance
UNAIDS	United Nations Programme on HIV/AIDS
VTF	Virologic Treatment Failure
WAZ	Weight for age Z score
3TC	Lamivudine

1 INTRODUCTION

HIV infection is one of the devastating epidemics that mankind has ever faced in the 21st century. The first cases of HIV have been reported in 1981 after young previously healthy gay men presented with rare cases of lung infections in the USA [1]. Afterwards, the HIV epidemic has grown very fast, and HIV has been responsible for claiming millions of lives over the past four decades. As of 2017, there are around 37 million people who are living with HIV, the number has been steadily increasing with slight plateauing over the past 15 years, which could be attributable to the prevention and control efforts by the global community. The incidence of new HIV infections has been decreasing over the past 20 years – with 3.4 million (range: 2.6 to 4.4 million) infections per year in 1995 to 1.8 million (range: 1.4 to 2.4 million) infections per year in 2017 [2].

Sub Saharan Africa has the highest burden of HIV/AIDS with significant regional and sub national variations in the prevalence and transmission dynamics of the virus [3]. The East and southern Africa (ESA) region is the hardest hit by the HIV epidemic. The majority of the HIV infected population lives in the ESA region – in the region, 19.6 million people live with HIV with 800 thousand new HIV infections per year and an adult prevalence of 6.8% [4]. The impact of HIV/AIDS in ESA is profound – HIV has created a huge burden on the health system and is also a significant social and economic hurdle to the governments and the society as a whole [5].

The overwhelming response by the global community and the huge expenditure by funders to the HIV epidemic are the reasons for success achieved in reducing HIV morbidity, mortality and control the spread of the epidemic. Funding for HIV has increased from around \$5 billion in 2000 to more than 20 billion in 2017 [2, 6]. A sustained support for HIV prevention and control from the Global Fund, especially targeting high burden and low-income countries, is one of the strategies that largely contributed to curbing the HIV epidemic in high burden settings. However, it was also noted that the most at risk population including children was not properly prioritized in most of these settings [7].

The overall HIV/AIDS related mortality halved over the past decade – it decreased from an average of 1.9 million deaths per year in 2005 to 940 thousand in 2017 [2, 8]. Effective combination antiretroviral therapy (cART) is key to sustained virological suppression and hence decreased morbidity and mortality. Access to treatment has improved significantly following global initiatives like 90-90-90 goals and the “ending the AIDS epidemic in 2030” [9, 10]. The 90-90-90 goals aim that 90% of patients who have HIV infection get diagnosed for HIV; 90% of those who know their status initiate cART; and, 90% of those who are on cART maintain virological suppression. In response to these global targets, the national and international efforts have led to a cART coverage of 79%, and 81% of those who have been initiated on cART are virologically suppressed globally [2].

Figures 1-3 show the overall prevalence, incidence and mortality of HIV infection among all ages and sexes, which were published in the Global Burden of Diseases report in August 2019 [11].

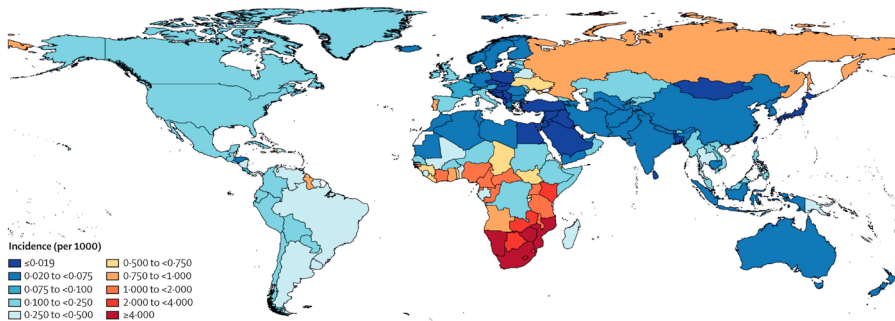


Figure 1. Global incidence of HIV infection among the general population as of 2017. Source: Global Burden of Diseases (GBD) report, 2019 [11].

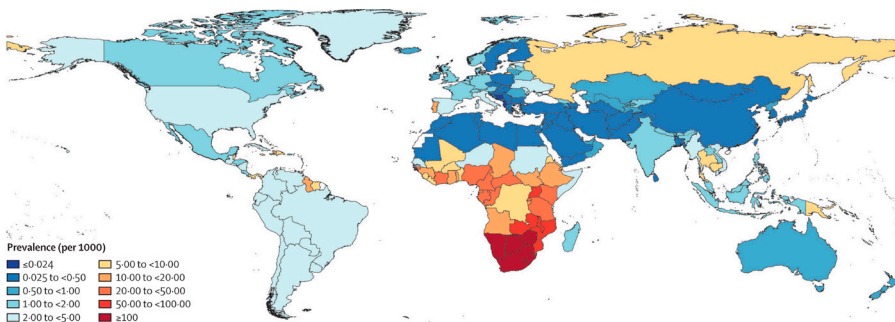


Figure 2. Global prevalence of HIV infection in all ages and sexes, 2017. Source: Global Burden of Diseases (GBD) report, 2019 [11].

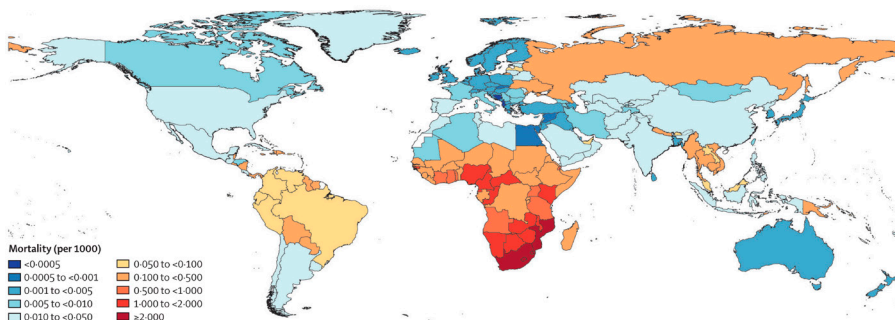


Figure 3. HIV/AIDS associated mortality among all ages and sexes, 2017. Source: Global Burden of Diseases (GBD) report, 2019 [11].

1.1 The HIV Epidemic in Children

1.1.1 Overview of Pediatric HIV infection

The history of pediatric HIV/AIDS starts back in 1982 when the CDC reports 22 cases of infants with HIV infection after receiving blood transfusion and with the first recommendations to prevent mother to child transmission of HIV coming out in the USA in 1985 [12]. Nearly three decades after the first cases of pediatric HIV have been reported, currently 1.7 million [1.3 million–2.2 million] children live with HIV [13]. The history of pediatric HIV in Ethiopia likely corresponds with the introduction of the HIV virus in the country where the virus might have been first introduced in the 1980s according to genetic diversification studies [14]. In the early 1980s when the burden of HIV in the general population was still low, one of the main ways of viral transmission was mother to child transmission (MTCT) leading to the first cases of pediatric HIV cases [15].

Children and adolescents constitute a unique group of the HIV infected population and require evidence-based approaches to care and treatment decisions [16]. Pediatric HIV care and treatment progress indicators often lag behind those for HIV infected adults and are associated with several bottlenecks, challenges and opportunities [17, 18]. More alarmingly, the UNAIDS further warns that the gains made among the pediatric population are slowing [17]. The fear is evidenced by the decline in new HIV infections from 2017 to 2018 of less than 10%; and, that only 52% of children who live with HIV infection had received combination antiretroviral therapy, which is lower than the adult figure (60%) [9, 17]. Moreover, there were 110 000 AIDS-related child deaths in 2017. In 2017, despite the high prevention of mother to child transmission (PMTCT) coverage of 80% – indicating the prevalence of pregnant women who had access to cART for PMTCT purposes, 180,000 children were newly infected with HIV either during pregnancy, birth or breastfeeding [17]. This figure is far from the UNAIDS target of 40 000 or fewer by the end of the year 2018 [17].

To improve the care and treatment of HIV infected children, the priority agenda for further action might include improving linkage to care, adherence to treatment and optimizing long and short-term outcomes of cART [19]. Management of antiretroviral therapy associated adverse events, retention to care, psychosocial support and improving disclosure of HIV status have also been key areas of focus for pediatric HIV care and treatment [19, 20].

1.1.2 Pathogenesis of HIV in children

HIV infection outcomes generally depend on integrity and maturity of the innate and adaptive immune systems. Children who acquire HIV infection vertically from an HIV infected mother have an immature immunity at an earlier age, specifically the adaptive/specific immunity is immature exhibiting no recall to previous

exposure of antigens [21]. Natural killer cells (NK), plasmacytoid and dendritic cells were reported to have significant impairments in both number and function among perinatally HIV infected children [21, 22]. The main players of the HIV infection pathogenesis are a sub set of the CD8+ T-lymphocyte population who produce cytokines and chemokines that have effect on viral infections; and, the CD4+ T-lymphocyte population which is critical for supporting antibody production and mediating the cytotoxic T-lymphocyte responses [23, 24]. This underlines the need for focusing on preserving these cell lines to ensure virologic suppression and low morbidity/mortality.

Among perinatally HIV infected children, a bimodal distribution of HIV progression and symptomatology was described in the developing world – with faster progression observed among those with in uterine infection compared to those who acquired infection postnatally [25, 26]. In addition to the time when HIV infection was acquired, gestational age and maternal health status [27], age appropriate CD4+ T-lymphocyte count, and HIV virus characteristics [28] were described as major predictors of death and disease progression among vertically HIV infected children [29].

The dynamics of HIV infection among perinatally infected children has been well described – with the availability of quantitative HIV-1 RNA polymerase chain reaction (PCR)[30-32]. Several studies documented the relevance of viral load on disease progression, early childhood mortality, and response to antiretroviral therapy where a higher baseline viral load is associated with poorer treatment outcomes [32-36].

Overall, the probability of vertical transmission of HIV without any PMTCT intervention is between 20% and 45%. From this aggregate, mother to child transmission (MTCT), 5-10% of the infections occur in utero; 10-20% happen intrapartum; and, 5-20% occur through breastfeeding [37]. The natural history of HIV also depends on the time of acquisition of HIV infection – whether, intrapartum or during breast feeding. Without early antiretroviral therapy, about 25-30% of perinatally HIV infected children are rapid progressors most of whom die before celebrating their first birthday. This group of children most likely acquires HIV infection in utero or during the early post-natal period. The majority of vertically HIV infected children become symptomatic during the early years of life and most (50-60%) die before celebrating their fifth birthday. A smaller group (5-25%) continue to become long term survivors and live beyond 8 years of age. These children tend to have lymphocytic interstitial pneumonitis and have poor anthropometric z scores [37].

1.1.3 Burden of Pediatric HIV in Ethiopia

Ethiopia is one of the high burden countries in the sub Saharan African region, which constitutes about 80% of the total HIV infections worldwide [38]. The overall prevalence of HIV infection in Ethiopia is around 1% (with a large variation by geographical area – 2.9% urban versus 0.4% rural prevalence); and, incidence rate of 0.33/1000 population. An estimated 610,000 (470 000 – 780 000) people live with HIV as of 2017 [39, 40]. In 2017, there are 62 000 (38 000 – 86 000) children younger than 14 years living with HIV; and, nearly 5 500 (2 600 – 8 800) new HIV infections occur annually in children less than 14 years of age [40]. Around 3 600 (1 800 – 5 800) deaths in children and 330 000 (220 000 – 460 000) orphans were attributed to HIV infection [40].

When we closely look at the trend of HIV infections in Ethiopia over the past 20 years, the prevalence of new infections declined by more than 80% during the first 10 years from 2000-2010. However, during the second decade, the HIV incidence rate started to increase by 10% yearly and the total number of new HIV infections increased by more than one third [41, 42]. The reversal of the gains in controlling the HIV pandemic in the country is basically associated with the weakening of national programs which were designed to control the epidemic. Particularly, in recent years, the surge in the prevalence and transmission of HIV in the townships has been reported to be worrisome [39, 41]. There is a need to strengthen and sustain the educational, advocacy and testing programs nationally to maintain the success achieved in controlling the HIV epidemic in the country. Overall, HIV still appears to be a huge burden as a cause of child morbidity and mortality, and the already weak health system and economy of the country are under pressure.

1.1.4 Diagnosis of HIV infection in children in Ethiopia

Diagnosis of HIV in children is complicated by the passage of HIV antibodies from the HIV infected mother to her baby; and, hence the presence of HIV antibodies in the baby cannot be used for diagnosis [43]. Routine rapid antibody testing is recommended for all infants and mothers living in a setting where the prevalence of HIV is >1% [44]. Virologic testing should be done for all infants who are exposed to HIV at 4 to 6 weeks of age, as these children will need to be on antiretroviral treatment as early as possible [45]. Nucleic acid tests [NATs] which include HIV RNA and HIV DNA PCR assays are key to diagnose HIV infection in infants. A positive first test requires a repeat virologic testing on another specimen as there could be false positive results with several RNA and DNA assays [46].

Diagnosis of HIV infection in infants and children can be done using clinical, immunologic and virological parameters (see Table 1). In the absence of virologic tests that can confirm HIV infection, in infants with HIV exposure, clinical criteria can

be used to make a diagnosis of presumptive severe HIV infection [47]. Specifically, an HIV exposed infant who develops severe sepsis, candida infection and chronic or persistent diarrhea is considered a case of severe presumptive HIV infection.

Table 1. Summary of the methods of diagnosis of HIV infection in children

Diagnostic Method	Advantage	Limitation
Clinical Suspicion of HIV diagnosis – diagnosis of Presumptive Severe HIV Infection	<ul style="list-style-type: none"> - Used in young infants who were exposed to HIV infection - Is used when there is limited access to virologic tests to confirm infection among <18 month children [47] - Easy to do, readily available and cost effective 	<ul style="list-style-type: none"> - Low sensitivity and secficity [48] - Leads to treatment discontinuation in children with other diagnosis
Antibody tests	<ul style="list-style-type: none"> - Can be used to confirm infeciton for children >18 months - Can also be sued to rule out infection among <18 month children [49]. - Cheap and easily accessible 	<ul style="list-style-type: none"> - Can not be used to make diagnosis of HIV in children <18 months
Virologic tests – HIV DNA or RNA PCR and other antigenic tests	<ul style="list-style-type: none"> - Can be used to confirm infection among HIV exposed infants and children <18 months - Has high sensitivity and specificity - Not affected by HIV antibodies transferred from the mother 	<ul style="list-style-type: none"> - Expensive and unavailable in most resource constrained settings

1.1.5 Pediatric HIV treatment in Ethiopia

Combination antiretroviral therapy (cART) has dramatically changed the natural history of HIV/AIDS, once a fatal disease has changed to a chronic illness. In 2014, UNAIDS set the 90-90-90 goals to be achieved by 2020 [50]. Following these efforts, the coverage of cART is increasing dramatically – as of 2018, nearly 80% of those who knew their HIV status could access treatment [40]. This is a significant progress compared to the 2016 UNAIDS report of around 50% accessing treatment [51].

Ethiopia has a remarkable progress towards the 90-90-90 targets, particularly in the first two 90’s. Seventy three percent of those who live with HIV know their status and 71% of those who know their status are on care and treatment [40]. However, the progress towards achieving the third 90 of achieving 90% virological

suppression among those who are on treatment is still lagging behind at around 32%. This figure is much lower than the international average of 81% virological suppression among those who initiated cART[40]. Even though the achievement is remarkable for adults, the coverage of cART access in children is only 34%, and only 21 385 children are on cART by 2017, which is around only a third [39, 40].

Based on guidelines from the World Health Organization (WHO), for children three years and older, the preferred first line combination includes two nucleoside reverse transcriptase inhibitors (NRTI) and one non-nucleoside reverse transcriptase inhibitors (NNRTI) – namely, Abacavir/Tenofovir, Lamivudine and Efavirenz (EFV) [52]. The current recommendation is to start all HIV infected children and adolescents on cART irrespective of clinical and laboratory parameters. The current recommendation by the WHO includes initiating cART with a protease inhibitor (PI)–based regimen for all children who are younger than three years since several studies indicated a high level of pre-treatment HIVDR, especially in children with PMTCT exposure [52, 53]. Specifically, the recommended first line cART for children 3 to 10 years is Abacavir (ABC), Lamivudine (3TC) and EFV. There are multiple alternative regimens for this age group – the 3TC backbone is usually the same with a combination of Zidovudine (AZT) or Tenofovir (TDF) replacing the NRTI by ABC. For the NNRTI wing, Nevirapine (NVP) can be used instead of EFV [52].

The adolescent first line cART regimen includes TDF as a preferred NRTI, 3TC as a backbone and EFV as a preferred NNRTI. As alternatives, Emtricitabine could be used as an alternative to 3TC, and Dolutegravir (DTG) and a lower dose of EFV (EFV400) instead of the higher dose (EFV600) – are considered as additional alternatives to the NNRTI. However, the guidelines highlight that safety and efficacy data are lacking for the use of DTG and EFV400 among pregnant women, in cases of HIV/TB co-infection and among adolescents <12 years of age [52]. DTG is considered a very promising alternative to NNRTIs owing to its low resistance potential, excellent safety, efficacy and low cost [54-56]. Similarly, for adults, EFV400 has been reported to be a compelling alternative compared to the standard dose of EFV (EFV600) owing to its non-inferior efficacy, few adverse events and its cost effectiveness [57-59].

1.2 Pediatric HIV Treatment Outcomes

Earlier studies have indicated the feasibility to do a largescale HIV care and treatment scale up in settings where there is limitation of resources [60]. HIV treatment has resulted in remarkable success in reducing HIV and opportunistic infection related child deaths [61, 62]. However, the effect of cART on child survival indicators could be compromised by poor adherence, high attrition and late diagnosis

of HIV infection [60, 63-65]. Children who are on long term cART who live in resource limited settings still have a higher mortality. Specifically, children who have malnutrition at treatment initiation and poor immunologic status were also associated with increased mortality while on cART[64, 66].

In Ethiopia, there has been a significant achievement in scaling up HIV treatment and care for infected children and adolescents but retention to care is still a big challenge [67]. As of 2013, more than 11,000 children were enrolled to HIV care and treatment while nearly two-third of them were initiated on antiretroviral therapy [67]. After scale of cART for HIV infected children, HIV associated mortality has decreased in Ethiopia – a reduction from 20.1 to 16.6 deaths per 100,000 population from 1990 to 2013[68]. However, reports also show that there is a high child mortality while on antiretroviral therapy [64].

1.2.1 Virological outcome following initiation of cART

Virological outcomes of children receiving antiretroviral therapy are one of the indicators of the 90-90-90 global goal by the year 2020. Briefly, the goal is for 90% of the children on cART to be virologically suppressed [50]. Virologic outcomes among HIV infected children on treatment from the developed settings were reported to be comparable to other populations [69]. However, even in such settings subgroups of children might suffer a poor outcome. For example, in a European cohort, young children below the age of 2 years were found to have the poorest virologic outcome [70]. Similar findings on the effect of age on virologic outcome was reported from Asia [69].

Early virologic outcomes among children on cART living in resource limited settings were reported to be optimal [71]. Even though, the varying thresholds used by studies for the definition of virological suppression could complicate comparisons, viral suppression of 70-90% has been reported by different studies [72, 73]. A Kenyan study reported that male sex and drug substitution were linked to poor virological suppression among HIV infected children [74].

