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Prevalence of baseline transaminitis in ARV naive HIV infected children and the role of routine LFT monitoring at The University Teaching Hospital, Lusaka Zambia

By

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A Dissertation submitted in partial fulfilment of the requirement for the award of the degree of Masters of Medicine in Paediatrics and Child Health

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Declaration

I hereby declare that this dissertation represents my own work and has not been presented either wholly or in part for a degree at the University of Zambia or any other University

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TABLE OF CONTENTS

CHAPTER THREE

CHAPTER FOUR

CHAPTER FIVE

CHAPTER SIX

CHAPTER SEVEN

References

Appendices

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CERTIFICATE OF APPROVAL

This dissertation of Dr Henry Mapala has been approved as partial fulfilment for the award of the Degree of Masters of Medicine in Paediatrics and Child Health by the University of Zambia

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Abbreviations

3TC	Lamuvidine
ABC	Abacavir
ACTG	AIDS Clinical Trials Group criteria
AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine aminotransferase
ANOVA	A One-Way Analysis of Variance
ART	Antiretroviral Therapy
ARV's	Antiretroviral Drugs
AST	Aspartate aminotransferase
ATT	Antituberculous Treatment
AZT	Zidovudine
DART	Development of Anti-Retroviral Therapy
EFV	Efavirenz
HAART	Highly Active Antiretroviral Therapy
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
LFT's	Liver Function Tests
NVP	Nevirapine
PCOE	Paediatric Centre of Excellence

RLS   Resource Limited Setting

SD   Standard Deviation

TB   Tuberculosis

ULN   Upper Limit of the Normal

WHO   World Health Organisation

LIST OF TABLES AND FIGURES

ABSTRACT

The advent of antiretroviral therapy (ART) has made an impressive impact in the management of HIV infection. However, ART can be responsible for a wide range of toxicities, from low-grade intolerance that may be self-limiting, to life-threatening side-effects. In assessing drug toxicity, mild elevations of serum transaminases are commonly seen and often improve despite continued administration of the same drug. Studies in adult patients have shown that first-line ART can be delivered safely without routine biochemistry monitoring for toxic effects, and therefore routine hepatic function monitoring during ART is no longer considered as essential. Elevations in serum liver enzyme levels have been described in relation to all the major classes of antiretroviral drugs. However, there are few data on liver toxicity among the paediatric population receiving ART in developing countries.

This was a cross sectional study involving review of patient medical files for 294 children initiated on ART in 2012 and follow-up from initiation to 6-months. The study was concluded at the University Teaching Hospital, Paediatric HIV/AIDS Centre of Excellence in Lusaka, Zambia.

Baseline prevalence of transaminitis (Grade ≥ 2) was 5.5%. At 3 months incidence of transaminitis was 2.9%. At 6 months incidence of transaminitis was 1.4%. Neither age nor CD4 count was significantly correlated with ALT at baseline, 3 months or 6 months. There was significant difference in mean ALT at baseline and 6 months, P-value = 0.037. There was no significant difference between mean ALT at baseline and 3 months, P-value = 0.19.

This study showed that the prevalence of baseline transaminitis in HIV infected children is low. Therefore, there will be no benefit in laboratory monitoring as a routine for liver toxic effects in HIV infected children receiving ART. The normalization of initial liver enzyme elevations despite continued therapy suggests that transaminase measurements should not be done frequently because, in most cases, the additional tests are unlikely to influence subsequent patient management. The indiscriminate use of these tests may confer additional costs to already constrained health systems in limited resource settings.

CHAPTER ONE

Introduction:

The roll-out of highly active anti-retroviral therapy (HAART) is proceeding rapidly in low and middle income countries, with the number of patients on HAART rising from about one quarter million at the end of 2002 to over five million at the end of 2009 (WHO 2010). This advent of antiretroviral therapy (ART) has made an impressive impact in the management of HIV infection; suppression of viral replication, reconstitution of immunological competence and reducing the morbidity and mortality associated with HIV/AIDS.