1.2.2 First-line treatment failure and predictors in Ethiopia

While initiation of cART has improved outcomes in HIV infected children [75], factors including HIV drug resistance, and long-term cART associated metabolic disorders which increase the risk of early treatment failure have emerged as threats to the long term durability. These factors compromise the impact of cART on child morbidity and mortality. Acute and chronic HIV adverse drug events and non-communicable diseases such as diabetics and cardiovascular disorders could negatively affect long term cART outcomes of HIV infected children [76].

In children, durability of cART regimens can be as short as six months to a couple of years and depends on several factors including adherence, genetics, prior cART exposure, HIV drug resistance (HIVDR) and age [66, 77]. In Ethiopia, local data on treatment outcomes reported a variable prevalence of 4-17.5% treatment failure among adults using immunological criteria [78]. In children, there is scarcity of data measuring treatment outcomes; however, the available data suggest that 5.9% and 6.7% clinical and immunologic cART failure rates were reported, respectively [66].

Treatment outcomes to cART can be measured using clinical, immunological or virological parameters at least 6 months after treatment has been initiated [45, 52]. Even though viral load testing has been considered as the gold standard in the diagnosis of treatment failure, the test remains to be costly, technically demanding which limits its applicability in resource poor settings. In resource-constrained settings, viral load determination cannot be readily used to make a diagnosis of treatment failure. Ethiopia has made significant progress in availing viral load testing at regional laboratories as part of the effort to meet the 90-90-90 goals in virological suppression. Short of viral load monitoring, cART outcomes are assessed using the imperfect clinical and immunologic criteria which are suggested by the WHO guidelines [45, 52].

Several sociodemographic, cART associated, and immunologic factors have been reported to predict the occurrence of first-line treatment failure among children and adolescents. From the sociodemographic predictors, child's age [66, 79-82], child's sex [66, 80], parental status, specifically being an orphan [83] have been found to predict occurrence of first line cART regimen failure. Moreover, cART related variables including duration of antiretroviral therapy [83], sub optimal adherence to antiretroviral therapy [66, 84-87], being on a nevirapine (NVP)-containing first line cART regimen [81, 88], drug substitution during therapy [66] and drug adverse events [66, 84, 85] were associated higher risk of treatment failure. Child related factors including undernutrition [66], low baseline CD4 count [66, 81, 82, 84, 87, 89], and advanced WHO clinical stage [66, 84, 87, 90] were also reported to increase the chance of failing while on first line cART. Presence of any form of tuberculosis co-infection also increased the likelihood of treatment failure among HIV infected children [87, 90].

1.2.3 Impact of HIV drug resistance on treatment success

The introduction and scale up of cART in children including for purposes of prevention of mother-to-child transmission (PMTCT) is a significant stride towards improving survival and quality of life of children in resource-limited countries [91, 92]. With the improving access to PMTCT, mother to child transmission rates have gone down significantly. However, children for whom the PMTCT interventions did not work and unfortunately get infected are at a particularly high risk of developing HIVDR [93, 94].

The ultimate goal of antiretroviral therapies is virological suppression and immunologic restoration. However, the current drugs cannot eradicate the virus from reservoir tissues – suboptimal exposure due to poor adherence would lead to evolution of selective mutation to the antiretroviral drugs [95-97]. The error prone reverse transcription of HIV virus, the very high replication and mutation rate make HIV prone to having frequent resistance mutations [95]. There are different approaches to resistance testing including phenotypic, genotypic, resistance testing in clinical practice and minority drug resistance variants testing [98]. Generally, genotypic resistance testing is considered a more sensitive method of detecting resistance mutations compared to the phenotypic method. Based on how and when it develops, HIV drug resistance can be acquired because of suboptimal treatments and medication nonadherence (ADC) or transmitted at the time of infection (TDR).

The pooled prevalence of HIV drug resistance mutations across African countries is 10.6% with the highest prevalence of 54.9% from central Africa [99]. The major NRTI inhibitor mutation in most African countries was found to be M184V. NNRTI inhibitor mutations were found to be the highest in the Eastern African region, reaching as high as half of the cases screened. On the other hand, the major NNRTI and PI inhibitor mutations varied by region [99].

There is a recent concern on the increment in the prevalence of pretreatment or transmitted HIV drug resistance among children especially those exposed to PMTCT. For instance, in a recent review on pre-treatment HIV drug resistance, it is indicated that there is an alarming increment in the rate of pre-treatment HIV drug resistance (PDR) among children over the past years [100]. The same review showed a much higher prevalence of resistance among those who had no PMTCT exposure (42.7%) compared to the unexposed ones (12.7%). Increment in pre-treatment drug resistance by more than a quarter was observed over the past ten years among PMTCT-unexposed children [100]. A 2014 review showed a resistance level in NRTIs to be 0% to 100%; NNRTIs, 3.3% to 100%; and PIs, 0% to 66.7% among pre-treated children globally [101].

A decade back data on HIV drug resistance in Ethiopia were limited [102]. Nevertheless, there are recent reports that indicate the burden of HIV drug resistance in the country. A recent analysis from the Northwest of the country showed a 6.0% PDR mutations prevalence among cART naïve adult patients (n=67) – all the mutations were associated with NNRTI [103]. Another nationwide surveillance study reported a lower prevalence of 3.9% TDR mutations among treatment naïve adult HIV patients [104]. In 2015, the prevalence of acquired drug resistance was reported to be low at around 5% despite many years of cART roll out. Major NNRTI resistance mutations including K103N, V106M and Y181M; and, major NRTI mutations like M184V and K65R were reported in the same study. There were no PI and Thymidine analogue resistance mutations observed in the cohort

[105]. However, a recent trend analysis indicated that the prevalence of acquired HIV drug resistance mutations increased steeply – it increased from 40% to 66% in 10 years. M184V was the most frequent NRTI-associated drug resistance mutation; thymidine analogue mutations like D67N, K70R and K219E; and, NNRTI associated drug resistance mutations like K103N, V106M and Y188C [106].

In settings where HIV drug resistance screening is an integral part of the routine care, HIVDR is useful for individual patient treatment; however, in LMICs where these services are not readily available, generating evidence on the mechanisms and implications of HIVDR might be useful for surveillance and for targeted individual patient treatment [107, 108]. There is still scarcity of data on the level and predictors of HIVDR among children and adolescents in LMICs especially in Africa.

1.2.4 Drug adverse events affecting treatment outcome

Renal and Liver Toxicity

HIV infection itself or the adverse effects related to the antiretroviral drugs could have deleterious effects on the kidneys and the liver. However, characterization of drug induced hepatotoxicity and drug-induced liver disease (DILI) among children with HIV infection is not well described with incompletely understood pathophysiologic processes. Even though elevated bilirubin and alkaline phosphatase indicate a cholestatic form of liver disease, the liver enzymes (aminotransferases) are often considered to be markers of liver injury [109, 110]. The definition requires meticulous follow-up based on the markers of liver injury, synthetic function and hepatic imaging when deemed necessary [111, 112]. The burden, pathophysiology and definition of renal adverse events among HIV-infected children also remains incompletely studied [113].

Liver abnormalities related to the HIV infection and/or antiretroviral therapy can present with high transaminase levels, jaundice and hyperbilirubinemia, abdominal symptoms like abdominal pain, or even fulminant liver failure [114, 115]. The magnitude of hepatotoxicity within the first three months of cART in adults were found to be low at 1-2%, with an incidence rate of 4 to 8 patients with hepatotoxicity per hundred person years. The same data also reported that there is no increased risk of mortality associated with development of hepatotoxicity within three months of initiation of cART [116]. Contrary to this, a much higher proportion (32%) of HIV infected adults on treatment had transaminase elevation in Ethiopia [117]. Adults with tuberculosis (TB) and HIV co-infection on cART and anti-TB had a much higher incidence of hepatotoxicity (30%) compared to HIV infected adults on cART only (15,7%) [118, 119].

Liver toxicity is the commonest adverse event in HIV infected children who are on cART [120]. Generally, children with HIV infection who are on treatment were

reported to have markedly higher prevalence (~16-20%) of liver toxicity compared to HIV infected adults taking cART [120-122]. Progressive improvement in elevated transaminases was reported as children were longer on cART. Resolution of hepatotoxicity marked by normalization of liver enzyme levels was shown to be at around 3 to 6 months of treatment [123]. The adverse events of antiretroviral therapy on hepatic functions in children are not clearly understood, especially for sub-Saharan African children where cART scale up is considered a recent success.

HIV infection and cART could also cause impaired kidney function and other problems – including nephrolithiasis, tubular acidosis and renal failure – among children and adolescents. The mechanism of kidney injury and renal function abnormalities could be related to immune mediated mechanisms, also called HIV-Associated Nephropathy (HIVAN); and, adverse events of cART which could also be responsible for kidney injury [124]. Hence, renal function of children and adolescents living with HIV could be compromised by both the HIV infection and cART drugs.

Dyslipidemia and Metabolic Adverse Events

Adverse events which could be acute or long term might lead to decreased HIV drug efficacy and hence increased child mortality. Lactic acidosis, lipodystrophy, dyslipidemia and insulin resistance have been described as potential metabolic adverse events among both children and adults who have been on long term cART [125], though data on the magnitude of the problem among children in resource limited settings is scanty [126]. In a cohort of adults from Australia, 46.3% had some level of dyslipidemia and observed more among those taking protease inhibitor containing regimens [127].

In children, dyslipidemia was reported in 38.3% while a majority (80.2%) had some form of lipodystrophy and no significant clinical associations were identified [128]. Longer time on HIV treatment was linked with higher rate of insulin resistance, and yet children on AZT or ABC containing cART were even more likely to have a higher probability of insulin resistance [129]. The general burden of dyslipidemia was reported to be 10-15% [129, 130]. These observations call for further studies to understand the magnitude of dyslipidemia and design preventive strategies as children live longer on cART.

2 AIMS OF THE RESEARCH

The general objective of the PhD research was optimizing antiretroviral therapy for children with HIV infection who live in resource limited settings through i) improved understanding of virologic outcome following initiation of cART and identifying factors associated with unfavorable treatment outcome, ii) assessing the prevalence and predictors adverse events associated with short and long term cART for targeted intervention, care, and implementation of preventive strategies. Overall, the PhD research aimed to assess the safety and efficacy of the recommended first line regimens among HIV-infected children.

2.1 Specific Aims

The specific objectives of the PhD research focused on efficacy and safety of cART regimens for children from a high HIV prevalence but with limited treatment options and scarce virologic monitoring settings. The specific objectives were:

1. To determine the prevalence and associations of pretreatment HIV drug resistance (PDR) among newly diagnosed HIV infected Ethiopian children (**Paper I**).
2. To assess virologic outcome and its predictors during the first year of cART among newly diagnosed HIV-infected children living in a resource limited setting (**Paper II**).
3. To determine the prevalence and correlates of HIV drug resistance as a cause of first line cART failure among HIV infected children on first line treatment regimen (**Paper III**).
4. To assess long and short term adverse events associated with cART among HIV infected children in a resource limited setting with limited viral load monitoring. We assessed two important potential adverse events under this specific objective:
 - 4.1. To determine renal and hepatic toxicity and associated factors among children who were on long term cART (**Paper IV**)
 - 4.2. To assess the prevalence and correlates of dyslipidemia associated with cART and HIV infection (**Paper V**).

3 MATERIALS AND METHODS

The data for the PhD research were obtained from two parallel HIV infected pediatric cohorts which enrolled a total of 870 children – the first was the Ethiopian Pediatric HIV Cohort (EPHIC) [131] that enrolled HIV infected children who started or were taking first line cART. The second cohort, EFV Pediatric Dose Optimization (EPDOS), enrolled only treatment naïve HIV-infected children newly diagnosed to have HIV infection.

3.1 Patient population

As shown in Figure 4, the patient population for this PhD project included HIV infected children from two concurrent cohorts – EPDOS and EPHIC. In the EPHIC cohort, children between the ages of 3 months to 18 years who were on first line cART at HIV centers across southern Ethiopia were included. Children who were diagnosed to have virologic treatment failure and those who were on second line cART or were critically ill were excluded from this cohort. Similarly, the EPDOS cohort included cART naïve children between the ages of 3 to 16 years who were newly diagnosed with HIV infection and starting an EFV based first line cART according to the WHO guidelines [45, 132]. The children in both cohorts were followed regularly to assess virologic outcome and cART associated adverse events (Figure 4).

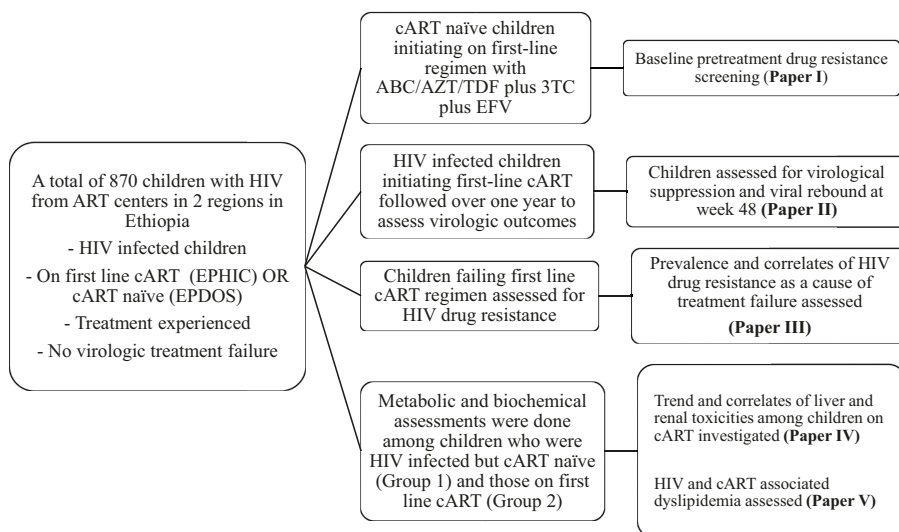


Figure 4. Summary of the patient population included in EPDOS and EPHIC cohorts.

3.2 Diagnosis and treatment of HIV Infection

The diagnosis of HIV infection in children was made according to the WHO guidelines as outlined in Figure 5 [133]. For children who are older than 18 months, including those who were newly diagnosed in the EPDOS cohort, HIV serology testing was employed to confirm diagnosis of HIV infection among the study subjects.

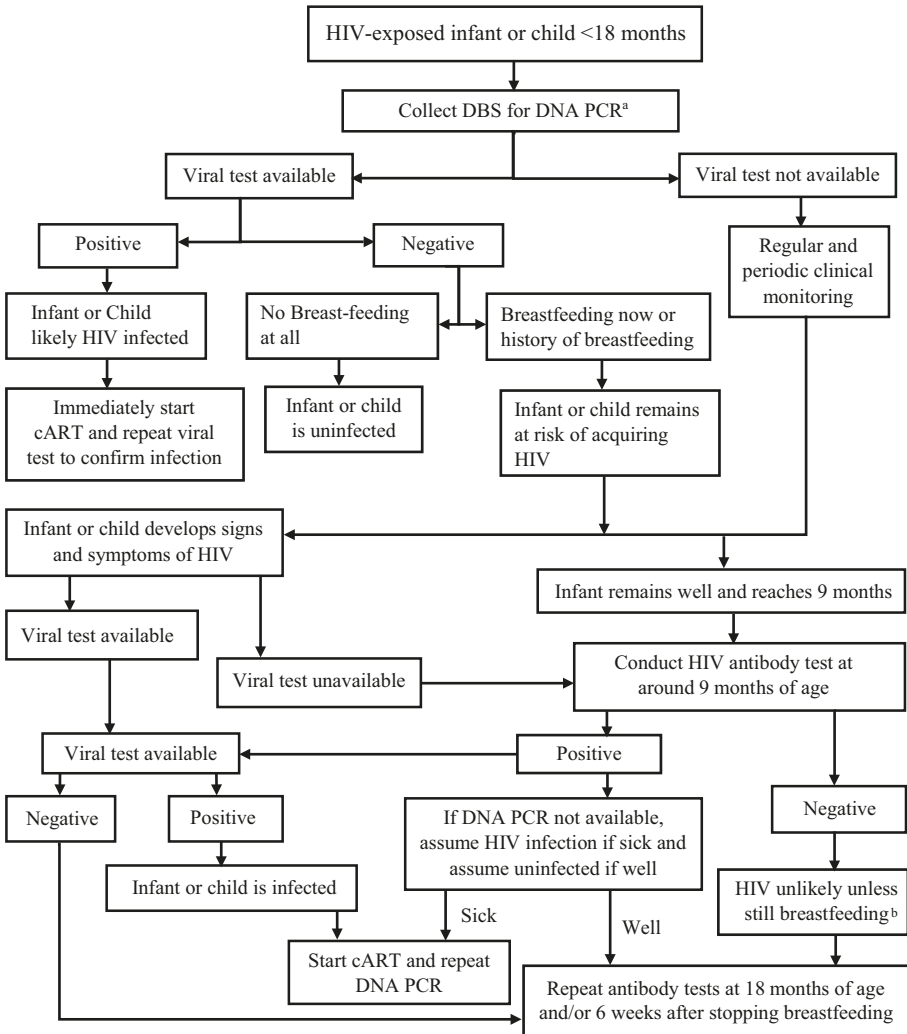


Figure 5. Infant testing algorithm for children less than 18 months of age.

Source: Adapted from WHO Guidelines on antiretroviral therapy for HIV infection in infants and children (https://www.who.int/hiv/pub/guidelines/arv2013/annexes/WHO_CG_annex_5.pdf) [133]. ^aFor newborns, test as early as possible – at or near the time of birth or at first postnatal visit (which is usually 4 to 6 weeks); ^bThere is a risk of HIV transmission until stopping breastfeeding.

3.3 Patient recruitment and follow up

Children in both EPHIC and EPDOS cohorts were followed using laboratory and clinical parameters at predefined follow up schedule as outlined in Table 2.

Table 2. Summary of patient recruitment and follow-up for both the EPHIC and EPDOS cohorts

Cohort	Follow up time	Evaluation and assessments
EPHIC	Baseline: at enrolment to cohort	<i>Clinical:</i> symptom screening, TB screening, WHO clinical staging, nutritional assessment, adherence assessment, clinical examination <i>Laboratory:</i> White blood cell, AST, ALT, CD4 count, platelet count, Creatinine, Blood Urea Nitrogen, Albumin, Hematocrit, plasma viral load, lipid profile assessment
	Follow up: every 6 months	<i>Clinical:</i> new symptoms, TB screening, WHO clinical staging, nutritional assessment, adherence assessment, focused clinical examination <i>Laboratory:</i> White blood cell, AST, ALT, CD4 count, platelet count, Creatinine, Blood Urea Nitrogen, Albumin, Hematocrit, plasma viral load, HIV drug resistance for those who have first line treatment failure, and lipid profile assessment
EPDOS	Baseline: at HIV diagnosis/ enrolment	<i>Clinical:</i> Symptom assessment, WHO clinical staging, nutritional assessment, screening for opportunistic infections <i>Laboratory:</i> Complete blood count, CD4 count, plasma viral load, lipid profile assessments, liver enzyme tests (AST, ALT), renal function tests, bilirubin, albumin and HIV drug resistance testing
	Weeks 8, 12, 24, and 48	<i>Clinical:</i> new symptom screening, nutritional assessment, TB screening, focused clinical examination, functional scoring <i>Laboratory:</i> Complete blood count, CD4 count, plasma viral load, lipid profile tests, liver enzyme tests (AST, ALT), renal function tests, bilirubin, albumin

3.4 Study procedures: laboratory and clinical parameters

Children were assessed at each follow up by a trained clinician on clinical signs and symptoms that show the control of HIV infection before and after initiation of treatment. The staging after treatment is referred to as t-staging (treatment staging) [52]. The clinical assessments included:

History and Physical Examination: detailed baseline history and physical examination with the purpose to identify any prevalent or incident opportunistic infections was done for each participant during enrolment to each cohort (EPDOS and EPHIC). After clinical assessment, all the participants were staged using the

WHO recommended clinical staging approach. The clinical stages correlate with the severity of the HIV infection – stage 1 indicates an asymptomatic stage while stage 4 indicates an advanced AIDS stage.

WHO clinical staging: using available clinical signs and symptoms, patients would clinically be staged as 1, 2, 3 or 4. The clinical stages are defined based on signs and symptoms/ diseases described in Table 3.

Table 3. The WHO clinical staging of HIV infection among children based on disease types

WHO Stage I	WHO Stage III	Stage IV
Asymptomatic Generalized lymphadenopathy	Persistent unexplained fever of >37.5 for more than 1 month	Severe malnutrition that doesn't not respond to treatment CNS toxoplasmosis
WHO Stage II	Unexplained moderate malnutrition which doesn't respond to standard therapy	Pneumocystis carinii pneumonia Cryptococcosis, extra-pulmonary
Popular itchy skin infections Persistent unexplained parotid gland enlargement Persistent unexplained hepatosplenomegaly Herpes zoster Extensive warts infection the skin Oral ulcerations (recurrent) Linear gingival erythema Recurrent or chronic respiratory tract infections Extensive molluscum contagiosum Fungal nail infections	Persistent oral candidiasis beyond the neonatal period Acute necrotizing ulcerative gingivitis or periodontitis Persistent unexplained diarrhea Oral hairy leukoplakia TB lymphadenopathy Pulmonary tuberculosis Anemia (<8gm/dl), neutropenia (<500/mm ³) or thrombocytopenia (<50,000/mm ³) (unexplained) Lymphoid interstitial pneumonitis Severe recurrent presumed bacterial pneumonia Chronic HIV associated lung disease, including bronchiectasis	Cytomegalovirus of an organ other than liver, spleen or lymph node Presumed severe recurrent bacterial infections Progressive multifocal leuko-encephalopathy Cryptosporidiosis associated chronic diarrhea Chronic diarrhea associated with isosporiasis Oropharyngeal or Esophageal candidiasis Chronic herpes simplex virus infection Disseminated endemic mycosis Atypical mycobacteriosis HIV associated cardiomyopathy or nephropathy Extra pulmonary tuberculosis except lymph nodes Lymphoma

Source: "Antiretroviral Therapy for HIV Infection in Infants and Children: Towards Universal Access: Recommendations for a Public Health Approach: 2010 Revision" [134].

HIV and Treatment Related Information: HIV infection and treatment related characteristics of the participants were collected at baseline and follow up as appropriate including if the participant was on treatment versus treatment naïve; time since HIV diagnosis and treatment; history related to preventive interventions like isoniazid preventive therapy (IPT) and cotrimoxazole preventive therapy (CPT); and prevention of mother-to-child transmission of HIV infection.

Anthropometric Assessment: weight, mid upper arm circumference, height and head circumference were assessed regularly. All anthropometrics were measured following standard procedures and using standardized/calibrated instruments. Anthropometric standardization was done using the WHO AnthroPlus software [135] and the WHO standards for categorization of anthropometrics to normal versus underweight. All the anthropometric z scores were categorized as under-nutrition when the Z scores (ZS) values were < -2 whereas values > -2 ZS were considered normal.

Assessment of Adherence to Treatment and Care: In the EPHIC cohort, which enrolled treatment experienced children and in the EPDOS cohort, which enrolled treatment naïve HIV infected children, adherence was regularly assessed using a composite of pill count, recall (24 hours and 1 week) and the visual analogue scale [136]. Adherence to treatment of more than 95% was considered optimal, while a level of less than 95% taken a sub optimal adherence to treatment.

Assessment of Clinical Staging and New Opportunistic Infections while on Follow up: symptom screening was done for the children enrolled in both cohorts to assess the occurrence of any new opportunistic infections, assessing treatment success and evaluation for any evidences of treatment failure – clinical, immunological and virologic treatment failure were assessed.

Assessment for Adverse Drug Reactions: adverse reactions associated with antiretroviral therapy were assessed using clinical and laboratory assessments.

Clinical Assessments: These were done using structured sign and symptom checklist. Signs and symptoms assessed included skin rash, jaundice, pallor, diarrhea or vomiting, dyspepsia, evidences for peripheral neuropathy, mentation and orientation or any other new symptoms during follow up.

Laboratory Assessments: These were done to assess adverse drug reactions and the success or failure of antiretroviral regimens. Scheduled laboratory tests including hematology (complete blood count – CBC, neutrophil and lymphocyte count, platelet count), chemistry tests (Creatinine, blood urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lipid profile tests (low density lipoprotein – LDLc, density lipoprotein – HDLc, total cholesterol, high and triglycerides), immunology status (CD4 and CD8 count), viral load and HIV drug resistance testing for those who failed first line cART and those who were naïve to treatment before starting cART.

3.4.1 HIV Drug Resistance Testing

Blood Specimen Collection and Storage

We collected blood spots (~ 50 µL each) from a finger-prick of participants on dried blood spot (DBS) cards (Labmate, Cape Town, South Africa) and were kept at room temperature overnight. A sealable plastic specimen bag with adequate desiccants was used to store the DBS cards individually until they were shipped to Simon Fraser University (SFU). DBS cards stayed at room temperatures for about one month before shipping and were stored at -80 °C freezer once in SFU.

Extraction and Genotyping

Nucleic acid extraction was done by transferring blood spots to sterile tubes using a standard 1/4" manual hole punch which was cleaned after each punch through punching of 10 holes into a clean paper to remove any residual material from the preceding participant DBS [137]. Two nucleic acid extraction attempts were made from each participant's blood spots. PureLink Kit (Invitrogen, Carlsbad, CA, USA) was used for total nucleic acid extraction from two blood spots. Additionally, NucliSENSEasyMAG which is an automated system for total nucleic (BioMerieux, Marcy-l'Étoile, France) was used for the majority (88) of the participants.

We used PCR to amplify Protease and Reverse Transcriptase (RT) of HIV-1 containing at least codons 1-234. Amplification was done with and without initial RT step. A maximum of four oligonucleotide primer sets – one primary and four alternate primer sets which were optimized to amplify HIV-1 group M subtypes were used. For an initial RT step, the RT-PCR and SuperScript III One-Step RT-PCR method was used. Moreover, nested PCR by an Expand HiFi System (Roche) was used for the without initial RT amplification step. Amplicons were directly sequenced on DNA sequencer (Applied Biosystems, Foster City, CA, USA), 3730xl or 3130xl. Sequencher version 5.0.1 (Gene Codes, Ann Arbor, MI, USA) was used to analyze chromatograms. Alternatively, automated base calling software, RECall was used for this purpose [138]. A quality control bioinformatics tool which is hosted by the Los Alamos HIV Sequence Database (LANL) (<https://www.hiv.lanl.gov/content/sequence/QC/index.html>) and the Stanford Drug Resistance Database were used for screening hypermutation, frame shift, deletions and stop codon. Alignment of sequences was done by HIVALign (options: MAFFT, codon-alignment) [139] and visualization was made by AliView [140]. The maximum likelihood by PhyML was used for phylogenetic inference under nucleotide substitution using General Time Reversible model [141]. To control for the effects of tree topology, phylogenies were generated from full alignments and codon stripped alignments based on HIV drug resistance surveillance mutations [142]. FigTree (version 1.3.1) was used to visualize phylogenies. Patristic sequences were extracted from Newicktree files

using PATRISTIC which were employed to extract parasitic phylogenetically inferred tip to tip pairwise distances [143]. HIVLANL's Recombinant Identification Program (RIP) was used to identify the HIV-1 subtype [144].

Drug Resistance Genotyping

The presence of drug resistance mutations which confer resistance to NRTI, Protease Inhibitors (PI) and NNRTI was confirmed based on Stanford University's HIV Drug Resistance Database – HIVdb Program (version 8.4, <https://hivdb.stanford.edu/hivdb>) [145, 146]. The Stanford University HIVdb program was also used to define individual drug resistance mutations. Genotypes having all degrees of reduced susceptibility to any of the drugs was defined as “resistant genotype” [145, 146]. Each sequence was appraised independently; moreover, participants who had >1 sequence an inclusive consensus was applied. This approach allowed us to improve capturing the breadth of drug resistance within individual patients. Moreover, it helped us understand the level of underestimation of drug resistance testing approaches – single versus replicate [147-150]. We excluded hypermutated and defective sequences from the drug resistance interpretation.

3.4.2 Blood Chemistry Tests

Liver enzyme, renal function tests and case definitions

Hematology tests, immunology (CD4, CD8), hepatic enzyme and kidney function tests were done according to standard procedures [151]. Recommended procedures to ensure quality were followed and tests were done by WHO accredited labs. Upper limits of normal for liver enzymes - AST, ALT levels were defined at 40 IU/L [111]; whereas, the ULN for BUN was 20 mg/dL and for serum creatinine was 1.06 mg/dL [152].

Lipid profile assessments and definition

We assessed the non-fasting levels of total cholesterol, HDLc, LDLc and TG for children enrolled in the EPDOS and EPHIC cohorts. Pediatric reference ranges previously published were used to define lipid profile abnormalities [124]. Accordingly, considering the highest value of each lipid profile parameter across age groups, > 200 mg/dL for total cholesterol; >150 mg/dL for triglyceride levels; >130 mg/dL for LDLc levels and <40 mg/dL for HDLc levels were defined as lipid profile level abnormalities.

Viral load assessment and clinical decision making

Plasma viral loads were determined using quantitative PCR machine (Abbott, Des Plaines, IL, USA). Results were reported in copies per milliliter and retesting or treatment decisions were made based on the WHO and Ethiopian national HIV

treatment guidelines (Figure 3) [45, 132]. A plasma viral load of <150 copies per mL was considered the lower limit of detection and lower limit of quantification for the machine at a volume of 0.2 ml of plasma. Plasma viral load results were, therefore, reported as undetectable, <150 copies per mL (but unquantifiable) and quantifiable at ≥ 150 copies per mL. Assessment of the success of antiretroviral therapy and diagnosis of virologic treatment failure were done according to the WHO and national guidelines as depicted in Figure 6.

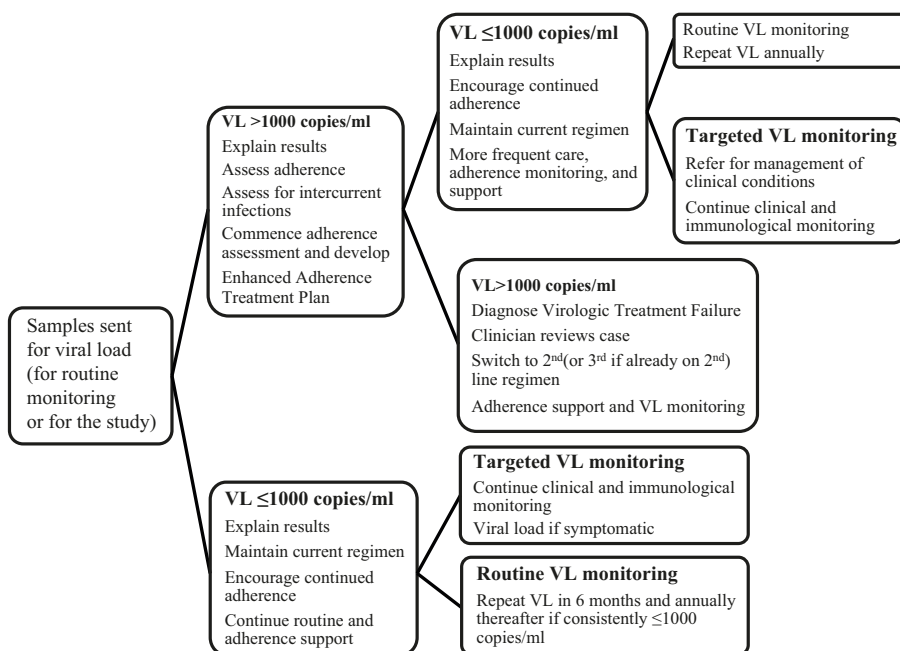


Figure 6. Diagnosis of virologic treatment failure and decision-making flow using viral load results. VL – viral load.

3.5 Statistical analysis and data management

Data were collected on paper and then entered in a database. REDCap and a dedicated database developed for EPDOS were used as databases for data collection and data quality control [153]. Data collection was done by health professionals who received trainings on standard operating procedures and principles of good clinical practice. Data quality in both projects was ensured by regular monitoring and evaluation visits at the implementation sites by the researchers and trained supervisors. Additional quality control measures were applied in the case reporting formats and the databases. A weekly random assessment of filled reports was done on 10% of the total, which enabled taking corrective measures in the process.

Characteristics of study participants and baseline/follow up data were summarized using descriptive statistics i.e, frequency (percentage), mean (standard deviation), and median (interquartile range). Data were also presented in tables and figures. Associations with the primary end points – PDR (Paper I), rate and correlates of virologic suppression (Paper II), HIV drug resistance mutations as a cause of treatment failure (Paper III) and drug adverse events (Papers IV and V) were assessed using both bivariate and multivariate analysis. For normally distributed data, association between the outcome variables and independent categorical variables was assessed using Chi square test. Whereas, for non-normally distributed data, Fisher’s exact test was used to assess presence of any association between the outcomes of interest and categorical independent variables. To assess associations between continuous independent variables and the primary endpoint, Mann Whitney U test was used.

Multivariate analysis was done to assess independent associations in Papers II to V. In paper II, the relationships between baseline demographic, clinical and laboratory variables and achieving virologic outcomes of interest were assessed using univariable and multivariable Cox proportional hazards regression. Kaplan-Meier survival analysis, with p-values computed using the log-rank test, were also undertaken to select baseline variables to graphically visualize relationships. The following variables were treated as binary or categorical variables, where the following reference categories were used. For sex, the reference category was “male”. Pretreatment drug resistance (PDR), tuberculosis (TB), IPT and CPT were also treated as binary variables (“yes” vs. “no”), where “yes” was the reference category. Categorical variables included cART regimen (where abacavir-based regimen was considered the reference category) and WHO clinical staging (where clinical stage I was the reference category). Hazards for continuous variables were defined as follows: – age (per year decrement), AST and ALT (per unit/L decrement), Albumin, BUN, Creatinine and bilirubin (per mg/dL decrement), baseline viral load (per log₁₀ copies/mL decrement), CD4 count (per cells/uL increment), hematocrit (per 1% increment), and total cholesterol, LDL, HDL, TG, WAZ, HAZ and BAZ (per mg/dL increment). In survival analyses, subjects who were lost to follow up or who died were censored at their last follow up timepoint. Covariates with a p-value of <0.2 in univariable analyses were included in the multivariable models.

In Paper V, binary logistic regression was employed to determine association between independent variables – decrease by 1 year of child’s age, decrease by 1mg/dL of the hemoglobin level, being clinical stage 3/4 disease, a decrease by 1 Z-score of weight-for-age and height-for-age z score (WAZ and HAZ) were included in the final multivariable regression model as these had a p-value <0.2 in the univariable model.

Finally, in the multivariate models, p value of <0.05 was set as the level of statistical significance. R software package was used for the analysis.

3.6 Ethical considerations

Ethical approvals were obtained from the SNNPR Regional Health Bureau (RHB) Institutional Review Board (IRB), Addis Ababa University, College of Health Sciences IRB, Karolinska Institutet and the National Research and Ethics Review Committee (NRERC) of Ethiopia (Reference numbers: 3-10/46/2018, 21 March 2018 for the EPHIC cohort and SHE/SM/14.3/0421/1/2019 (renewal) for the EPDOS cohort). Parents/guardians of participating children gave written informed consent, while children <12 years gave assent additionally. All data collection forms were made anonymous to ensure confidentiality and stored in secure locations accessed by only limited personnel. Participation was completely on voluntary basis and participants were informed that they were free to withdraw from the studies at any time without any consequences.

4 RESULTS AND DISCUSSION

A total of 870 study participants were enrolled in two cohorts – EPHIC and EPDOS from January 2016 to January 2019. From these, 111 (12.8%) were newly diagnosed HIV infected children who were treatment naïve from the EPDOS cohort, while the rest 769 children were on first line cART from the EPHIC cohort [131]. Children enrolled from EPDOS cohort were followed for 48 weeks, while those from EPHIC for 18 months.

Children in the EPDOS cohort who were assessed for baseline pretreatment HIV drug resistance (**Paper I**) were prospectively followed to assess virological outcomes (**Paper II**). Among children who failed first line combination antiretroviral therapy, HIV drug resistance was assessed as a cause of the failure (**Paper III**). Children who have been on cART were also prospectively followed for renal and hepatic toxicities (**Paper IV**). We also assessed for and compared HIV infected cART naïve children from the EPDOS cohort with cART experienced HIV infected children from the EPHIC cohort for metabolic derangements (**Paper V**). Paper I assessed the burden of pretreatment HIV drug resistance while paper II assessed virologic outcomes including virologic suppression and rebound during the first year of cART in a cohort of children followed for 48 weeks (Figure 1 or Paper II). It also explored the predictors and trend of virologic response during the first year of treatment included pretreatment drug resistance using the data from Paper I.

Children in the EPHIC cohort were assessed for virological treatment failure at enrollment. A total of 94 children who had virological treatment failure and had a viral load of >2500 copies per mL underwent HIV drug resistance testing (Paper III). The rest with no treatment failure were followed for 18 months to assess renal and hepatic drug adverse events (Paper IV) and a subset of children underwent screening for dyslipidemia (Paper V).

4.1 Pretreatment HIV Drug Resistance among HIV infected Children (paper I)

A total of 93 children with a median age of 9 years (Interquartile range (IQR): 5-12) were included in the analysis for paper I. The children had a median CD4+ T-lymphocyte cell (T-cell) count of 319 (IQR: 141-615) cells/mm³ and median viral load of 4.3 log₁₀ copies/mL (IQR: 3.7-4.9 log₁₀ copies/mL).

Prevalence of PDR and Drug Resistance Mutation Types

From all the 93 participants, we collected DBS; N=22 or Dried Plasma Spots (DPS; N=71). Genotyping for HIV drug resistance succeeded for 57/93 (61.3%) of the samples, which is comparable to reports which used dried blood products

for genotyping [154-156]. Furthermore, success rates did not significantly differ between DPS (14 of 22; 63.6%) and DBS (43 of 71; 60.6%) ($p=1.0$) specimens. Additionally, genotyping success rate was similar across all patient characteristic which are described in Table 2, except the weight-for-age Z-score where children with successful genotyping had median Z-score of -1.9 (IQR -2.9-(-0.9)) compared to -1 (IQR -1.7-(-0.1)) for those in whom genotyping was unsuccessful ($p=0.03$).

Consistent with the reported national HIV epidemic which Ethiopia experienced with subtype C [157-162], 54/57(94.7%) children had HIV subtype C, 1/57(1.7%) each of AG recombinant type, ACG recombinant and A subtypes were identified in the included children (Figure-1 of Paper I). No phylogenetically-linked infections were found except a pair of siblings who had closely clustering sequences. This finding is consistent with most pediatric cohorts.

From those participants who were successfully genotyped, 8/57 (14%) HIV DRMs (Figure 1 of Paper I). All 8 children with PDR harbored resistance to NNRTIs – 5(62.5%) had NNRTI DRMs only while 3(37.5%) were found to have dual class resistance. PI drug resistance mutations were not observed in any of the participants. Five of 43 and 3/14 children from DBS and DBS groups respectively harbored HIV drug resistance mutations, indicating that there are no significant differences in the level of PDR between the groups ($p = 0.4$).