However, antiretroviral agents can be responsible for a wide range of toxicities, from low-grade intolerance that may be self-limiting, to life-threatening side-effects. The risk of specific side effects varies from drug to drug, from drug class to drug class and from patient to patient (WHO 2010).

Adverse hepatic events caused by drugs can be considered to be either predictable (high incidence) or unpredictable (low incidence). Drugs that produce predictable liver injury, such as paracetamol, usually do so within a few days and are generally a result of direct liver toxicity of the parent drug or its metabolites (Pham T-V, 1997). Unpredictable events manifest as overt or symptomatic disease and can occur with intermediate (1–8 weeks) or long (1 year) periods of latency. The majority of adverse drug-induced hepatic events are unpredictable and are either immune-mediated hypersensitivity reactions or are idiosyncratic.

The pathogenesis of drug-induced liver injury usually involves the participation of a toxic drug or metabolite that either elicits an immune response or directly affects the biochemistry of the cell. In either case, the resultant cell death is the event that leads to the clinical manifestation of hepatitis. Metabolism of chemicals takes place largely in the liver, which accounts for the organ's susceptibility to metabolism-dependent, drug-induced injury (Kaplowitz N. 2002).

The risk of developing hepatotoxicity involves a complex interplay between the chemical properties of the drug, environmental factors (e.g., the use of concomitant drugs or alcohol), age, sex, underlying diseases (e.g., HIV or diabetes), and genetic factors (DeLeve L, 2000) .

The most extensively documented risk factors are concomitant drug use and diseases. There is evidence of drug-induced liver disease among patients with HIV, hepatitis B virus, and hepatitis C virus infections.

Genetic factors include genes that control the handling of the drug (metabolism, detoxification, and transport), as well as those that influence cell injury and repair.

Very few drugs currently in clinical use are associated with predictable dose-related liver toxicity (Pham T-V, 1997). . Most instances of drug-induced liver disease are unpredictable, and symptoms occur either with intermediate or long periods of latency before onset. Low-frequency, unpredictable reactions, either immune-mediated hypersensitivity or idiosyncratic, often occur on a background of a higher incidence of mild, asymptomatic, and usually transient liver injury (Kaplowitz N. 2002).

In unpredictable and idiosyncratic cases, monitoring of liver enzyme levels may be helpful in identifying a population with toxic potential, although issues of cost-effectiveness and compliance with therapy render this approach problematic. This is not the case, however, with predictable or immune-mediated reactions that have a short latency period and a rapid onset of symptoms. Management of these events include the cessation of treatment with the drug, where appropriate.

Liver toxicity caused by ART can be inflicted through several mechanisms. Five different categories are proposed for the mechanisms of ART-related liver toxicity by antiretroviral class: hypersensitivity reactions, direct mitochondrial inhibition, disturbances of lipid/sugar metabolism and steatosis, direct cell stress, and immune reconstitution in the presence of viral hepatitis co-infection. These hepatotoxicity events are more often idiosyncratic; they are unpredictable and occur with variable latency and low incidence (Verma 2009).

Despite the limitations of the classification of liver injury, which ultimately is merely descriptive, it may be useful in clinical practice because it describes typical clinical characteristics of hepatotoxicity for specific drugs or ARVs or classes of drugs and might give hints on the mechanism, ultimately helping the management of patients (Russmann 2009).

Explanations for an observed toxicity could include a concurrent infectious process (e.g. common childhood illnesses, including hepatitis A in a child with symptoms of hepatitis, or malaria in a child with severe anaemia), or a reaction to medications other than ARVs (e.g.

isoniazid-induced hepatitis in a child on treatment for tuberculosis or a rash induced by co-trimoxazole) (WHO 2010).

The association of liver enzyme elevation and hepatic toxicity with anti-tuberculous therapy (ATT) when co-administered with ART is not unexpected, since drug-induced hepatitis is a common complication of ATT and may be due to the initial induction of liver enzymes by rifampicin. Studies have reported low rates of hepatotoxicity when ART is administered along with rifampicin (Padmapriyadarsini 2013). It has been argued that liver damage exists in HIV patients independent of ART exposure (Mendes-Correa 2008). HIV per se may influence the ultra-structural architecture of the liver. The changes are associated with the endoplasmic reticulum, representing a cellular response to virus-induced injury.