Table 3 of Paper I depicts the DRM profiles among the included participants. Overall, G190A and Y181C were the most common NNRTI drug resistance mutations. Moreover, K103S, K103N and Y188L were identified in one participant each. L210W, M184V, M184I, T215Y and K219N were observed NRTI drug resistance mutations. EPDOS_8 and EPDOS_53 (GeneBank) harbored HIV drug resistance observed in more than one drug class.

Associated Factors with Pretreatment HIV Drug Resistance

We next sought to explore the correlates occurrence of PDR among (Table 4 of Paper I). Drug resistance as an outcome was categorized as yes or no for the purpose of this analysis. Children who harbored PDR were younger than those without, but the association did not reach statistical significance (median 5 [IQR 0.3-10] versus 8 [5-12]) ($p=0.06$). Furthermore, lower albumin level predicted PDR even though there was only a modest reduction (2.9 IQR: 2.5-3.4 mg/dL among children harboring resistance versus 3.8 3.2-4.2 mg/dL among those without; $p=0.04$). Low albumin levels could implicate undernutrition as a consequence of drug resistance causing poor treatment outcomes; however, children with and without PDR did not have statistically significant differences in their anthropometric. It is known that albumin is one of most abundant proteins in human blood plasma and plays role in drug transport and metabolism [163, 164]. However, mechanism of the link between lower albumin level and presence of PDR in the current cART-naïve

pediatric population, and the potential clinical implications of the link, are currently not clear and require confirmation in larger, independent studies.

None of the clinical and laboratory variables assessed predicted PDR. Only three children exhibited resistance to NRTIs as part of a dual class resistance which limited the plan to assess correlates of resistance in this group of patients. Participants who exhibited NNRTI/NRTI dual class PDR were younger than those who had single or no drug class resistance (0.2 [0.1-0.3] versus 8 [5-12]) ($p=0.02$).

These figures of PDR among HIV infected newly diagnosed Ethiopian children represent a substantially higher burden of PDR in this population than newly diagnosed HIV infected adults where the rate of PDR was around 3.9% [104]. Reports on the burden of PDR among adults and children from other similar settings with high HIV prevalence support our findings from Ethiopia on the rate and predictors of pretreatment drug resistance among both adults and children [165]. PMTCT exposure in children could explain the relatively higher prevalence of PDR among children as compared to adults [166].

Reports show that the prevalence of PDR within several sub-Saharan African countries is increasing over the past two decades. In some settings, the increment occurred at alarming levels [100, 167]. The observations in this analysis further underscore the concern that HIVDR could be a major threat to the HIV epidemic control efforts in resource-constrained settings [168] and identify the pediatric population particularly to be at risk of poor treatment outcomes owing to the high HIV PDR levels. In settings where the NNRTI pretreatment resistance exceeds 10%, the current WHO treatment guidelines recommend against using NNRTI-containing regimens as first-line treatment [169]. Based on WHO guidelines, for children >3 years of age, the recommended first line regimens contain a combination of two NRTI drugs (3TC with one of ABC, TDF, or AZT) and one NNRTI drug (either EFV or NVP) while second line regimens include two NRTI drugs (3TC and one other NRTI which was not part of the failing first line regimen) plus PI [41, 42, 45, 170].

4.2 HIV treatment outcomes among hiv infected children (Paper II)

Baseline characteristics of the children who were enrolled for this sub study are described in table 1 of paper II. Briefly, a total of 111 cART naïve HIV infected children were initially enrolled in the EPDOS cohort [171]. However, follow-up was limited to the 110 (99.1%) children as one child did not participate in the baseline and follow up assessments. During the study period, a total of 16 children did not complete follow up –13 children (11.8%) were either lost to follow up or were transferred out to another center out of the study catchment region and

three children died: a 13 year boy died at home of unknown cause, and two girls, aged 6 and 10 years, who had initially lost to follow up, were later confirmed to have died when the study team attempted to trace them (Figure 1 in Paper II). In total, 97 (88.2%) participants successfully completed all 48 weeks of follow-up.

Of the 110 HIV infected treatment naïve children who entered follow-up, 94/110 (85.5%) achieved undetectable viremia, defined as pVL<150 copies per mL, at some point during the study period. The level of virological suppression rate in this study is comparable to reports by other studies [71, 172-174]. The finding gives an important insight in towards the progress in the global targets in Ethiopia. In Ethiopia, there is no disaggregated countrywide pediatric data on the rate of sustained virological suppression among HIV infected children who initiated cART (that is, the statistic represented by the third “90” of the UNAIDS 90-90-90 goal) [175]. Only a collective estimate of 86% virological suppression has been reported in 2016, which is higher than the ESA regional average of 52% [41, 176, 177].

We further explored clinical and laboratory associates of virological suppression to pVL<150, using Cox proportional Hazards regression. In bivariate analyses, several variables were weakly associated with attaining undetectable viremia at p-values <0.2. Each decrease of one integer unit of body mass index for age Z-score (BAZ) was associated with a 10% higher hazard of achieving undetectable viremia (Hazard Ratio(HR) 1.1 [95%CI 0.9-1.3], (p=09). Being on a TDF-containing cART regimen was associated with a 40% increased chance of achieving undetectable viremia (HR 1.4 [95% CI 0.9-2.1] compared to participants on other regimen types (p=0.18). The relationship between virological suppression to undetectable levels and NRTI drug type contained in first line cART is also depicted using Kaplan-Meier curves in Figure 2. Moreover, lacking pre-treatment drug resistance (PDR) was associated with a 2.1-fold increased hazard of achieving undetectable viremia (Hazard Ratio 2.1 [95% CI 0.7-5.9]) compared to participants with PDR (p=0.16). The relationship between PDR and time to achieving pVL<150 copies/ml is also graphically illustrated in Figure 2. On the other hand, not taking isoniazid preventive therapy (IPT) was associated with 30% decreased hazard of achieving virological suppression (HR 0.7 [95% CI 0.5-1.2], p=0.19). Similarly, being classified into WHO clinical stage 2 or stage 3 was associated with 30% decreased hazard of achieving undetectable viremia (HR 0.8 - [95% CI 0.7-1.1]) as compared to being classified into stage 1 (p=0.08, data not shown). Each decrease in baseline viral load by one log₁₀ copies per ml was associated with a 20% decreased hazard of achieving undetectable viremia (HR 0.8 - [95%CI 0.7-1.1]) (p=0.13). Virological suppression to undetectable levels stratified by baseline viral load is graphically illustrated using Kaplan-Meier curves in Figure 2. Each decrease in baseline low density lipoprotein (LDL) by one mg/dl was associated with a 10% decreased hazard of achieving undetectable viremia (HR 0.9 - [95% CI 0.9-1.0], p=0.17).

A multivariable model was constructed using all baseline parameters with p-values of <0.2 in the univariable analyses, with the exception of body-mass-index-for-age Z-score (BAZ). The latter was excluded to avoid the possibility of collinearity, as both height-for-age Z-score (HAZ) and weight-for-age Z-score (WAZ) were included in the model, and BAZ is a function of weight and height. After adjustment for all baseline factors included in the model, the only variables that remained independently associated with virological suppression <150 copies per mL were pretreatment drug resistance and type of cART regimen (Table 2). Lacking pretreatment HIV drug resistance was associated with an 11 times higher chance of virologic suppression to undetectable levels compared to having PDR (HR: 11.1 95% CI: 1.3–94.7, p-value= 0.028). Being on a TDF based cART regimen was associated with 3.1 times higher chance of virological suppression compared to being on an ABC based regimen (HR: 3.1; 95%CI: 1.0-9.6; p-value=0.049).

The impact of PDR on virologic outcomes among newly-diagnosed HIV infected children is not well established in resource-limited settings [82, 104, 178-180]. In theory, PDR could compromise the efficacy of first line cART regimens, thereby reducing rates of virological suppression and increasing subsequent rates of virological treatment failure. However, previous studies have reported conflicting results. Several African and multinational studies reported a positive association between PDR and virologic suppression, while other studies reported the lack of any association between PDR and virological suppression [82, 181]. Our study showed that presence of any PDR was associated with reduced rates of virological suppression, after adjusting for other baseline factors.

The difference in virological suppression between HIV infected children with PDR and those without is significant after the third month of cART indicating that more children with PDR continue to have high viral load. Interestingly our result indicates no significant association of PDR with risk of viral rebound. Despite several studies which reported the relevance of baseline viral load as a predictor of virologic outcome [182-184], our study demonstrated no association between the level of baseline viral load and virologic response.

Antiretroviral regimens types have different safety and efficacy outcomes among children with HIV infection. All the children who were included in the current cohort were started on an EFV based cART regimen based on the WHO 2016 consolidated guideline [52], which limited our ability to compare the impact of NNRTI drug types on virological outcomes. A tenofovir based regimen was found to be associated with better virological suppression compared to an abacavir based cART regimen. However, studies have shown comparable antiviral activity between tenofovir/lamivudine and abacavir/lamivudine combined with dolutegravir and abacavir/lamivudine versus tenofovir/ emtricitabine [185-187]. The similar findings in previous reports could be because of using different third agent in the cART combination and warrants more investigation.

We next assessed rates of virologic rebound our cohort. We defined virologic rebound as registering at least one pVL ≥ 150 copies/mL after having first achieved a pVL of <150 copies/mL; as such, this analysis was limited to the 94 children who had achieved virological suppression at least once during follow up. Of these 94 children, 36 (31.9%) subsequently experienced virological rebound. Specifically, of these 36 children, 11 (30.6%), who were suppressed at week 8 had a rebound at week 12; another 11 children (30.6%) who had achieved suppression at weeks 8 and/or 12 had rebound at week 24; and, 14 (38.9%) children who had achieved one or more virological suppression at weeks 8, 12 and/or 24 had viral rebound at week 48.

We next employed Cox proportional Hazards regression to characterize the relationship between participant clinical and sociodemographic characteristics and risk of virologic rebound. Of note, the variables investigated in the present analysis were those measured at baseline, not at the time of initial virologic suppression. On univariable analysis, a decrease by one year of age was associated with a 10% lower risk of viral rebound (HR 0.9 - [95% CI 0.8-1.0], $p=0.12$). Similarly, taking a TDF-containing first line regimen resulted in a 60% reduction in the hazard of virological rebound (HR 0.4 - [95% CI 0.2-0.9], $p=0.027$). The trend of virological rebound stratified by the NRTI drug contained in first line regimen is depicted using Kaplan-Meier curves (Figure 3 in Paper II). Not taking CPT was also associated with a 60% reduction in the hazard of virological rebound (HR 0.4 - [95% CI 0.2-0.9], $p=0.04$). On the other hand, being WHO clinical stage 4 increased the hazard of virological suppression by 4-fold (HR 4.0 - [95% CI 1.1-14.3], $p=0.036$). The relationship between CPT status and WHO clinical stage with the trend of virological rebound is described using Kaplan-Meier curves in Figure 3. Not taking IPT also increased the risk of virological rebound by 1.7-fold (HR 1.7 - [95% CI 0.8-3.6], $p=0.18$) (Table 3 in Paper II).

Multivariable cox proportional hazards model was constructed using all the variables with p -value <0.2 . After controlling for confounders, all the covariates did not reach statistical significance except WHO clinical stage which showed a weak association. Specifically, being classified as WHO clinical stage 4 disease resulted in a 3.6 times increased hazard of virologic rebound (HR 3.6 - [95% CI 0.7-18.8], $p=0.13$) (Table 3).

Of note, of the 36 children who experienced viral rebound following initial suppression according to the above definition, 30 (83.3%) registered only a single pVL result ≥ 150 copies per mL after which they later re-achieved virological suppression to < 150 copies/ml. The remaining 6 children (16.7%) never re-achieved suppression, and fulfilled the WHO definition of virological treatment failure (VTF), defined as two successive pVL results ≥ 1000 copies per mL, recorded at least 3 months apart, after at least 6 months on cART [52]. Indeed, 9 of the original 110 study participants (8.2%) had fulfilled the definition of VTF according

to the WHO guidelines: these included the latter 6 participants as well as three additional children who never achieved pVL suppression < 150 copies/ml at any point during study follow up.

Even though a high proportion of children initially achieved virological suppression, almost one-third (31.9%) proportion subsequently registered at least one pVL \geq 150 copies per mL, a rate that is similar to a study from South Africa [177]. Those who were classified as being WHO clinical stage 4 had an increased hazard of virologic rebound. Promisingly however, the majority (~80%) of participants who experienced viral rebound went on to re-achieve pVL suppression, suggesting that most were transient viral “blips” rather than an indication of virologic treatment failure (VTF). Indeed, VTF rates were relatively low: only 9 of the original 110 study participants (8.2%) fulfilled the WHO definition of VTF at the end of the study period. Taken together, the findings show that newly diagnosed HIV infected children who are started on standard cART regimes, as recommended by the WHO guidelines [52], generally do well virologically at 48 weeks of treatment.

4.3 Prevalence and Correlates of Acquired HIV Drug Resistance (Paper III)

In paper III, we evaluated the rate of HIV drug resistance among treatment experienced HIV infected children who failed first line cART. Resistance analysis was done based on inclusive consensus sequence which was assembled from all existing intact HIV-1 sequences every participant to avoid the possibility of underestimating the degree of in individual children (Figure 1A of Paper III)[147-150, 188-191]. A high prevalence of HIVDR was recorded – for 73/90 (81%) at least one drug resistance mutation was recorded (Figure 1B of Paper IV). The most common form of resistance was dual class resistance where 62 (69%) children had resistance to both of NRTI and NNRTI. Only one child had resistance to three drug classes – PI, NRTI and NNRTI. Nine (10%) children had resistance to NNRTI while one child had isolated resistance to NRTIs (1%). In 17 (19%) of the children, no drug resistance mutations (DRMs) were observed which indicates towards the significance of other factors for treatment failure.

A broad range of DRMs were observed among participants who harbored drug resistance mutations. Forty-five NRTI DRMs which are described in the Stanford database were used to interpret our results [146, 192]. We also referred to the IAS-USA list while interpreting our findings [193]. Twenty-eight of the 45 (which occurred at 15 codons) were observed during this analysis. M184V was the most frequent NRTI DRM, 61/64 (95%) of children with NRTI DRM, which is consistent with the rapid selection by 3TC (Figure 2A of Paper IV) [194-197]. The next most frequent DRMs were those for AZT and d4T; namely, T215Y, D67N, K70R, M41L, and L210W [146]. Each of these were recorded in 23%, 25%, 22%, 20% and 20% of children with NRTI resistance, respectively.

Thirty six of the NNRTI DRMs which occurred at 18 RT codons are defined [146, 192, 193]; from these, 25 which occur at 17 codons, were recorded among the children in our study. Y181C DRM which is known to confer resistance to all of the NNRTIs, and K103N DRM which is attributed to high-level resistance to EFV and NVP, were recorded in about a third of the participants with NNRTI resistance, respectively (Figure 2B of Paper IV) [146, 198]. Moreover, K101E and G190A which cause resistance to NVP and EFV [146], were recorded among 17% (13/72) and 28% (20/72) of participants with NNRTI resistance, respectively. Overall, our results show the diverse drug resistance mutation profiles among HIV-1-infected children who experience virologic first-line treatment failure. The findings broadly reflect the specific antiretrovirals used as first-line drugs in the region, with their relative mutational barriers [146, 194-198].

Impact of the findings for first and second line WHO-recommended regimens in the region

According to WHO and national guidelines, Ethiopia currently recommends two NRTIs (3TC plus one of AZT or ABC or TDF) with one NNRTI (one of NVP or EFV) as first-line treatment for children three years and older [132, 170]. Further, two NRTIs (3TC and one other NRTI not included in the failing regimen) and a PI constitute a preferred second line regimen [132]. All the participants with NNRTI resistance had resistance for both NVP and EFV which is consistent with high levels of cross-resistance in the NNRTI group (Figure 3A of Paper III)[198]. This finding leaves the PIs as the main second-line options in such settings which is in-line with the available guidelines. However, the implications of the NRTI resistance profile are more worrisome indicating that the second line regimes might not be as effective. Specifically, 62/64 (97%) participants exhibited resistance to 3TC (including ABC and Emtricitabine [FTC]) because of the M184I/V mutation carriage (Figure 3A of Paper IV). This finding indicates that the inclusion of 3TC in second line regimens will compromise the efficacy of the regimens. Furthermore, 45% (29/64) and 53% (34/64) children who had NRTI resistance mutation confer resistance to TDF and AZT, respectively. Our findings show that resistance mutations which conferred resistance to two of the NRTIs which are recommended for use in first- and second-line cART regimens by WHO and National Guidelines in Ethiopia were observed in 27/64 (42%) of the participants. Nine children (13%, 9/64) had resistance to three of the NRTI drugs while 42% (27/64) had resistance mutation to all four (Figure 3B of Paper IV) [132, 170]. In summary, the success of recommended second-line regimens could be significantly compromised due to the high burden of HIV drug resistance observed in this study. This is especially worrying as there are no third line options for children in Ethiopia [132], and more importantly, in sub-Saharan Africa, access to alternative treatment regimens is significantly limited [199, 200].

Predictors of drug resistance among HIV infected children failing first line treatment

Finally, we explored factors that are associated with drug resistance as shown in Table 2 of Paper IV. Longer duration of cART was associated with more resistance to NNRTI resistance (median 50.5 [IQR 23-72 months] versus 20.5 [6.4-46.5] months, $p=0.02$). The same trend was observed for NRTI resistance and any resistance. Drug substitution predicted a higher probability of NRTI mutation (57% among those who underwent drug substitution versus 30% among those without, $p=0.04$), a trend that was also observed among participants with any resistance and NNRTI resistance. On the other hand, EFV containing regimens exhibited a significantly less common resistance: 47% of children with no resistance were taking EFV-containing regimens as opposed to only 21% of children with any resistance, $p=0.03$. This observation is in line with the higher antiviral activity of EFV than NVP containing regimen [201, 202]. The findings show that closer follow up is warranted for children who have been on treatment longer and those who underwent drug substitution. Additionally, NNRTI regimens which contain EFV might be protective against drug resistance-associated virologic failure.

4.4 Liver and renal toxicities associated with long term cART use (paper IV)

For the analysis in this sub study, we used a total of 705 children, from the EPHIC cohort, who had serial measurement of markers of liver and kidney damage. The children included those who had at least one hepatic enzyme and renal function tests performed during the follow up period. At enrolment, 6, 12 and 18 month follow up, data were available for 705, 615, 554 and 490 children, respectively. All four tests done for a total of 450 children. The children were a median age of 12 years (Q1-Q3: 8-14 years); more than half (53.3%) were male. Participants were a median of 3.3 years (Q1-Q3: 1.1 – 6.1 years) on first line cART and were on different regimens at enrolment including AZT,3TC,NVP 343(44.1%); D4T,3TC,NVP 162 (20.8%); AZT,3TC,EFV 109(14.0%); TDF,3TC,EFV or NVP 74(9.5%), D4T,3TC,EFV 45(5.3%); ABC,3TC, EFV or NVP or Lopinavir/ritonavir, 41(5.3%) and AZT or D4T,3TC,Lopinavir/ritonavir 3(0.39%).

Liver enzyme abnormalities among children taking first line antiretroviral therapy

A quarter of the children 177(25.1%) had elevated AST, while a tenth 83(11.8%) exhibited high ALT levels. Younger age at enrolment was associated with elevation of AST levels ($p=0.008$), being NVP or AZT based cART regimens ($p=0.001$), shorter time on cART ($p=0.02$) and a $pVL>1000$ copies/mL ($p=0.03$) (Table 1 of Paper IV). Shorter treatment duration was associated with increased risk of having abnormal AST levels 6 months into enrolment ($p=0.03$) (Table 2 of Paper IV).

Similarly, for ALT levels, young age ($p=0.003$), shorter duration of treatment ($p=0.03$) and having $pVL>1000$ copies/mL ($p=0.03$) predicted abnormal levels (Table 1 in Paper IV).

Further, we assessed the trend of liver enzyme abnormalities over the duration of the follow up period (Figure 1 of Paper II). We used linear mixed model regression to investigate the trend of AST abnormalities. Over the follow up period, an estimate of 1.4 (95% CI: 0.4-2.5) IU/L per each follow-up time ($p=0.01$). Similarly, the ALT decreased by 1.4 (95% CI: 0.2-2.6) IU/L at each follow-up visit ($p=0.01$). However, the trend of the change in AST and ALT stratified by the cART regimen did not reach statistical significance except for those taking a NVP containing first-line regimen where those taking NVP containing regimen had a significantly higher change than those taking EFV based regimen (p -value=0.04) (Figure 2 and Figure 3 in Paper II).

Our findings in this analysis are in line with results reported by other studies in different settings. For instance, a four year follow up study found similar findings with regards to the pattern of liver enzyme changes [203]. The decreasing pattern in the level of liver enzymes could be interpreted differently. It could indicate stabilization of the liver damage in the earlier phases of cART initiation. On the other end, it could also signal a deteriorating liver damage where signs of liver failure could also manifest. Ultrasonography and liver synthetic tests could help conclusively determine if this trend suggests deteriorating liver function [204].

In order to assess if liver enzyme abnormalities observed in the study suggest progressive normalization or deterioration, we further assessed the burden of liver fibrosis using noninvasive markers – the APRI score and FIB-4 index. From those screened, a total of 76(10.2%) at enrolment, 56(9.6%) at 6 months, 29(9.6%) at 12 months and 18(6.3%) at 18 months had a score of >0.5 , which suggests liver fibrosis($p=0.03$). Moreover, based on the FIB-4 index, 8(1.2%) at enrolment, 3(0.6%) at 6 months, 3(0.9%) at 12 months and 0 at 18 months had FIB-4 index >1.5 which also suggests fibrosis ($p=0.05$). An APRI score of >1.5 suggests cirrhosis – from the EPHIC cohort, 9(0.9%), 5(0.8%), 3(0.8%), and 1(0.3%) had levels suggesting cirrhosis at respective follow up times of 6, 12 and 18 months. There was a statistically significant reduction in the APRI score over the course of the follow up – a reduction of 0.02 units per follow-up visit was recorded (95% CI: -0.04 – (-0.006)) ($p=0.01$). However, the reduction in the FIB-4 score was not statistically significant ($p=0.44$). These findings support the possibility that normalization of the liver enzymes could likely be an indication of stabilization following cART initiation, where there could be acute liver toxicity associated with some of the antiretroviral regimen drugs.

Abnormalities in renal function tests among HIV-infected children taking first line cART

To assess renal function of the enrolled HIV infected children, we assessed creatinine and BUN. In most of the analysis, GFR was used instead of creatinine clearance since creatinine value would vary by age, height and weight [205]. Reassuringly, baseline derangement in renal function were less common in the studied cohort. Elevated creatinine (above upper limit of normal for age) was observed in 24(3.4%) and while 84(12.1%) had elevated BUN levels. Elevated BUN level at enrolment was significantly more common among young children. Only six children had a mild reduction in GFR (60-90 mL/min per 1.73 m²) (Table 1 of Paper IV). There was a significant increment of the BUN level during the course of the follow up. However, the worrying finding in this analysis was that there was a significant increment in BUN and a significant decrement in GFR. Specifically, the BUN increased by a median of 1.6(95% CI: 0.4-2.7) mg/dL for every 6 month increase in treatment duration (p=0.01) and the GFR decreased by 35.6 (95% CI: 17.7-53.4) mL/min per 1.73 m² (p-value <0.0001) for every 6 month follow up (Figure 1 of Paper II). Of note, cART regimen type was not associated with the change in both BUN and GFR (Figure 2 and 3 in Paper II). This finding is in contrast to other studies which reported that TD-containing regimens increase the risk of renal function derangements for example an increased risk of persistent renal disease (PRD) [206-209]. However, we noted a difference in the median GFR between cART regimen types – children who were taking D4T and TDF -containing regimens had lower GFR than the other groups. Likely because of the small sample size in the sub groups particularly those taking a TDF-containing first line regimen, the differences did not reach statistical significance.

The trend of the GFR showed a down-hill pattern which could indicate a progressive renal disease. Similar findings were reported using serum creatinine levels in a four years follow up among a small group of HIV infected children and adolescents [203]. The progressive increment in BUN and decrement in GFR both warrant a closer follow up for an earlier systematic prevention and management of the renal complications of effects of cART or the HIV infection – HIV associated nephropathy (HIVAN) [210].

4.5 Metabolic drangements among HIV infected children on treatment (paper V)

Data of 320 children from the EPHIC and EPDOS cohorts, with median age of 11.0 (Q1-Q3: 7.01 – 14.0) years were used for this analysis. Nearly half, 165 (49 %) of the children were male. From the total, 105 (32.8%) were treatment naïve HIV infected participants (Group 1) while 215 (67.2%) participants were taking first-line treatment (Group 2), who were taking D4T, 3TC, NVP – 54(23.5%); AZT, 3TC,

EFV – 27(11.7%); AZT, 3TC, NVP – 90(39.1%); D4T, 3TC, EFV – 12(5.2%); TDF, 3TC, NVP or EFV – 15(6.5%) and ABC, 3TC with EFV, NVP or PI – 4(1.7%). The median duration on cART was 54.0(Q1-Q3: 23.0 – 86.5) months.

From the children in Group 1, 41(39.0%) were WHO clinical stage 1 – 23(21.9%); WHO clinical stage 2 – 31(29.5%); WHO clinical stage 3, and 9 (8.6%) WHO clinical stage 4. Whereas, from Group 2, 35(16.0%), 61(25.6%), 77(33.5%) and 11(5.5%) were WHO clinical stage 1, 2, 3 and 4, respectively. Irrespective of treatment status, more children from Group 2 had a WHO clinical stage 3 or 4 disease compared to Group 1 ($p=0.04$).

Group 1 children were younger than children in Group 2 ($p<0.001$). Children taking antiretroviral therapy had better immunologic recovery evidenced by significantly higher median CD4 count and virological suppression ($p<0.001$) as compared to those who were cART naïve. Similarly, anthropometric indices were better among Group 2 children than Group 1 (Table 1 of Paper V).

Dyslipidemia among HIV infected children by treatment status

We compared Group 1 and 2 children for dyslipidemia. Median total cholesterol in Group 1 was higher than those in Group 2 (median=120.0 mg/dL, IQR: 97.8 mg/dL – 150.0 mg/dL as compared to median=102.5 mg/dL, IQR: 80.0 mg/dL – 127.8 mg/dL, respectively; $p=0.001$). Conversely, HDLc was lower among Group 2 than in Group 1 (median=45.0 mg/dL, IQR: 30.3 mg/dL – 48.0 mg/dL and median=48.5 mg/dL, IQR: 41.0 mg/dL – 66.9 mg/dL, respectively) ($p=0.002$).

We then dichotomized lipid parameters in to normal and dyslipidemia based on the suggested lower and upper limits of normal. More children from Group 2 (40.2%) compared to those from Group 1 (23.4%) had abnormal HDLc levels ($p=0.006$). Triglyceride level was also higher among Group 2 than Group 1 even though not statistically significant ($p=0.16$). Overall, any lipid profile abnormality was more common among children in Group 2 (70.2%) than in Group 1 (58.1%) ($p=0.033$) (Table 2 of Paper V).

Whilst lingering medical conditions including obesity and HIV infection are possibly linked to dyslipidemia, normal children with normal anthropometrics from the sub Saharan African region demonstrate a very low burden of dyslipidemia [211]. Previous reports documented a high burden of dyslipidemia associated with HIV infections – findings which are in line with the our analysis [128, 212-214]. Similar the trend observed in our analysis, studies from India, which included HIV infected children with and without treatment experience, similarly high prevalence of hypertriglyceridemia and low HDLc levels [213] [214]. Moreover, a study in Uganda among treatment experienced HIV-infected children reported similar dyslipidemia profile [212]. Treatment status as a risk for dyslipidemia was also similarly reported among a cohort of adult HIV infected patients in Ethiopia [215].

In this analysis (Paper V), presence of treatment experience was associated with lower total cholesterol values possibly indicating differential lipid type abnormalities with treatment experience, for example abnormalities in hypertriglyceridemia and low HDLc levels were more common among children in Group 2. As there are limited data investigating the pattern of dyslipidemia stratified by HIV infection status, more data on the specific types of dyslipidemia and correlates would shade light on the detailed burden of the problem for possible preventive and therapeutic action.

Even in the absence of HIV infection, undernutrition and obesity are linked to dyslipidemia [216-218]. Our analysis demonstrated that undernutrition was related to risk of dyslipidemia within Group 1 children, while there was no significant association among children in Group 2. The beneficial effects of treatment leading to improvements in anthropometrics which was the case for children who received treatment for at least six months could explain this finding.

The finding of more dyslipidemia among children with treatment experience and duration on treatment compared to cART naïve children warrants a special attention as these children need to be followed with regular biochemical assessments. With the introduction of efficacious cART, the life expectancy of HIV infected children in resource limited settings is expected to increase, which necessitates minimizing long and short-term adverse reactions as an important component of the care and treatment package. In HIV infected children, cardiovascular risks (CVD) have been linked to persistence of dyslipidemia [219, 220]. CVD could possibly threaten the accomplishments gained in cutting child mortality and morbidity associated with HIV infection [221]. Even though these complications are less common and a relatively smaller group of children would require therapeutic interventions, risk of CVD could be minimized through integration of routine screening in the HIV care and treatment package, practicing recommended modifications in life style and lipid lowering interventions, when needed [222].

The deleterious consequences of persistent dyslipidemia in children were investigated [223]. Our findings in this analysis add to the existing body of literature on the risk posed by persistent dyslipidemia including neurologic and cardiovascular accidents in the absence of treatments which need to be administered to lower the lipid level in the early stages of abnormality. Treatment experience was shown to be an important predictor of dyslipidemia among children with HIV infection. These findings emphasize the demand for a targeted follow up with emphasis on those with treatment status and timely institution of treatments in cases where lipid profile abnormalities persist.

Correlates of dyslipidemia among HIV infected children by treatment status

Correlates of dyslipidemia among children with HIV infection was assessed by treatment status using univariable and multivariable logistic regression models. Children on treatment had better anthropometric indices as compared to treatment naïve counterparts (Table 3 in Paper V). HAZ was the only independent predictor of dyslipidemia among treatment naïve children (Table 4 of Paper V).

Furthermore, duration of treatment was briefer in those with treatment experience and who had dyslipidemia (median=49 months, Q1-Q3: (21.0 – 78.0) as compared to those without (median= 60 months, Q1-Q3: (30.3 – 90.8) months, which was not statistically different ($p=0.253$). Total cholesterol and HDLc values were however significantly different with treatment experience (Figure 1 of Paper V). Children who took cART for more than 5 years had a significantly lower total cholesterol when compared to treatment naïve subjects ($p=0.003$).

5 CONCLUSIONS AND RECOMMENDATION

Pretreatment HIV drug resistance was observed to be less common in Ethiopian HIV infected children, but its presence predict treatment failure during the first year of cART. The rates of PDR observed in our study population is similar to reports from sub Saharan African children with no PMTCT exposure [100]. Our findings of the burden of PDR can be taken as representative figures for the whole of Ethiopia as our study included HIV-infected children having different geographic locations, ethnic and sociodemographic attributes across two of the largest administrative regions of Ethiopia.

Overall, our findings reveal that a high proportion of children with HIV infection from resource scarce settings can achieve virological suppression within the first year of cART. PDR is associated with worse virological suppression while a tenofovir based cART regimen is associated with better virological suppression within one year of treatment. The high prevalence of viral rebound in more than a third of the included children is worrying, but fewer patients exhibited sustained high viremia suggesting virologic treatment failure. We also demonstrated a considerable (81%) burden of HIVDR among children who were on a failing first line regimen in Ethiopia. This is especially important as it seriously limits potential treatment options. More alarmingly, for about a third of the children included in the study, none of the four locally available NRTIs were fully active to be used as second line.

Regarding the safety of antiretroviral therapy, it was observed that abnormalities in liver enzyme tests tend to improve with time on treatment, while kidney function, assessed by GFR and BUN, tends to deteriorate progressively. Nevirapine based regimens are associated with more liver enzyme abnormalities but is not the case for renal function. Another safety parameter assessed in this PhD thesis was HIV and cART associated dyslipidemia. Low HDLc dyslipidemia and hypertriglyceridemia were observed to be common among both treatment experienced and naïve children and adolescents. However, treatment experience was associated with a significantly higher prevalence of any dyslipidemia.

Based on our results, an individualized patient care decision approach based on presence of pretreatment drug resistance mutations is recommended. Furthermore, the findings indicate the need for more studies with larger sample size to understand the relationship between PDR, treatment regimen and virologic response.

The use of PI based regimens as first line drugs for children is supported by our findings of high burden of resistance to nevirapine and EFV which are the NNRTIs currently accessible for pediatric first line treatment in Ethiopia [41, 42]. On the other hand, we observed a relatively low burden of resistance (~5%) to NRTI drugs and none of the PI class drugs showed resistance mutations, which supports

using NRTIs in first line regimens. Nonetheless, affordable and expanded access to alternative treatment options including integrase inhibitors is an urgent need in resource limited settings, given the emergence of dual class resistance, notwithstanding in a minority (5%) of cases.

To accelerate the fight to end the HIV/AIDS epidemic, an integrated and effective clinical care of HIV infected children living in resource constrained settings is very important. Expanding access to HIV treatment options and to routine virologic resistance testing in resource poor settings like Ethiopia is greatly needed. Such services would improve meeting basic human rights needs and improve prognosis of vulnerable children in such settings.

The safety related analysis and findings of the project emphasizes the need for routine monitoring of hepatic and renal function tests as well as screening for metabolic disorders. Monitoring children for adverse events of long-term cART, including dyslipidemia are of utmost importance to ensure better quality of life for HIV infected children. It is important to consider institution of The presence of lifestyle modifications and lipid lowering drugs in a small group of children to reduce risks of cardiovascular accidents.

6 ACKNOWLEDGMENTS

My PhD education has indeed been quite a long and exciting journey. It has also been so educative giving me the key life skills for my future career as a scientist. I am very honored and humbled to be part of Karolinska Institutet as a PhD student. Of course, many people have contributed to the successful making of this thesis, and I would like to extend my deep gratitude to you all for being part of this endeavor in one or another way.

To my Supervisors;

First and for most I would like to express my deepest gratitude to my main supervisor, **Associate Prof. Eleni Aklillu** who tirelessly coached me, encouraged me and showed me the way to success. I always remain indebted to you for all the scientific guidance, encouragement and technical support throughout my PhD study. Your close supervision, expertise, valuable constructive comments and suggestions were indispensable to improve my understanding and skills of scientific undertakings. More importantly, your skills in engaging your PhD students in reasoning and scientific arguments, which you have done in a very friendly collegial approach was one big lesson for me to learn. I just would like to say – Thank you!!

My co-supervisor **Prof. Eyasu Makonnen** who was very friendly and whom I always call when I have any doubts is where my huge gratitude goes to. Prof Eyasu, you have taught me courses as a medical student and now you have helped me grow as a research scientist with your calm and mature suggestions and comments. I am always in appreciation of your very friendly and humble approach in solving problems encountered during the journey. I am honored to have learnt fine skills of decision making, interpersonal communications and problem solving.

I would also like to thank my co-supervisor **Dr. Degu Jerene**, who helped me to adjust my expectations as a PhD student as I move from a primarily clinical practice perspective to a PhD studentship. Degu, you have been a great mentor with a rich research expertise and yet in keeping the harmony with clinical practice and integrating the skills of clinical decision making.

To my friend and collaborator **Dr. Thomas Brune** and the whole family, thank you very much for encouraging me, hosting me at your house and supporting me during my stay in Stockholm. You very much helped me to cope with the times I had to be away from my family. To friends Dr. Rajabu Hussein MnKugwe (PhD student), Mr Adugna Chala (PhD Candidate), Mr Sintayehu Tadesse, Mr Gettahun H/Meskel, Dr Tolossa Chaka and all the data collectors in the sites, I would like to thank you for making my time easier and enjoyable. You have been fantastic friends and collaborators throughout the journey. I hope to continue working with you in the next chapter of my research career too.

I would also like to thank my long-time collaborators **Dr. Foster, B. Alex** of Oregon Health and Science University; and, **Professor Zabrina Brumme** and her research group at Simon Fraser University for supporting me during the research work related to this PhD project.

To the IAS-CIPHER pediatric HIV research grant and the Swedish Research Council, I would like to express my gratitude for funding the two clinical studies. I also would like to thank Hawassa University, specifically Dr. Abel Gedefaw, Dr. Efreem G/Hana and Dr. Negash Tagesse for supporting and encouraging me during the PhD.

To the **study participants**, I would like to say that you have contributed a lot in the search for knowledge for a good cause. Any sacrifice you have made is honored and I remain indebted to you all. To the participants who died during the study, may you Rest in Peace.

To the Department of Laboratory Medicine; my special thanks go to Prof. Matti Sällberg, Prof. Antony Wright, Dr Jenny Degerholm Langsmo, Yvonne Elliman and Arja Kramsu for your unreserved support throughout training. I am grateful for all you did to make my defense go through as planned. I am honored to have been trained in the Department of Laboratory.

Finally, my sincere and deep gratitude to my family (Freshwork Ayalew, Yohanna Birkneh and Brook Birkneh) for their unparalleled and continuous help, love and support. I am grateful to my brothers and sisters for always being there for me as family and friends. I am forever indebted to my mother, Meseret Birhane Zeamanuel for her perseverance to give me the opportunities that have made me who I am today. I happily dedicate this milestone to her. This journey would not have been possible if not for my family and friends, and I would like to thank them all!

7 REFERENCES

1. AVERT: Global information and education on HIV and AIDS: History of HIV AIDS review.<https://www.wavertorg/professionals/history-hiv-aids/overview2017>, Accessed on July 9, 2019.
2. UNAIDS: Data on people living with HIV/AIDS.<http://aidsinfounaidsorg/2017>, Accessed on July 8, 2019.
3. Dwyer-Lindgren L, Cork MA, Sligar A, Steuben KM, Wilson KF, Provost NR, Mayala BK, VanderHeide JD, Collison ML, Hall JB, et al: Mapping HIV prevalence in sub-Saharan Africa between 2000 and 2017.*Nature* 2019, 570:189-193.
4. AVERT: HIV and AIDS in East and southern Africa regional overview.<https://www.wavertorg/professionals/hiv-around-world/sub-saharan-africa/overview2017>, Accessed on July 8, 2019.
5. Rabkin M, El-Sadr WM, De Cock KM: The impact of HIV scale-up on health systems: A priority research agenda.*J Acquir Immune Defic Syndr* 2009, 52 Suppl 1:S6-11.
6. AVERT: Global information and education on HIV and AIDS: Funding for HIV and AIDS.<https://www.wavertorg/professionals/hiv-around-world/global-response/funding2017>, Accessed on July 8, 2019.
7. Avdeeva O, Lazarus JV, Aziz MA, Atun R: The Global Fund's resource allocation decisions for HIV programmes: addressing those in need.*J Int AIDS Soc* 2011, 14:51.
8. Survival of HIV-positive patients starting antiretroviral therapy between 1996 and 2013: a collaborative analysis of cohort studies.*Lancet HIV* 2017, 4:e349-e356.
9. 90-90-90: Treatment for all.<https://www.unaidsorg/en/resources/9090902017>, Accessed on July 8, 2019.
10. UNAIDS: Fast-Track strategy to end the AIDS epidemic by 2030.<https://www.unaidsorg/en/resources/campaigns/World-AIDS-Day-Report-20142017>, Accessed on July 8, 2019.
11. Global, regional, and national incidence, prevalence, and mortality of HIV, 1980-2017, and forecasts to 2030, for 195 countries and territories: a systematic analysis for the Global Burden of Diseases, Injuries, and Risk Factors Study 2017.*Lancet HIV* 2019.