Liver disease is often reflected by biochemical abnormalities of one of two different hepatic systems of liver function. The most common alterations in liver enzyme levels encountered in clinical practice can be divided into two major subgroups: hepatocellular predominant and cholestatic predominant. The tests that measure the level of serum liver enzymes usually reflect hepatocyte integrity or cholestasis rather than liver function; although they are commonly referred to as liver function tests (Dufour 2000).

Injury to the liver, whether acute or chronic, eventually results in an increase in serum concentrations of aminotransferases, both aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Other than in the liver, AST is also diffusely represented in the heart, skeletal muscle, kidneys, brain and red blood cells. ALT has low concentrations in skeletal muscle and kidney (Dufour DR 2000).

In the liver, ALT is localized solely in the cellular cytoplasm, whereas AST is both cytosolic (20% of total activity) and mitochondrial (80% of total activity) (Giannini 2005). An increase in ALT serum levels is, therefore, more specific for liver damage.

An abnormal level of aminotransferases is usually defined as a value exceeding the upper reference limit, since there is no clinical significance to the occurrence of low levels of biochemical markers for liver injury, except for serum albumin. This elevation of transaminase levels (transaminitis) is a sensitive signal for liver injury. However, it may not be specific or even clinically relevant, because most instances of transaminitis improve despite the continuation of the drug therapy. On the other hand, hyperbilirubinaemia, an elevation in the serum bilirubin levels, is a more specific signal for hepatic injury. However,

it is unclear from available data whether these bilirubin elevations are isolated or are accompanied by transaminitis (Diana 2007).

Assessment of enzyme abnormalities involves careful evaluation of the predominant pattern of enzyme alteration (hepatocellular vs. cholestatic); the magnitude of enzyme alteration in the case of aminotransferases (< 5 times, 5–10 times or > 10 times the upper reference limit, or mild, moderate or marked) respectively; the rate of change (increase or decrease over time); and the nature of the course of alteration (e.g., mild fluctuation vs. progressive increase) (Giannini 2005). Regardless of the severity of the hepatotoxicity, the interplay of the effects of the ART drugs and of associated risk factors, such as underlying diseases and concomitant drugs influences the patient's susceptibility to the hepatotoxic effects of all drugs (DeLeve 2004).

However, studies have shown that less severe cases of ART-related hepatotoxicity are typically reversible, although characterized by abnormal serum ALT and/or AST levels in the presence or absence of clinical symptoms of liver injury (Zimmerman 2000). This reversal of initial liver enzyme elevations despite continued therapy suggests that transaminase measurements should not be done routinely because, in most cases, the tests are unlikely to influence subsequent patient management. Moreover, indiscriminate use of these tests confers additional costs to already constrained health systems in Resource Limited Settings (RLS) (Kalyesubula 2011).

Background:

There are few data on liver toxicity among the paediatric population receiving antiretroviral treatment in developing countries. Most information relating to ART toxicity comes from the settings of developed countries. However, the full spectrum of ART toxicities observed in adults has also been reported in children. It has been observed that some toxicities are less common in children (e.g. Nevirapine-related symptomatic hepatotoxicity is rare in children), while others are more commonly reported in children than adults (e.g. Efevarenz-related rash or Tenofovir-related loss of bone density (McComsey 2004)

In resource-rich countries, patients receiving ART have routine tests (typically every 3 months) to monitor efficacy and toxic effects. This testing, although practised, is not mandated in public health ART roll-out due to resource limitation (Ekpini 2010).

In Zambia, liver function tests are done at initiation of ART, at two weeks, at three months, and then monitored ideally, every six months (Zambia Paediatric ART national guidelines: Towards Universal Access 2007)

In assessing drug toxicity, mild elevations of serum transaminases are commonly seen and often improve despite administration of the same drug. Majority of the studies have used an elevated alanine (ALT) or aspartate transaminase (AST) of 3 times the upper limit of normal range (ULN) with symptoms (abdominal pain, nausea, vomiting, unexplained fatigue or jaundice) attributable to liver injury or 5 times ULN of ALT or AST without symptoms to define hepatotoxicity (Tostmann A, 2008).

Studies done in adults and infants by the Development of Anti-Retroviral Therapy in Africa Trial 1 (DART Trial 1) have shown that first-line ART can be delivered safely without routine biochemistry and haematology monitoring for toxic effects (The DART Trial 1 2010). The DART Trial 1 was designed to investigate whether routine toxicity and efficacy monitoring of HIV-infected adult and infant patients receiving ART had an important long-term effect on clinical outcomes in Africa. It was an open, non-inferiority trial conducted in three centres in Uganda and one in Zimbabwe.

Following the results of the DART trial 1, routine hepatic function monitoring during ART is no longer considered as essential by the World Health Organisation (WHO) in the management of HIV-infected infants and children (WHO 2010). WHO states that routine laboratory monitoring, although desirable, is not mandatory, recognizing that it may not be available in all situations for the management of ART drug toxicity.

Long-term clinical experience with drugs such as isoniazid has helped to shift the primary focus away from laboratory monitoring to increased emphasis on patient education and medical assessment. In resource-poor countries like Zambia, regular laboratory monitoring in some centres is often not feasible owing to cost constraints; therefore, patient education takes on even greater importance (WHO 2010).

CHAPTER TWO

<u>Literature Review:</u>

The expansion of antiretroviral therapy (ART) in Africa has been achieved in settings with poor health infrastructure, and often without access to routine laboratory monitoring for toxic effects or efficacy. Whether treatment programmes should provide laboratory monitoring or focus resources on continuing to expand access to first-line and second-line ART is a crucial debate in the present day economic crisis in resource limited settings countries, like Zambia. (WHO, 2010)

In these resource-poor settings, there is limited capacity for laboratory monitoring of ART toxicity. Establishing this infrastructure—building laboratories, purchasing and maintaining laboratory equipment, training technicians, and establishing quality assurance and quality control programs—requires substantial financial and human resources. Evidence-based guidelines are needed to focus scarce resources on tests that will have an impact on care and require studies that evaluate the clinical benefits and costs of individual tests (Subbaraman R, 2007).

The spectrum of adverse effects associated with ART may vary between developed and developing countries for several reasons. First, economic constraints limit the repertoire of accessible antiretroviral medications, making a handful of drugs responsible for most toxicities in developing countries (Kumarasamy N, 2004). Second, prohibitory laboratory monitoring costs may occasionally delay the diagnosis of specific toxicities, thereby increasing their severity. Third, comorbid conditions that are more prevalent in resource-limited regions, such as anaemia and malnutrition; initial presentation with advanced immunosuppression; use of concomitant anti-tuberculous therapy (ATT); and use of herbal medications may influence the incidence of adverse effects (Mills E, 2005).

 Finally, host genetics may be associated with drug toxicities; this is a relevant issue, because most antiretroviral drugs have been validated in developed countries (primarily in white populations) but are now being widely used in developing countries, where the vast majority of HIV-infected people live (Cutrell A, 2001).

Because of the complex nature of HIV and AIDS, and the complexity associated with the interaction of the drugs with the virus, the causal relationship between therapy and toxicity is often difficult to determine definitively.

As clinical experience with the ARV drugs continues to increase, the understanding of the pathophysiologic and biochemical mechanisms of their clinical toxicities will continue to evolve particularly, the factors which predispose patients to ARV-associated toxicities (Carr A, 2000).

However, toxic reactions during an immune reconstitution inflammatory syndrome, which, itself, is due to rapid rise of CD4+ lymphocytes soon after ART initiation can explain the significant role of this fact (Shelburne SA, 2005).

Liver enzyme elevations are common in HIV-infected patients, especially those on ART. Analysis of the events surrounding liver enzyme elevations is limited, because HIV infected patients present several risk factors for biochemical abnormalities, and a precise etiology is rarely defined clearly. Other than ART-derived hepatotoxicity, some liver diseases are often associated with HIV infection and should also be ruled out (Pol 2004).