12. Elizabeth Glaser Pediatric AIDS Foundation: The History of HIV/AIDS in Children: <http://www.pedaids.org/2014/04/16/the-history-of-hiv-aids-in-children/>.
13. UNAIDS 2019 data - State of the HIV epidemic: https://www.unaids.org/sites/default/files/media_asset/2019-UNAIDS-data_en.pdf.
14. Abebe A, Lukashov VV, Rinke De Wit TF, Fisseha B, Tegbaru B, Kliphuis A, Tesfaye G, Negassa H, Fontanet AL, Goudsmit J, Pollakis G: Timing of the introduction into Ethiopia of subcluster C' of HIV type 1 subtype C. *AIDS Res Hum Retroviruses* 2001, 17:657-661.
15. Kloos H: HIV/AIDS in Ethiopia: The Epidemic and Social, Economic, and Demographic Impacts. *International Conference on African Development Archives* 2001, <https://pdfs.semanticscholar.org/3ddc/77d812c57307503cd54213d221e6a020b.pdf>.
16. Gray ME, Nieburg P, Dillingham R: Pediatric Human Immunodeficiency Virus Continuum of Care: A Concise Review of Evidence-Based Practice. *Pediatr Clin North Am* 2017, 64:879-891.
17. UNAIDS warns that progress is slowing and time is running out to reach the 2020 HIV targets. <https://www.unaids.org/en/resources/presscentre/pressreleaseandstatementarchive/2018/july/miles-to-go>.
18. Kisesa A, Chamla D: Getting to 90-90-90 targets for children and adolescents HIV in low and concentrated epidemics: bottlenecks, opportunities, and solutions. *Curr Opin HIV AIDS* 2016, 11 Suppl 1:S1-5.
19. Penazzato M, Irvine C, Vicari M, Essajee SM, Sharma A, Puthanakit T, Abrams EJ, Doherty M: A Global Research Agenda for Pediatric HIV. *J Acquir Immune Defic Syndr* 2018, 78 Suppl 1:S10-s15.
20. Tadesse BT, Foster BA, Berhan Y: Cross Sectional Characterization of Factors Associated with Pediatric HIV Status Disclosure in Southern Ethiopia. *PLoS One* 2015, 10:e0132691.
21. Tiemessen CT, Kuhn L: Immune pathogenesis of pediatric HIV-1 infection. *Curr HIV/AIDS Rep* 2006, 3:13-19.
22. Azzoni L, Rutstein RM, Chehimi J, Farabaugh MA, Nowmos A, Montaner LJ: Dendritic and natural killer cell subsets associated with stable or declining CD4+ cell counts in treated HIV-1-infected children. *J Infect Dis* 2005, 191:1451-1459.
23. Scott-Algara D, Buseyne F, Porrot F, Corre B, Bellal N, Rouzioux C, Blanche S, Riviere Y: Not all tetramer binding CD8+ T cells can produce cytokines and chemokines involved in the effector functions of virus-specific CD8+ T-lymphocytes in HIV-1 infected children. *J Clin Immunol* 2005, 25:57-67.

24. Chakraborty R, Morel AS, Sutton JK, Appay V, Ripley RM, Dong T, Rostron T, Ogola S, Palakudy T, Musoke R, et al: Correlates of delayed disease progression in HIV-1-infected Kenyan children.*J Immunol* 2005, 174:8191-8199.
25. Blanche S, Tardieu M, Duliege A, Rouzioux C, Le Deist F, Fukunaga K, Caniglia M, Jacomet C, Messiah A, Griscelli C: Longitudinal study of 94 symptomatic infants with perinatally acquired human immunodeficiency virus infection. Evidence for a bimodal expression of clinical and biological symptoms.*Am J Dis Child* 1990, 144:1210-1215.
26. Mayaux MJ, Burgard M, Teglas JP, Cottalorda J, Krivine A, Simon F, Puel J, Tamalet C, Dormont D, Masquelier B, et al: Neonatal characteristics in rapidly progressive perinatally acquired HIV-1 disease. The French Pediatric HIV Infection Study Group.*Jama* 1996, 275:606-610.
27. Blanche S, Mayaux MJ, Rouzioux C, Teglas JP, Firtion G, Monpoux F, Ciraru-Vigneron N, Meier F, Tricoire J, Courpotin C, et al.: Relation of the course of HIV infection in children to the severity of the disease in their mothers at delivery.*N Engl J Med* 1994, 330:308-312.
28. Spencer LT, Ogino MT, Dankner WM, Spector SA: Clinical significance of human immunodeficiency virus type 1 phenotypes in infected children.*J Infect Dis* 1994, 169:491-495.
29. Kourtis AP, Ibegbu C, Nahmias AJ, Lee FK, Clark WS, Sawyer MK, Nesheim S: Early progression of disease in HIV-infected infants with thymus dysfunction.*N Engl J Med* 1996, 335:1431-1436.
30. De Rossi A, Masiero S, Giaquinto C, Ruga E, Comar M, Giacca M, Chieco-Bianchi L: Dynamics of viral replication in infants with vertically acquired human immunodeficiency virus type 1 infection.*J Clin Invest* 1996, 97:323-330.
31. Dickover RE, Dillon M, Leung KM, Krogstad P, Plaeger S, Kwok S, Christopherson C, Deveikis A, Keller M, Stiehm ER, Bryson YJ: Early prognostic indicators in primary perinatal human immunodeficiency virus type 1 infection: importance of viral RNA and the timing of transmission on long-term outcome.*J Infect Dis* 1998, 178:375-387.
32. Shearer WT, Quinn TC, LaRussa P, Lew JF, Mofenson L, Almy S, Rich K, Handelsman E, Diaz C, Pagano M, et al: Viral load and disease progression in infants infected with human immunodeficiency virus type 1. Women and Infants Transmission Study Group.*N Engl J Med* 1997, 336:1337-1342.
33. Palumbo PE, Raskino C, Fiscus S, Pahwa S, Fowler MG, Spector SA, Englund JA, Baker CJ: Predictive value of quantitative plasma HIV RNA and CD4+ lymphocyte count in HIV-infected infants and children.*Jama* 1998, 279:756-761.

34. Mofenson LM, Korelitz J, Meyer WA, 3rd, Bethel J, Rich K, Pahwa S, Moye J, Jr., Nugent R, Read J: The relationship between serum human immunodeficiency virus type 1 (HIV-1) RNA level, CD4 lymphocyte percent, and long-term mortality risk in HIV-1-infected children. National Institute of Child Health and Human Development Intravenous Immunoglobulin Clinical Trial Study Group. *J Infect Dis* 1997, 175:1029-1038.
35. Biggar RJ, Janes M, Pilon R, Miotti P, Taha TE, Broadhead R, Mtimivalye L, Kumwenda N, Cassol S: Virus levels in untreated African infants infected with human immunodeficiency virus type 1. *J Infect Dis* 1999, 180:1838-1843.
36. Taha TE, Kumwenda NI, Hoover DR, Biggar RJ, Broadhead RL, Cassol S, van der Hoven L, Markakis D, Liomba GN, Chipangwi JD, Miotti PG: Association of HIV-1 load and CD4 lymphocyte count with mortality among untreated African children over one year of age. *Aids* 2000, 14:453-459.
37. Manual on Paediatric HIV Care and Treatment for District Hospitals: Addendum to the Pocket Book of Hospital Care of Children. Geneva: World Health Organization; 2011. 5, Introduction: Infants, children and HIV-infection. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK304128/>.
38. Kharsany AB, Karim QA: HIV Infection and AIDS in Sub-Saharan Africa: Current Status, Challenges and Opportunities. *Open AIDS J* 2016, 10:34-48.
39. EDHS: Ethiopian Demographic and Health Survey - HIV Prevalence report. <https://dhsprogram.com/pubs/pdf/FR328/FR328HIVpdf2016>, (Accessed October 22, 2018).
40. UNAIDS: Progress towards the 90-90-90 targets. <http://aidsinfo.unaids.org/2018>.
41. Girum T, Wasie A, Worku A: Trend of HIV/AIDS for the last 26 years and predicting achievement of the 90-90-90 HIV prevention targets by 2020 in Ethiopia: a time series analysis. *BMC Infect Dis* 2018, 18:320.
42. Levi J, Raymond A, Pozniak A, Vernazza P, Kohler P, Hill A: Can the UNAIDS 90-90-90 target be achieved? A systematic analysis of national HIV treatment cascades. *BMJ Glob Health* 2016, 1:e000010.
43. Creek TL, Sherman GG, Nkengasong J, Lu L, Finkbeiner T, Fowler MG, Rivadeneira E, Shaffer N: Infant human immunodeficiency virus diagnosis in resource-limited settings: issues, technologies, and country experiences. *Am J Obstet Gynecol* 2007, 197:S64-71.
44. Manual on Paediatric HIV Care and Treatment for District Hospitals: Addendum to the Pocket Book of Hospital Care of Children. Geneva: World Health Organization; 2011. 6, Diagnosis of HIV infection in infants and children. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK304129/>.

45. World Health Organization (WHO). Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection, Recommendations for a Public Health Approach; World Health Organization: Geneva, Switzerland, 2013.
46. Read JS: Diagnosis of HIV-1 infection in children younger than 18 months in the United States.*Pediatrics* 2007, 120:e1547-1562.
47. Grundmann N, Iliff P, Stringer J, Wilfert C: Presumptive diagnosis of severe HIV infection to determine the need for antiretroviral therapy in children less than 18 months of age.*Bull World Health Organ* 2011, 89:513-520.
48. Mutesu-Kapembwa K, Andrews B, Kapembwa K, Chi BH, Banda Y, Mulenga V, Kankasa C: Performance of modified WHO presumptive criteria for diagnosis of HIV infection in children <18 months admitted to University Teaching Hospital in Lusaka.*Med J Zambia* 2010, 37:64-70.
49. Buchanan AM, Nadjm B, Amos B, Mtove G, Sifuna D, Cunningham CK, Crump JA, Reyburn H: Utility of rapid antibody tests to exclude HIV-1 infection among infants and children aged <18 months in a low-resource setting.*J Clin Virol* 2012, 55:244-249.
50. UNAIDS: 90-90-90: An ambitious treatment target to help end the AIDS epidemic. http://www.unaids.org/sites/default/files/media_asset/90-90-90_enpdf2014.
51. UNAIDS: Global AIDS update, 2016. http://www.unaids.org/sites/default/files/media_asset/global-AIDS-update-2016_enpdf2016, Accessed on March 4, 2017.
52. WHO: Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection - Recommendations for a public health approach - Second edition. <https://www.who.int/hiv/pub/arv/arv-2016/en/2016>, [Accessed on May 28, 2019].
53. Kityo C, Sigaloff KC, Sonia Boender T, Kaudha E, Kayiwa J, Musiime V, Mukuye A, Kiconco M, Nankya I, Nakatudde-Katumba L, et al: HIV Drug Resistance Among Children Initiating First-Line Antiretroviral Treatment in Uganda.*AIDS Res Hum Retroviruses* 2016, 32:628-635.
54. Phillips AN, Cambiano V, Nakagawa F, Revill P, Jordan MR, Hallett TB, Doherty M, De Luca A, Lundgren JD, Mhangara M, et al: Cost-effectiveness of public-health policy options in the presence of pretreatment NNRTI drug resistance in sub-Saharan Africa: a modelling study.*Lancet HIV* 2018, 5:e146-e154.
55. Bruzzese E, Lo Vecchio A, Smarrazzo A, Tambaro O, Palmiero G, Bonadies G, Guarino A: Dolutegravir-based anti-retroviral therapy is effective and safe in HIV-infected paediatric patients.*Ital J Pediatr* 2018, 44:37.

56. Briand C, Dollfus C, Faye A, Kantor E, Avettand-Fenoel V, Caseris M, Descamps D, Schneider V, Tabone MD, Vaudre G, et al: Efficacy and tolerance of dolutegravir-based combined ART in perinatally HIV-1-infected adolescents: a French multicentre retrospective study.*J Antimicrob Chemother* 2017, 72:837-843.
57. Dickinson L, Amin J, Else L, Boffito M, Egan D, Owen A, Khoo S, Back D, Orrell C, Clarke A, et al: Comprehensive Pharmacokinetic, Pharmacodynamic and Pharmacogenetic Evaluation of Once-Daily Efavirenz 400 and 600 mg in Treatment-Naive HIV-Infected Patients at 96 Weeks: Results of the ENCORE1 Study.*Clin Pharmacokinet* 2016, 55:861-873.
58. Carey D: Efavirenz 400 mg daily remains non-inferior to 600 mg: 96 week data from the double-blind, placebo-controlled ENCORE1 study.*J Int AIDS Soc* 2014, 17:19523.
59. Yang SP, Liu WC, Lee KY, Wu BR, Su YC, Wu PY, Zhang JY, Luo YZ, Sun HY, Chang SY, et al: Effectiveness of a reduced dose of efavirenz plus 2 NRTIs as maintenance antiretroviral therapy with the guidance of therapeutic drug monitoring.*J Int AIDS Soc* 2014, 17:19524.
60. Sauvageot D, Schaefer M, Olson D, Pujades-Rodriguez M, O'Brien DP: Antiretroviral therapy outcomes in resource-limited settings for HIV-infected children <5 years of age.*Pediatrics* 2010, 125:e1039-1047.
61. Stanecki K, Daher J, Stover J, Akwara P, Mahy M: Under-5 mortality due to HIV: regional levels and 1990-2009 trends.*Sex Transm Infect* 2010, 86 Suppl 2:ii56-61.
62. Danforth K, Granich R, Wiedeman D, et al. Global Mortality and Morbidity of HIV/AIDS. In: Holmes KK, Bertozzi S, Bloom BR, et al., editors. Major Infectious Diseases. 3rd edition. Washington (DC): The International Bank for Reconstruction and Development / The World Bank; 2017 Nov 3. Chapter 2. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK525184/> doi: 10.1596/978-1-4648-0524-0/ch2.
63. Mutanga JN, Mutembo S, Ezeamama AE, Song X, Fubisha RC, Mutesu-Kapembwa K, Sialondwe D, Simuchembu B, Chinyonga J, Thuma PE, Whalen CC: Long-term survival outcomes of HIV infected children receiving antiretroviral therapy: an observational study from Zambia (2003–2015). *BMC Public Health* 2019, 19:115.
64. Arage G, Assefa M, Worku T, Semahegn A: Survival rate of HIV-infected children after initiation of the antiretroviral therapy and its predictors in Ethiopia: A facility-based retrospective cohort.*SAGE Open Med* 2019, 7:2050312119838957.

65. Endalamaw A, Tezera N, Eshetie S, Ambachew S, Habtewold TD: Adherence to Highly Active Antiretroviral Therapy Among Children in Ethiopia: A Systematic Review and Meta-analysis. *AIDS Behav* 2018, 22:2513-2523.
66. Bacha T, Tilahun B, Worku A: Predictors of treatment failure and time to detection and switching in HIV-infected Ethiopian children receiving first line anti-retroviral therapy. *BMC Infectious Diseases* 2012, 12:197.
67. Melaku Z, Lulseged S, Wang C, Lamb MR, Gutema Y, Teasdale CA, Ahmed S, Gadisa T, Habtamu Z, Bedri A, et al: Outcomes among HIV-infected children initiating HIV care and antiretroviral treatment in Ethiopia. *Trop Med Int Health* 2017, 22:474-484.
68. Deribew A, Tessema GA, Deribe K, Melaku YA, Lakew Y, Amare AT, Abera SF, Mohammed M, Hiruye A, Teklay E, et al: Trends, causes, and risk factors of mortality among children under 5 in Ethiopia, 1990–2013: findings from the Global Burden of Disease Study 2013. *Population Health Metrics* 2016, 14:42.
69. Predictors of faster virological suppression in early treated infants with perinatal HIV from Europe and Thailand. *Aids* 2019, 33:1155-1165.
70. Sabin CA, Smith CJ, d'Arminio Monforte A, Battegay M, Gabiano C, Galli L, Geelen S, Gibb D, Guiguet M, Judd A, et al: Response to combination anti-retroviral therapy: variation by age. *Aids* 2008, 22:1463-1473.
71. Ateba Ndongo F, Texier G, Ida Penda C, Tejiokem MC, Tetang Ndiang S, Ndongo JA, Guemkam G, Sofeu CL, Kfutwah A, Faye A, et al: Virologic Response to Early Antiretroviral Therapy in HIV-infected Infants: Evaluation After 2 Years of Treatment in the PEDIACAM Study, Cameroon. *Pediatr Infect Dis J* 2018, 37:78-84.
72. Chandrasekaran P, Shet A, Srinivasan R, Sanjeeva GN, Subramanian S, Sunderesan S, Ramesh K, Gopalan B, Suresh E, Poornagangadevi N, et al: Long-term virological outcome in children receiving first-line antiretroviral therapy. *AIDS Research and Therapy* 2018, 15:23.
73. Barth RE, Tempelman HA, Smelt E, Wensing AM, Hoepelman AI, Geelen SP: Long-term outcome of children receiving antiretroviral treatment in rural South Africa: substantial virologic failure on first-line treatment. *Pediatr Infect Dis J* 2011, 30:52-56.
74. Kadima J, Patterson E, Mburu M, Blat C, Nyanduko M, Bukusi EA, Cohen C, Oyaro P, Abuogi L: Adoption of routine virologic testing and predictors of virologic failure among HIV-infected children on antiretroviral treatment in western Kenya. *PLoS One* 2018, 13:e0200242.