The way in which hepatotoxicity is defined is fundamental to the incidence of hepatotoxicity. There are many ways in which hepatotoxicity may be defined, and, even when an apparently similar definition has been used, there may be subtle differences in its application.

The most common approach to the definition of hepatotoxicity is to consider elevations in Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels above a certain threshold, as proposed by the AIDS Clinical Trials Group (ACTG) criteria (ACTG, 1996) for use in its trials. Under this definition, significant hepatotoxicity (grade 2, 3 or 4) is defined as an increase in ALT and/or AST levels (whichever is higher) to above 10 times the Upper limit of normal (ULN).

The AIDS Clinical Trials Group (ACTG) was initially established in 1987 to broaden the scope of the AIDS research effort of the National Institute of Allergy and Infectious Diseases (NIAID). The ACTG established and supports the largest Network of expert clinical and translational investigators and therapeutic clinical trials units in the world, including sites in

resource-limited countries. These investigators and units serve as the major resource for HIV/AIDS research, treatment, care, and training/education in their communities.

The work accomplished by the ACTG has had a profound impact on the well-being of persons infected with HIV-1. Clinical trials and laboratory studies conducted by the ACTG have made major contributions to optimizing antiretroviral therapy (ART), managing drug resistance, preventing and treating co-infections, evaluating acute and long-term toxicities, and demonstrating the importance of pharmacogenomics in predicting drug toxicities (ACTG, 1996). Results of these studies have helped establish the paradigm for the management of HIV disease and form the basis of current treatment guidelines. This progress in the treatment of HIV-1-infected individuals has resulted in dramatic reductions in AIDS mortality in the U.S. and other countries of the developed world.

An increase in serum ALT, formerly known as serum glutamate pyruvate transaminase (SGPT), is more specific for hepatocellular injury than an increase in aspartate aminotransferase (AST or serum glutamic oxaloacetic transaminase [SGOT]).

Serum enzyme concentrations are measured by functional catalytic assays with normal values established from "healthy" populations. The normal range lies within 2 standard deviations of the mean of the distribution, with 2.5% of persons who are otherwise healthy having concentrations above and below the limits of normal on a single measurement (ACTG, 1996).

 Populations used to set standard values in the past probably included individuals with occult liver disease, whose exclusion has led to decreases in the upper limit of normal (ULN) (Prati D, 2002). Interlaboratory variation in assay results can be substantial. Consequently, comparison of multiples of the ULN has become standard (Dufour, 2000).

In an individual, transaminases may vary as much as 45% on a single day, with the highest levels occurring in the afternoon, or 10 to 30% on successive days. ALT and AST elevation may occur after exercise, hemolysis, or muscle injury.

A recent retrospective review of healthy volunteers participating in drug trials who received placebo found that 20% had at least one ALT value greater than the ULN, and 7% had one

value at least two times the ULN (Rosenzweig P, 1999). Serum hepatic transaminase concentration tends to be higher in men and in those with greater body mass index.

Other standardized toxicity assessment scales have been widely used to evaluate products treating specific diseases. However, toxicity grading scales for these laboratory abnormalities should consider the local laboratory reference values when the parameter limit values are defined.

Table 1. STANDARDISED TOXICITY GRADING SCALE TABLE

Serum *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)**
Sodium – Hyponatremia mEq/L	132 – 134	130 – 131	125 – 129	< 125
Sodium – Hypernatremia mEq/L	144 – 145	146 – 147	148 – 150	> 150
Potassium – Hyperkalemia mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – Hypokalemia mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	< 3.1
Glucose – Hypoglycemia mg/dL	65 – 69	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia Fasting – mg/dL Random – mg/dL	100 – 110 110 – 125	111 – 125 126 – 200	>125 >200	Insulin requirements or hyperosmolar coma
Blood Urea Nitrogen BUN mg/dL	23 – 26	27 – 31	> 31	Requires dialysis
Creatinine – mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
Calcium – hypocalcemia mg/dL	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	< 7.0
Calcium – hypercalcemia mg/dL	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	> 12.0
Magnesium – hypomagnesemia mg/dL	1.3 – 1.5	1.1 – 1.2	0.9 – 1.0	< 0.9
Phosphorous – hypophosphatemia mg/dL	2.3 – 2.5	2.0 – 2.2	1.6 – 1.9	< 1.6
CPK – mg/dL	1.25 – 1.5 x ULN***	1.6 – 3.0 x ULN	3.1 –10 x ULN	> 10 x ULN
Albumin – Hypoalbuminemia g/dL	2.8 – 3.1	2.5 – 2.7	< 2.5	--
Total Protein – Hypoproteinemia g/dL	5.5 – 6.0	5.0 – 5.4	< 5.0	--
Alkaline phosphate – increase by factor	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	3.1 – 10 x ULN	> 10 x ULN
Liver Function Tests –ALT, AST increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	> 1.75 x ULN
Bilirubin – when Liver Function Test is normal; increase by factor	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
Cholesterol	201 – 210	211 – 225	> 226	---
Pancreatic enzymes – amylase, lipase	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	> 5.0 x ULN

Table adopted from the DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS VERSION 1.0, DECEMBER, 2004; CLARIFICATION AUGUST 2009

The laboratory values provided in the table served as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges were provided to demonstrate that they were appropriate.

However, antiretroviral therapy has been found to be associated with low level hepatotoxicity at initiation, regardless of drug class or combination. In HIV-infected children, the early occurrence of liver enzyme elevations after the recent introduction of an antiretroviral drug

mainly corresponds with the development of drug-related hepatitis. This has been observed to resolve after discontinuation of the causative drug (Pol 2004).

In a study done in adults in Haiti, drug-induced hepatitis was reported to be uncommon in their population, and therefore, it was not cost-effective to monitor with liver function tests. The incidence of drug-induced hepatitis in this study was similar to rates reported in other resource-poor settings but was lower than those in middle and high-income nations with higher rates of injection drug use and chronic hepatitis B and C (Serena P. 2010).

Of note, patients in their cohort receiving tuberculosis medications were at increased risk for drug-induced hepatitis; therefore, the study concluded that targeted laboratory monitoring of patients receiving concurrent ART and tuberculosis medications may be cost-effective (Duncombe CJ, 2003)

In a study done in Kampala, Uganda, the Incidence of significant hepatotoxicity within three months of first-line ART was low, with a background prevalence of transaminitis of 1.7% and an incidence of 4.2% at 14 weeks (Kiragga 2010). The Kampala study was designed to observe the baseline prevalence and incidence of transaminitis as well as its related risk factors following ART initiation, based on clinical and laboratory findings and to evaluate the role of transaminases in monitoring liver toxicity in Resource limited settings (RLS). The study suggested that routine measurement of transaminases may not be necessary in all patients initiating ART in RLS. However, the study observed that routine measurement may be important in following patients on ART and concurrent TB treatment as well as those with jaundice to avoid missing hepatotoxicity.

The low incidence of significant transaminitis (grade 2-4) in the Kampala study was similar to that in a cohort of patients who were initiated on a nevirapine-based regimen in another centre in Kampala (2.2%) and in a 5-year cohort study in Botswana (1.1%) (Puvimanasinghe, 2008). This low incidence in the Kampala study could have been due to the low prevalence of other risk factors for hepatotoxicity in their study population.

In the Kampala study, the only sign that was statistically significantly related to transaminitis was jaundice. This is supported by studies that have found jaundice to be a good predictor of severity of liver disease (Navarro VJ, 2006).

In contrast, a randomised trial in South Africa found a high incidence of transaminitis (17%) among patients on a nevirapine-based regimen (Maartens 2005). The patients in South Africa were mainly on nevirapine-based regimens, which have been shown to cause early hepatotoxicity with increased frequency at higher baseline CD4 counts (Errea 2009).

The results of the Kampala study are also in line with the evidence from the DART trial; which showed comparable outcomes between clinically and laboratory monitored patients on ART (Sayana 2010). The results of the trial clearly showed that first-line ART could be delivered safely without routine biochemistry and haematology monitoring for toxic effects, but that routine CD4-cell count monitoring has a small but significant benefit in terms of disease progression and mortality. This is probably owing to slightly earlier switching to second-line ART (Peter T, 2010).