75. Musoke PM, Mudiope P, Barlow-Mosha LN, Ajuna P, Bagenda D, Mubiru MM, Tylleskar T, Fowler MG: Growth, immune and viral responses in HIV infected African children receiving highly active antiretroviral therapy: a prospective cohort study.*BMC Pediatr* 2010, 10:56.
76. Willig AL, Overton ET: Metabolic Complications and Glucose Metabolism in HIV Infection: A Review of the Evidence.*Curr HIV/AIDS Rep* 2016, 13:289-296.
77. Yihun BA, Kibret GD, Leshargie CT: Incidence and predictors of treatment failure among children on first-line antiretroviral therapy in Amhara Region Referral Hospitals, northwest Ethiopia 2018: A retrospective study.*PLoS One* 2019, 14:e0215300.
78. Teshome Yimer Y, Yalew AW: Magnitude and Predictors of Anti-Retroviral Treatment (ART) Failure in Private Health Facilities in Addis Ababa, Ethiopia. *PLoS One* 2015, 10:e0126026.
79. Castro H, Judd A, Gibb DM, Butler K, Lodwick RK, van Sighem A, Ramos JT, Warszawski J, Thorne C, Noguera-Julian A, et al: Risk of triple-class virological failure in children with HIV: a retrospective cohort study.*Lancet* 2011, 377:1580-1587.
80. Bunupuradah T, Puthanakit T, Kosalaraksa P, Kerr S, Boonrak P, Prasitsuebsai W, Lumbiganon P, Mengthaisong T, Phasomsap C, Pancharoen C, et al: Immunologic and virologic failure after first-line NNRTI-based antiretroviral therapy in Thai HIV-infected children.*AIDS Res Ther* 2011, 8:40.
81. Emmett SD, Cunningham CK, Mmbaga BT, Kinabo GD, Schimana W, Swai ME, Bartlett JA, Crump JA, Reddy EA: Predicting virologic failure among HIV-1-infected children receiving antiretroviral therapy in Tanzania: a cross-sectional study.*J Acquir Immune Defic Syndr* 2010, 54:368-375.
82. Ngo-Giang-Huong N, Wittkop L, Judd A, Reiss P, Goetghebuer T, Duiculescu D, Noguera-Julian A, Marczyńska M, Giacchino C, Ene L, et al: Prevalence and effect of pre-treatment drug resistance on the virological response to antiretroviral treatment initiated in HIV-infected children – a EuroCoord-CHAIN-EPPICC joint project.*BMC Infect Dis* 2016, 16:654.
83. Janssens B, Raleigh B, Soeung S, Akao K, Te V, Gupta J, Vun MC, Ford N, Nouhin J, Nerrienet E: Effectiveness of highly active antiretroviral therapy in HIV-positive children: evaluation at 12 months in a routine program in Cambodia.*Pediatrics* 2007, 120:e1134-1140.
84. Yassin S, Gebretekla GB: Magnitude and predictors of antiretroviral treatment failure among HIV-infected children in Fiche and Kuyu hospitals, Oromia region, Ethiopia: a retrospective cohort study.*Pharmacol Res Perspect* 2017, 5:e00296.

85. Koye DN, Ayele TA, Zeleke BM: Predictors of mortality among children on Antiretroviral Therapy at a referral hospital, Northwest Ethiopia: a retrospective follow up study.*BMC Pediatr* 2012, 12:161.
86. Sebunya R, Musiime V, Kitaka SB, Ndeezi G: Incidence and risk factors for first line anti retroviral treatment failure among Ugandan children attending an urban HIV clinic.*AIDS Res Ther* 2013, 10:25.
87. Kokeb M, Degu G: Immunological Response of Hiv-Infected Children to Highly Active Antiretroviral Therapy at Gondar University Hospital, North-Western Ethiopia.*Ethiop J Health Sci* 2016, 26:25-30.
88. Mgelea EM, Kisenge R, Aboud S: Detecting virological failure in HIV-infected Tanzanian children.*S Afr Med J* 2014, 104:696-699.
89. Sisay MM, Ayele TA, Gelaw YA, Tsegaye AT, Gelaye KA, Melak MF: Incidence and risk factors of first-line antiretroviral treatment failure among human immunodeficiency virus-infected children in Amhara regional state, Ethiopia: a retrospective follow-up study.*BMJ Open* 2018, 8:e019181.
90. Costenaro P, Penazzato M, Lundin R, Rossi G, Massavon W, Patel D, Nabachwa S, Franceschetto G, Morelli E, Bilardi D, et al: Predictors of Treatment Failure in HIV-Positive Children Receiving Combination Antiretroviral Therapy: Cohort Data From Mozambique and Uganda.*J Pediatric Infect Dis Soc* 2015, 4:39-48.
91. Chi BH, Adler MR, Bolu O, Mbori-Ngacha D, Ekouevi DK, Gieselman A, Chipato T, Luo C, Phelps BR, McClure C, et al: Progress, challenges, and new opportunities for the prevention of mother-to-child transmission of HIV under the US President's Emergency Plan for AIDS Relief.*J Acquir Immune Defic Syndr* 2012, 60 Suppl 3:S78-87.
92. Gibb DM, Giaquinto C: Children with HIV infection: special cases.*Lancet* 2000, 356 Suppl:s34.
93. Paredes R, Marconi VC, Lockman S, Abrams EJ, Kuhn L: Impact of anti-retroviral drugs in pregnant women and their children in Africa: HIV resistance and treatment outcomes.*J Infect Dis* 2013, 207 Suppl 2:S93-100.
94. Ton Q, Frenkel L: HIV drug resistance in mothers and infants following use of antiretrovirals to prevent mother-to-child transmission.*Curr HIV Res* 2013, 11:126-136.
95. Chen TK, Aldrovandi GM: Review of HIV antiretroviral drug resistance.*Pediatr Infect Dis J* 2008, 27:749-752.

96. Maggiolo F, Airoidi M, Kleinloog HD, Callegaro A, Ravasio V, Arici C, Bombana E, Suter F: Effect of adherence to HAART on virologic outcome and on the selection of resistance-conferring mutations in NNRTI- or PI-treated patients.*HIV Clin Trials* 2007, 8:282-292.
97. Maggiolo F, Ravasio L, Ripamonti D, Gregis G, Quinzan G, Arici C, Airoidi M, Suter F: Similar adherence rates favor different virologic outcomes for patients treated with nonnucleoside analogues or protease inhibitors.*Clin Infect Dis* 2005, 40:158-163.
98. Tang MW, Shafer RW: HIV-1 antiretroviral resistance: scientific principles and clinical applications.*Drugs* 2012, 72:e1-25.
99. Ssemwanga D, Lihana RW, Ugoji C, Abimiku A, Nkengasong J, Dakum P, Ndembu N: Update on HIV-1 acquired and transmitted drug resistance in Africa.*AIDS Rev* 2015, 17:3-20.
100. Boerma RS, Sigaloff KC, Akanmu AS, Inzaule S, Boele van Hensbroek M, Rinke de Wit TF, Calis JC: Alarming increase in pretreatment HIV drug resistance in children living in sub-Saharan Africa: a systematic review and meta-analysis.*J Antimicrob Chemother* 2017, 72:365-371.
101. Rojas Sanchez P, Holguin A: Drug resistance in the HIV-1-infected paediatric population worldwide: a systematic review.*J Antimicrob Chemother* 2014, 69:2032-2042.
102. Abegaz WE, Grossman Z, Wolday D, Ram D, Kaplan J, Sibide K, Wuhib T, Ismael S, Nkengasong J, Mekonen T, et al: Threshold survey evaluating transmitted HIV drug resistance among public antenatal clinic clients in Addis Ababa, Ethiopia.*Antivir Ther* 2008, 13 Suppl 2:89-94.
103. Arimide DA, Abebe A, Kebede Y, Adugna F, Tilahun T, Kassa D, Assefa Y, Balcha TT, Bjorkman P, Medstrand P: HIV-genetic diversity and drug resistance transmission clusters in Gondar, Northern Ethiopia, 2003-2013.*PLoS One* 2018, 13:e0205446.
104. Telele NF, Kalu AW, Gebre-Selassie S, Fekade D, Abdurahman S, Marrone G, Neogi U, Tegbaru B, Sonnerborg A: Pretreatment drug resistance in a large countrywide Ethiopian HIV-1C cohort: a comparison of Sanger and high-throughput sequencing.*Sci Rep* 2018, 8:7556.
105. Mulu A, Maier M, Liebert UG: Low Incidence of HIV-1C Acquired Drug Resistance 10 Years after Roll-Out of Antiretroviral Therapy in Ethiopia: A Prospective Cohort Study.*PLoS One* 2015, 10:e0141318.
106. Mulu A, Maier M, Liebert UG: Upward trends of acquired drug resistances in Ethiopian HIV-1C isolates: A decade longitudinal study.*PLoS One* 2017, 12:e0186619.

107. Clutter DS, Jordan MR, Bertagnolio S, Shafer RW: HIV-1 drug resistance and resistance testing.*Infect Genet Evol* 2016, 46:292-307.
108. Bussmann H, de la Hoz Gomez F, Roels TH, Wester CW, Bodika SM, Moyo S, Taffa N, Anderson MG, Mine M, Bile EC, et al: Prevalence of transmitted HIV drug resistance in Botswana: lessons learned from the HIVDR-Threshold Survey conducted among women presenting for routine antenatal care as part of the 2007 national sentinel survey.*AIDS Res Hum Retroviruses* 2011, 27:365-372.
109. Nunez M: Clinical syndromes and consequences of antiretroviral-related hepatotoxicity.*Hepatology* 2010, 52:1143-1155.
110. Nunez M, Soriano V: Hepatotoxicity of antiretrovirals: incidence, mechanisms and management.*Drug Saf* 2005, 28:53-66.
111. Pokorska-Spiewak M, Stanska-Perka A, Popielska J, Oldakowska A, Coupland U, Zawadka K, Szczepanska-Putz M, Marczyńska M: Prevalence and predictors of liver disease in HIV-infected children and adolescents.*Sci Rep* 2017, 7:12309.
112. Mugusi S, Ngaimisi E, Janabi M, Minzi O, Bakari M, Riedel KD, Burhenne J, Lindquist L, Mugusi F, Sandstrom E, Aklillu E: Liver enzyme abnormalities and associated risk factors in HIV patients on efavirenz-based HAART with or without tuberculosis co-infection in Tanzania.*PLoS One* 2012, 7:e40180.
113. Achhra AC, Nugent M, Mocroft A, Ryom L, Wyatt CM: Chronic Kidney Disease and Antiretroviral Therapy in HIV-Positive Individuals: Recent Developments.*Curr HIV/AIDS Rep* 2016, 13:149-157.
114. Price JC, Thio CL: Liver disease in the HIV-infected individual.*Clin Gastroenterol Hepatol* 2010, 8:1002-1012.
115. AIDSInfo: Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV: Adverse Events.<https://aidsinfo.nih.gov/guidelines2017>.
116. Chu KM, Boulle AM, Ford N, Goemaere E, Asselman V, Van Cutsem G: Nevirapine-associated early hepatotoxicity: incidence, risk factors, and associated mortality in a primary care ART programme in South Africa.*PLoS One* 2010, 5:e9183.
117. Mulu W, Gidey B, Chernet A, Alem G, Abera B: Hepatotoxicity and associated risk factors in HIV-infected patients receiving antiretroviral therapy at Felege Hiwot Referral Hospital, Bahirdar, Ethiopia.*Ethiop J Health Sci* 2013, 23:217-226.

118. Yimer G, Ueda N, Habtewold A, Amogne W, Suda A, Riedel KD, Burhenne J, Aderaye G, Lindquist L, Makonnen E, Aklillu E: Pharmacogenetic & pharmacokinetic biomarker for efavirenz based ARV and rifampicin based anti-TB drug induced liver injury in TB-HIV infected patients. *PLoS One* 2011, 6:e27810.
119. Yimer G, Amogne W, Habtewold A, Makonnen E, Ueda N, Suda A, Worku A, Haefeli WE, Burhenne J, Aderaye G, et al: High plasma efavirenz level and CYP2B6*6 are associated with efavirenz-based HAART-induced liver injury in the treatment of naive HIV patients from Ethiopia: a prospective cohort study. *Pharmacogenomics J* 2012, 12:499-506.
120. Saulela S, Martorell M, Esteban JI, Tural C, Ruiz I, Puig L, Esteban R, Guardia J, Vargas V, Shah I: The Prevalence Liver Function and Immunologic Status of Children With HIV and Hepatitis B Virus Coinfection in Enugu, Nigeria. *Haemophilia* 2006, 12:228-236.
121. Gil AC, Lorenzetti R, Mendes GB, Morcillo AM, Toro AA, Silva MT, Vilela MM: Hepatotoxicity in HIV-infected children and adolescents on antiretroviral therapy. *Sao Paulo Med J* 2007, 125:205-209.
122. Shah I: Adverse effects of antiretroviral therapy in HIV-1 infected children. *J Trop Pediatr* 2006, 52:244-248.
123. Ajulo MO, Omole MK, Moody JO, Dixon-Umo OT, Salami OL: Liver aminotransferases in under-five HIV-positive children on HAART. *Afr J Med Med Sci* 2015, 44:197-204.
124. Bhimma R, Purswani MU, Kala U: Kidney disease in children and adolescents with perinatal HIV-1 infection. *J Int AIDS Soc* 2013, 16:18596.
125. Kim RJ, Rutstein RM: Impact of antiretroviral therapy on growth, body composition and metabolism in pediatric HIV patients. *Paediatr Drugs* 2010, 12:187-199.
126. Musoke PM, Fergusson P: Severe malnutrition and metabolic complications of HIV-infected children in the antiretroviral era: clinical care and management in resource-limited settings. *Am J Clin Nutr* 2011, 94:1716s-1720s.
127. Schulte-Hermann K, Schalk H, Haider B, Hutterer J, Gmeinhardt B, Pichler K, Brath H, Dorner TE: Impaired lipid profile and insulin resistance in a cohort of Austrian HIV patients. *J Infect Chemother* 2016, 22:248-253.
128. Mandal A, Mukherjee A, Lakshmy R, Kabra SK, Lodha R: Dyslipidemia in HIV Infected Children Receiving Highly Active Antiretroviral Therapy. *Indian J Pediatr* 2016, 83:226-231.

129. Dirajlal-Fargo S, Musiime V, Cook A, Mirembe G, Kenny J, Jiang Y, Debanne S, Klein N, McComsey GA: Insulin Resistance and Markers of Inflammation in HIV-Infected Ugandan Children in the CHAPAS 3 Trial.*Pediatr Infect Dis J* 2017.
130. Innes S, Abdullah KL, Haubrich R, Cotton MF, Browne SH: High Prevalence of Dyslipidemia and Insulin Resistance in HIV-infected Prepubertal African Children on Antiretroviral Therapy.*Pediatr Infect Dis J* 2016, 35:e1-7.
131. Tadesse BT, Foster BA, Jerene D, Ruff A: Cohort profile: improving treatment of HIV-infected Ethiopian children through better detection of treatment failure in southern Ethiopia.*BMJ Open* 2017, 7:e013528.
132. Ministry of Health Federal Democratic Republic of Ethiopia. National Guidelines for Comprehensive HIV Prevention, Care and Treatment; Ethiopian Ministry of Health: Addis Ababa, Ethiopia, 2014.
133. Antiretroviral therapy for HIV infection in infants and children: towards universal access. Recommendations for a public health approach. 2010 revision. Geneva, World Health Organization, 2010.http://whqlibdoc.who.int/publications/2010/9789241599801_eng.pdf.
134. Antiretroviral Therapy for HIV Infection in Infants and Children: Towards Universal Access: Recommendations for a Public Health Approach: 2010 Revision. Geneva: World Health Organization; 2010. ANNEX C, WHO CLINICAL STAGING OF HIV FOR INFANTS AND CHILDREN WITH ESTABLISHED HIV INFECTION. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK138574/>.
135. WHO Anthro Survey Analyser and other tools: <https://www.who.int/childgrowth/software/en/>.
136. Amico KR, Fisher WA, Cornman DH, Shuper PA, Redding CG, Konkle-Parker DJ, Barta W, Fisher JD: Visual analog scale of ART adherence: association with 3-day self-report and adherence barriers.*J Acquir Immune Defic Syndr* 2006, 42:455-459.
137. Buckton AJ, Prabhu DP, Cane PA, Pillay D: No evidence for cross-contamination of dried blood spots excised using an office hole-punch for HIV-1 drug resistance genotyping.*J Antimicrob Chemother* 2009, 63:615-616.
138. Woods CK, Brumme CJ, Liu TF, Chui CK, Chu AL, Wynhoven B, Hall TA, Trevino C, Shafer RW, Harrigan PR: Automating HIV drug resistance genotyping with RECall, a freely accessible sequence analysis tool.*J Clin Microbiol* 2012, 50:1936-1942.

139. Gaschen B, Kuiken C, Korber B, Foley B: Retrieval and on-the-fly alignment of sequence fragments from the HIV database.*Bioinformatics* 2001, 17:415-418.
140. Larsson A: AliView: a fast and lightweight alignment viewer and editor for large datasets.*Bioinformatics* 2014, 30:3276-3278.
141. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O: New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0.*Syst Biol* 2010, 59:307-321.
142. Shafer RW, Rhee SY, Pillay D, Miller V, Sandstrom P, Schapiro JM, Kuritzkes DR, Bennett D: HIV-1 protease and reverse transcriptase mutations for drug resistance surveillance.*Aids* 2007, 21:215-223.
143. Fourment M, Gibbs MJ: PATRISTIC: a program for calculating patristic distances and graphically comparing the components of genetic change.*BMC Evol Biol* 2006, 6:1.
144. Siepel AC, Halpern AL, Macken C, Korber BT: A computer program designed to screen rapidly for HIV type 1 intersubtype recombinant sequences.*AIDS Res Hum Retroviruses* 1995, 11:1413-1416.
145. Liu TF, Shafer RW: Web resources for HIV type 1 genotypic-resistance test interpretation.*Clin Infect Dis* 2006, 42:1608-1618.
146. Bennett DE, Camacho RJ, Otelea D, Kuritzkes DR, Fleury H, Kiuchi M, Heneine W, Kantor R, Jordan MR, Schapiro JM, et al: Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update.*PLoS One* 2009, 4:e4724.
147. Harrigan PR, Wynhoven B, Brumme ZL, Brumme CJ, Sattha B, Major JC, de la Rosa R, Montaner JS: HIV-1 drug resistance: degree of underestimation by a cross-sectional versus a longitudinal testing approach.*J Infect Dis* 2005, 191:1325-1330.
148. Steegen K, Luchters S, Demecheleer E, Dauwe K, Mandaliya K, Jaoko W, Plum J, Temmerman M, Verhofstede C: Feasibility of detecting human immunodeficiency virus type 1 drug resistance in DNA extracted from whole blood or dried blood spots.*J Clin Microbiol* 2007, 45:3342-3351.
149. Le T, Chiarella J, Simen BB, Hanczaruk B, Egholm M, Landry ML, Dieckhaus K, Rosen MI, Kozal MJ: Low-abundance HIV drug-resistant viral variants in treatment-experienced persons correlate with historical antiretroviral use.*PLoS One* 2009, 4:e6079.