The Development of AntiRetroviral Therapy in Africa (DART) trial was therefore designed to investigate whether delivery of ART with or without routine monitoring of CD4-cell counts for efficacy, and haematology and biochemistry for safety, led to similar outcomes in HIV infected patients receiving ART who had already fulfilled clinical and CD4-count criteria to start ART. DART also showed that routine efficacy monitoring with CD4 tests every 12 weeks had no discernible effect in the first year on ART, but resulted in higher rates of switch to second-line therapy from the second year on ART, small but significant decreases in the proportion of person-years spent with low CD4-cell counts, and lower rates of HIV disease progression and death from the third year on ART

Therefore, HIV disease staging and classification are critical tools for tracking and monitoring the disease and for providing clinicians with important information about HIV disease stage and clinical management.

The U.S. Centers for Disease Control and Prevention (CDC) disease staging system assesses the severity of HIV disease by CD4 cell counts and by the presence of specific HIV-related conditions. The definition of AIDS includes all HIV-infected individuals with CD4 counts of <200 cells/μL (or CD4 percentage <14%) as well as those with certain HIV-related conditions and symptoms (CDC, 1999)

Table 2. CDC immunological classification system

Immune category	Age-related CD4 values by percentage (CD4 cells)		
	<12 months	**1 - 5 years**	**6 -12 years**
1. no suppression	≥25 (>1500)	≥25 (≥1000)	≥25 (≥500)
2. moderate suppression	15 - 24 (750-1499)	15 -24 (500-999)	15 - 24 (200-499)
3. severe suppression	<15 (<750)	<15 (<500)	<15 (<200)

Table adopted from WHO, 2007

The WHO system classifies HIV disease on the basis of clinical manifestations that can be recognized and treated by clinicians in diverse settings, including resource-constrained settings, and by clinicians with varying levels of HIV expertise and training (WHO, 2007)

Table 3. WHO immunological classification system

HIV-associated immunodeficiency	Age-related CD4 values by percentage			
	≤11 months (%)	**12-35 months (%)**	**35-59 months(%)**	**≥5 years (cells/mm^3)**
None or not significant	>35	>30	>25	>500
Mild	30 - 35	25 - 30	20 - 25	350 – 499
Advanced	25 - 29	20 -24	15 – 19	200 – 349
Severe	<25	<20	<15	<200 *or* <15%

Table adopted from WHO 2008

Large cohort studies have shown that CD4% is less variable and less age-dependent than absolute CD4 count in early life (Dunn D, 2003); therefore, CD4% is preferred in treatment guidelines for children younger than 5 years of age.

Antiretroviral therapy initiation thresholds for children ages 12 months and older vary among guidelines (WHO, 2008) Risk of HIV disease progression to AIDS is predicted by both CD4% and CD4 count 3 and is highest in younger children.

The 12-month AIDS risk for children having CD4 < 20% at ages 1, 2, 5 and 10 years are 21%, 12%, 4.7% and 2.2% respectively (Dunn D, 2003). This emphasizes the importance of correct criteria for starting ART especially among younger children with the highest risk for AIDS.

In a study done in Thailand showed that Initiation criteria using CD4% is superior to using CD4 count because of the natural decline in CD4 count especially in early life.
Poor to fair correlation between CD4% and CD4 count especially in young children have been reported and raised the need for further investigations to define the CD4 count to initiate ART across age groups (Dunn D, 2008).

Large cohort analysis suggest that children age 5 years or older have similar HIV disease progression rate as young adults, and that similar CD4 count criteria for ART initiation can be used in both groups (Dunn D, 2003).
Some studies have suggested a greater risk of the development of hepatotoxicity among patients with low CD4 cell counts, high viral loads, and older age. Children advanced immunosuppression may have modestly elevated amino-transferases at baseline (Manosuthi W, 2008).
It is observed that the development of hepatotoxicity has a significant association with immunosuppression of the patients as measured by CD4 cell counts. One reason could be that patients with low CD4 cell counts are more prone to acquire opportunistic infections, necessitating consumption of different drugs, leading to subclinical liver damage and thereby increasing susceptibility to hepatotoxicity (Towner WJ 2012).