150. Garcia F, Alvarez M, Fox Z, Garcia-Diaz A, Guillot V, Johnson M, Chueca N, Phillips A, Hernandez-Quero J, Geretti AM: Predicting antiretroviral drug resistance from the latest or the cumulative genotype. *Antivir Ther* 2011, 16:373-382.
151. WHO. Guidelines on Standard Operating Procedures for Clinical Chemistry. New Delhi, Regional Office for South-East Asia, WHO, 2000.
152. Gregory GA, Andropoulos DB. Gregory's Pediatric Anesthesia. Oxford, Wiley-Blackwell, 2012: 1300–1315.
153. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG: Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009, 42:377-381.
154. Calcagno A, Motta I, Milia MG, Rostagno R, Simiele M, Libanore V, Fontana S, D'Avolio A, Ghisetti V, Di Perri G, Bonora S: Dried plasma/blood spots for monitoring antiretroviral treatment efficacy and pharmacokinetics: a cross-sectional study in rural Burundi. *Br J Clin Pharmacol* 2015, 79:801-808.
155. Garrido C, Zahonero N, Fernandes D, Serrano D, Silva AR, Ferraria N, Antunes F, Gonzalez-Lahoz J, Soriano V, de Mendoza C: Subtype variability, virological response and drug resistance assessed on dried blood spots collected from HIV patients on antiretroviral therapy in Angola. *J Antimicrob Chemother* 2008, 61:694-698.
156. Rodriguez-Auad JP, Rojas-Montes O, Maldonado-Rodriguez A, Alvarez-Muñoz MT, Muñoz O, Torres-Ibarra R, Vazquez-Rosales G, Lira R: Use of Dried Plasma Spots for HIV-1 Viral Load Determination and Drug Resistance Genotyping in Mexican Patients. *Biomed Res Int* 2015, 2015:240407.
157. Delatorre EO, Bello G: Phylodynamics of HIV-1 subtype C epidemic in east Africa. *PLoS One* 2012, 7:e41904.
158. Hemelaar J, Gouws E, Ghys PD, Osmanov S, Isolation W-UNfH, Characterisation: Global trends in molecular epidemiology of HIV-1 during 2000-2007. *AIDS* 2011, 25:679-689.
159. Kalu AW, Telele NF, Gebreselasie S, Fekade D, Abdurahman S, Marrone G, Sonnerborg A: Monophylogenetic HIV-1C epidemic in Ethiopia is dominated by CCR5-tropic viruses-an analysis of a prospective country-wide cohort. *BMC Infect Dis* 2017, 17:37.
160. Kassu A, Fujino M, Matsuda M, Nishizawa M, Ota F, Sugiura W: Molecular epidemiology of HIV type 1 in treatment-naive patients in north Ethiopia. *AIDS Res Hum Retroviruses* 2007, 23:564-568.

161. Mulu A, Lange T, Liebert UG, Maier M: Clade homogeneity and Pol gene polymorphisms in chronically HIV-1 infected antiretroviral treatment naive patients after the roll out of ART in Ethiopia. *BMC Infect Dis* 2014, 14:158.
162. Tully DC, Wood C: Chronology and evolution of the HIV-1 subtype C epidemic in Ethiopia. *AIDS* 2010, 24:1577-1582.
163. Yang F, Bian C, Zhu L, Zhao G, Huang Z, Huang M: Effect of human serum albumin on drug metabolism: structural evidence of esterase activity of human serum albumin. *J Struct Biol* 2007, 157:348-355.
164. Baba T, Touchi A, Ito K, Yamaguchi Y, Yamazoe Y, Ohno Y, Sugiyama Y: Effects of serum albumin and liver cytosol on CYP2C9- and CYP3A4-mediated drug metabolism. *Drug Metab Pharmacokinet* 2002, 17:522-531.
165. WHO: HIV drug resistance report 2017. <https://apps.who.int/iris/bitstream/handle/10665/255896/9789241512831-engpdf2017>, Accessed on July 7, 2019.
166. Firdu N, Enquesselassie F, Jerene D: HIV-infected adolescents have low adherence to antiretroviral therapy: a cross-sectional study in Addis Ababa, Ethiopia. *Pan Afr Med J* 2017, 27:80.
167. Chimukangara B, Lessells RJ, Rhee SY, Giandhari J, Kharsany ABM, Naidoo K, Lewis L, Cawood C, Khanyile D, Ayalew KA, et al: Trends in Pretreatment HIV-1 Drug Resistance in Antiretroviral Therapy-naive Adults in South Africa, 2000-2016: A Pooled Sequence Analysis. *EClinicalMedicine* 2019, 9:26-34.
168. Ngo-Giang-Huong N, Aghokeng AF: HIV Drug Resistance in Resource-limited Countries: Threat for HIV Elimination. *EClinicalMedicine* 2019, 9:3-4.
169. WHO: Guidelines on the public health response to pretreatment HIV drug resistance. <https://apps.who.int/iris/bitstream/handle/10665/255880/9789241550055-engpdf2017>, Accessed on July 7, 2019.
170. WHO: Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection, Recommendations for a Public Health Approach. 2013.
171. Tadesse BT, Foster BA, Chala A, Chaka TE, Bizuayehu T, Ayalew F, G HM, Tadesse S, Jerene D, Makonnen E, Aklillu E: HIV and cART-Associated Dyslipidemia Among HIV-Infected Children. *J Clin Med* 2019, 8.
172. Chandrasekaran P, Shet A, Srinivasan R, Sanjeeva GN, Subramanyan S, Sunderesan S, Ramesh K, Gopalan B, Suresh E, Poornagangadevi N, et al: Long-term virological outcome in children receiving first-line antiretroviral therapy. *AIDS Res Ther* 2018, 15:23.

173. Amogne W, Aderaye G, Habtewold A, Yimer G, Makonnen E, Worku A, Sonnerborg A, Aklillu E, Lindquist L: Efficacy and Safety of Antiretroviral Therapy Initiated One Week after Tuberculosis Therapy in Patients with CD4 Counts < 200 Cells/ μ L: TB-HAART Study, a Randomized Clinical Trial. *PLoS One* 2015, 10:e0122587.
174. Habtewold A, Makonnen E, Amogne W, Yimer G, Aderaye G, Bertilsson L, Burhenne J, Aklillu E: Is there a need to increase the dose of efavirenz during concomitant rifampicin-based antituberculosis therapy in sub-Saharan Africa? The HIV-TB pharmagene study. *Pharmacogenomics* 2015, 16:1047-1064.
175. Bain LE, Nkoke C, Noubiap JJN: UNAIDS 90-90-90 targets to end the AIDS epidemic by 2020 are not realistic: comment on “Can the UNAIDS 90-90-90 target be achieved? A systematic analysis of national HIV treatment cascades”. *BMJ Glob Health* 2017, 2:e000227.
176. UNAIDS: Eastern southern Africa: Data. <https://www.unaids.org/en/regions-countries/easternandsouthernafrika2017>, Accessed on July 2, 2019.
177. Teasdale CA, Sogaula N, Yuengling KA, Wang C, Mutiti A, Arpadi S, Nxele M, Pepeta L, Mogashoa M, Rivadeneira ED, Abrams EJ: HIV viral suppression and longevity among a cohort of children initiating antiretroviral therapy in Eastern Cape, South Africa. *J Int AIDS Soc* 2018, 21:e25168.
178. Boender TS, Hoenderboom BM, Sigaloff KC, Hamers RL, Wellington M, Shamu T, Siwale M, Labib Maksimos EE, Nankya I, Kityo CM, et al: Pretreatment HIV drug resistance increases regimen switches in sub-Saharan Africa. *Clin Infect Dis* 2015, 61:1749-1758.
179. Kantor R, Smeaton L, Vardhanabhuti S, Hudelson SE, Wallis CL, Tripathy S, Morgado MG, Saravanan S, Balakrishnan P, Reitsma M, et al: Pretreatment HIV Drug Resistance and HIV-1 Subtype C Are Independently Associated With Virologic Failure: Results From the Multinational PEARLS (ACTG A5175) Clinical Trial. *Clin Infect Dis* 2015, 60:1541-1549.
180. Kityo C, Boerma RS, Sigaloff KCE, Kaudha E, Calis JCJ, Musiime V, Balinda S, Nakanjako R, Boender TS, Mugenyi PN, Rinke de Wit TF: Pretreatment HIV drug resistance results in virological failure and accumulation of additional resistance mutations in Ugandan children. *J Antimicrob Chemother* 2017, 72:2587-2595.
181. Su B, Zheng X, Liu Y, Liu L, Xin R, Lu H, Huang C, Bai L, Mammano F, Zhang T, et al: Detection of pretreatment minority HIV-1 reverse transcriptase inhibitor-resistant variants by ultra-deep sequencing has a limited impact on virological outcomes. *J Antimicrob Chemother* 2019.

182. Di Biagio A, Rusconi S, Marzocchetti A, Signori A, Schiavetti I, Bruzzone B, Monno L, Punzi G, Colao MG, Penco G, et al: The role of baseline HIV-1 RNA, drug resistance, and regimen type as determinants of response to first-line antiretroviral therapy.*J Med Virol* 2014, 86:1648-1655.
183. Raffi F, Hanf M, Ferry T, Khatchatourian L, Joly V, Pugliese P, Katlama C, Robineau O, Chirouze C, Jacomet C, et al: Impact of baseline plasma HIV-1 RNA and time to virological suppression on virological rebound according to first-line antiretroviral regimen.*J Antimicrob Chemother* 2017, 72:3425-3434.
184. Paredes R, Mocroft A, Kirk O, Lazzarin A, Barton SE, van Lunzen J, Katzenstein TL, Antunes F, Lundgren JD, Clotet B: Predictors of virological success and ensuing failure in HIV-positive patients starting highly active antiretroviral therapy in Europe: results from the EuroSIDA study.*Arch Intern Med* 2000, 160:1123-1132.
185. Derache A, Iwuji CC, Danaviah S, Giandhari J, Marcelin AG, Calvez V, de Oliveira T, Dabis F, Pillay D, Gupta RK: Predicted antiviral activity of tenofovir versus abacavir in combination with a cytosine analogue and the integrase inhibitor dolutegravir in HIV-1-infected South African patients initiating or failing first-line ART.*J Antimicrob Chemother* 2019, 74:473-479.
186. Cruciani M, Mengoli C, Malena M, Serpelloni G, Parisi SG, Moyle G, Bosco O: Virological efficacy of abacavir: systematic review and meta-analysis.*J Antimicrob Chemother* 2014, 69:3169-3180.
187. Sax PE, Tierney C, Collier AC, Fischl MA, Mollan K, Peeples L, Godfrey C, Jahed NC, Myers L, Katzenstein D, et al: Abacavir-lamivudine versus tenofovir-emtricitabine for initial HIV-1 therapy.*N Engl J Med* 2009, 361:2230-2240.
188. Reuter S, Oette M, Sichtig N, Kaiser R, Balduin M, Jensen B, Haussinger D: Changes in the HIV-1 mutational profile before first-line HAART in the RESINA cohort.*J Med Virol* 2011, 83:187-195.
189. Badri SM, Adeyemi OM, Max BE, Hota BN, Barker DE: Utility of repeat genotypic resistance testing and clinical response in patients with three class resistance and virologic treatment failure.*AIDS Patient Care STDS* 2007, 21:544-550.
190. Contreras GA, Delbianco G, Bell CS, Kleinosky MT, Murphy JR, Heresi GP: Single genotypes underestimate the prevalence of antiretroviral resistance in patients with perinatally acquired HIV.*J Infect* 2012, 64:125-126.
191. Punyacam P, Iemwimangsa N, Chantratita W, Sukasem C, Sungkanuparph S: HIV drug resistance interpreted by cumulative versus last genotypes in HIV-infected patients with multiple treatment failures.*Curr HIV Res* 2012, 10:271-274.

192. Rhee SY, Jordan MR, Raizes E, Chua A, Parkin N, Kantor R, Van Zyl GU, Mukui I, Hosseinipour MC, Frenkel LM, et al: HIV-1 Drug Resistance Mutations: Potential Applications for Point-of-Care Genotypic Resistance Testing.*PLoS One* 2015, 10:e0145772.
193. Wensing AM, Calvez V, Gunthard HF, Johnson VA, Paredes R, Pillay D, Shafer RW, Richman DD: 2017 Update of the Drug Resistance Mutations in HIV-1.*Top Antivir Med* 2017, 24:132-133.
194. Schuurman R, Nijhuis M, van Leeuwen R, Schipper P, de Jong D, Collis P, Danner SA, Mulder J, Loveday C, Christopherson C, et al.: Rapid changes in human immunodeficiency virus type 1 RNA load and appearance of drug-resistant virus populations in persons treated with lamivudine (3TC).*J Infect Dis* 1995, 171:1411-1419.
195. Wainberg MA, Salomon H, Gu Z, Montaner JS, Cooley TP, McCaffrey R, Ruedy J, Hirst HM, Cammack N, Cameron J, et al.: Development of HIV-1 resistance to (-)2'-deoxy-3'-thiacytidine in patients with AIDS or advanced AIDS-related complex.*Aids* 1995, 9:351-357.
196. Wainberg MA, Lewis L, Salomon H, Gu Z, Keller A, Cammack N, Goldsmith J, Church J, Spira B, Wheeler S, Pizzo P: Resistance to (-)-2',3'-dideoxy-3'-thiacytidine (3TC) in HIV-1 isolated from paediatric patients.*Antivir Ther* 1996, 1:98-104.
197. Petrella M, Oliveira M, Moisi D, Detorio M, Brenner BG, Wainberg MA: Differential maintenance of the M184V substitution in the reverse transcriptase of human immunodeficiency virus type 1 by various nucleoside antiretroviral agents in tissue culture.*Antimicrob Agents Chemother* 2004, 48:4189-4194.
198. Shafer RW, Schapiro JM: HIV-1 Drug Resistance Mutations: an Updated Framework for the Second Decade of HAART.*AIDS Rev* 2008, 10:67-84.
199. Meintjes G, Dunn L, Coetsee M, Hislop M, Leisegang R, Regensberg L, Maartens G: Third-line antiretroviral therapy in Africa: effectiveness in a Southern African retrospective cohort study.*AIDS Res Ther* 2015, 12:39.
200. Assefa Y, Gilks CF, Lynen L, Williams O, Hill PS, Tolera T, Malvia A, Van Damme W: Performance of the Antiretroviral Treatment Program in Ethiopia, 2005-2015: strengths and weaknesses toward ending AIDS.*Int J Infect Dis* 2017, 60:70-76.
201. Tang MW, Kanki PJ, Shafer RW: A review of the virological efficacy of the 4 World Health Organization-recommended tenofovir-containing regimens for initial HIV therapy.*Clin Infect Dis* 2012, 54:862-875.

202. Keiser P, Nassar N, White C, Koen G, Moreno S: Comparison of nevirapine- and efavirenz-containing antiretroviral regimens in antiretroviral-naive patients: a cohort study.*HIV Clin Trials* 2002, 3:296-303.
203. Melvin AJ, Warshaw M, Compagnucci A, Saidi Y, Harrison L, Turkova A, Tudor-Williams G: Hepatic, Renal, Hematologic, and Inflammatory Markers in HIV-Infected Children on Long-term Suppressive Antiretroviral Therapy.*J Pediatric Infect Dis Soc* 2017, 6:e109-e115.
204. Opiyo WO, Ng'Wena AG, Ofulla AV: Liver Function Markers And Associated Serum Electrolytes Changes In Hiv Patients Attending Patient Support Centre Of Jaramogi Oginga Odinga Teaching And Referral Hospital, Kisumu County, Kenya.*East Afr Med J* 2013, 90:276-287.
205. Stevens LA, Nolin TD, Richardson MM, Feldman HI, Lewis JB, Rodby R, Townsend R, Okparavero A, Zhang YL, Schmid CH, Levey AS: Comparison of drug dosing recommendations based on measured GFR and kidney function estimating equations.*Am J Kidney Dis* 2009, 54:33-42.
206. Verhelst D, Monge M, Meynard JL, Fouqueray B, Mougenot B, Girard PM, Ronco P, Rossert J: Fanconi syndrome and renal failure induced by tenofovir: a first case report.*Am J Kidney Dis* 2002, 40:1331-1333.
207. Creput C, Gonzalez-Canali G, Hill G, Piketty C, Kazatchkine M, Nochy D: Renal lesions in HIV-1-positive patient treated with tenofovir.*Aids* 2003, 17:935-937.
208. Turret J, Deray G, Isnard-Bagnis C: Tenofovir effect on the kidneys of HIV-infected patients: a double-edged sword?*J Am Soc Nephrol* 2013, 24:1519-1527.
209. Cuzin L, Pugliese P, Allavena C, Rey D, Chirouze C, Bani-Sadr F, Cabie A, Huleux T, Poizot-Martin I, Cotte L, et al: Antiretroviral therapy as a risk factor for chronic kidney disease: Results from traditional regression modeling and causal approach in a large observational study.*PLoS One* 2017, 12:e0187517.
210. Bunupuradah T, Phupitakphol T, Sophonphan J, Prasitsuebsai W, Anugulruengkitt S, Jantarabekjakul W, Sopa B, Ruxrungtham K, Chanakul A, Puthanakit T: Prevalence of Persistent Renal Dysfunction in Perinatally HIV-infected Thai Adolescents.*Pediatr Infect Dis J* 2018, 37:66-70.
211. Chedjou-Nono E, Sap S, Choukem SP, Ngosso Tetanye I, Nebongo D, Koki Ndombo O: Cardiometabolic profile of obese children in a sub-Saharan African setting: a cross-sectional study.*BMC Pediatr* 2017, 17:129.
212. Nampijja D, Kumbakumba E, Bajunirwe F, Kiwanuka J: Dyslipidemia and its Correlates among HIV Infected Children on HAART Attending Mbarara Regional Referral Hospital.*Int Clin Pathol J* 2017, 4.

213. Padmapriyadarsini C, Shet A, Srinivasan R, Ramachandran G, Sanjeeva GN, Devi P, Ramesh K, Bhavani PK, Reddy D, Suresh E, et al: High Prevalence of Lipid Abnormalities and Insulin Resistance Among Antiretroviral Naive HIV-infected Children in India.*Pediatr Infect Dis J* 2018, 37:253-257.
214. Kumar P, Suri D, Vignesh P, Verma Attri S, Kumar Bhalla A, Singh S: Metabolic Abnormalities and Clinical Lipodystrophy in Children With Human Immunodeficiency Virus Infection: A Report From a Tertiary Care Center in India.*Pediatr Infect Dis J* 2017, 36:e313-e316.
215. Tadewos A, Addis Z, Ambachew H, Banerjee S: Prevalence of dyslipidemia among HIV-infected patients using first-line highly active antiretroviral therapy in Southern Ethiopia: a cross-sectional comparative group study. *AIDS Res Ther* 2012, 9:31.
216. Cook S, Kavey RE: Dyslipidemia and pediatric obesity.*Pediatr Clin North Am* 2011, 58:1363-1373, ix.
217. Fernandes VP, de Faria EC, Bellomo-Brandao MA, Nogueira RJ: Malnutrition causing neonatal dyslipidemia.*Nutr Clin Pract* 2011, 26:440-444.
218. Veiga GR, Ferreira HS, Sawaya AL, Calado J, Florencio TM: Dyslipidaemia and undernutrition in children from impoverished areas of Maceio, state of Alagoas, Brazil.*Int J Environ Res Public Health* 2010, 7:4139-4151.
219. Furtado JM, Almeida SM, Mascarenhas P, Ferraz ME, Ferreira JC, Vilanova M, Monteiro MP, Ferraz FP: Anthropometric features as predictors of atherogenic dyslipidemia and cardiovascular risk in a large population of school-aged children.*PLoS One* 2018, 13:e0197922.
220. Juonala M, Magnussen CG, Berenson GS, Venn A, Burns TL, Sabin MA, Srinivasan SR, Daniels SR, Davis PH, Chen W, et al: Childhood adiposity, adult adiposity, and cardiovascular risk factors.*N Engl J Med* 2011, 365:1876-1885.
221. MR BL, Drouin O, Bartlett G, Nguyen Q, Low A, Gavriilidis G, Easterbrook P, Muhe L: Incidence and Prevalence of Opportunistic and Other Infections and the Impact of Antiretroviral Therapy Among HIV-infected Children in Low- and Middle-income Countries: A Systematic Review and Meta-analysis. *Clin Infect Dis* 2016, 62:1586-1594.
222. Zachariah JP, Johnson PK: Pediatric lipid management: an earlier approach. *Endocrinol Metab Clin North Am* 2014, 43:981-992.
223. Sultan S, Dowling M, Kirton A, DeVeber G, Linds A, Elkind MSV: Dyslipidemia in Children With Arterial Ischemic Stroke: Prevalence and Risk Factors.*Pediatr Neurol* 2018, 78:46-54.