This observation suggests that clinicians in RLS should routinely look for jaundice as a marker of significant transaminitis in patients on ART. Once jaundice is detected, the measurement of transaminases should be done to assess the severity of hepatotoxicity, as clinically-driven testing. This is supported by studies that have found jaundice to be a good predictor of severity of liver disease (Navarro 2006).

A study done in Yaonde, Cameroun, in adults concluded that ART is associated with low level hepatotoxicity at therapy initiation, regardless of drug class or combination. The study reported a different pattern of drug injury with nevirapine use, with onset of liver enzyme

elevations occurring beyond 16 weeks of therapy, consistent with direct or idiosyncratic host-mediated liver injury (Soriano 2001). The occurrence of hepatotoxicity ranged from 1% to 9.5%, and few patients showed serious liver related outcomes. Also in this study, the biggest percentage of patients who presented with elevated levels of transaminases (76.81% for AST and 53.33% for ALT) were found to present with first degree hepatotoxicity which corresponds to low level liver toxicity, based on the WHO toxicity scale (Sulkowski 2004).

The understanding of the shared routes of HIV infection and blood-borne hepatotropic viruses such as hepatitis B virus (HBV) and hepatitis C virus (HCV) has raised concern of the possibility co-infection of HIV with these viruses, and adverse effects of such co-infection on the manifestations and outcome of these infections (Zhou S, 2010).

Studies have reported that HIV/HBV and HIV/HCV co-infection have a negative impact on liver disease caused by these viruses. Hepatitis C virus infection has been found to accelerate the evolution and progression of liver disease in HIV-infected individuals (Koziel M, 2007)

In a study done in Sao Paulo, Brazil, mild liver toxicity was observed in 19.7% of the HIV-infected children and adolescents on ART. The Sao Paulo study was designed to determine liver enzyme elevation frequencies in HIV-infected children and adolescents receiving ART, and their association with risk factors (Lorenzetti. 2007). The study results suggest that this population tolerated ART well, probably because of an absence of risk factors that would contribute towards the development of severe liver injury in adults such as hepatitis C virus and/or hepatitis B virus co-infection, older age, high alcohol intake and use of illicit drugs (Nunez 2005).

There is growing recognition that children receiving ART face serious adverse effects, resulting in lack of adherence when faced with a lack of food in the household. Undernutrition increases the probability of developing hepatic toxicity to antiretroviral drugs, especially Nevirapine. Adequate food intake facilitates the absorption and effectiveness of drugs, and increased appetite is an intended and desirable effect of drug therapy, one that is needed to promote recuperation and enhanced immune function (Sanne I, Mommeja-Marin, Hinkle J, et al, 2005)

There are three different systems by which a child or a group of children can be compared to the reference population: Z-scores (standard deviation scores), percentiles, and percent of median. For population-based assessment- including surveys and nutritional surveillance- the

14

Z-score is widely recognized as the best system for analysis and presentation of anthropometric data because of its advantages compared to the other methods (Briend A, 1990). At the individual level, however, although there is substantial recognition that Z-score is the most appropriate descriptor of malnutrition, health and nutrition centres (e.g. supplementary feeding programmes in refugee camps) have been in practice reluctant to adopt its use for individual assessment (Grantham-McGregor SM, 1991).

Both HIV/AIDS and undernutrition affect immune function, with lack of essential micronutrients leading to nutritionally acquired immune-dysfunction syndrome (Bachou H, 2006). Compromised immune defences increase susceptibility to infectious diseases and complicate case management (Heikens GT, 2008).
 Under such conditions, case fatality rates in children are prone to increase even under the standard treatment guidelines of the World Health Organization (WHO) (Ndondoki C, 2011).

Initiation of antiretroviral therapy (ART) improves immunity, enables the body to fight opportunistic infections, and reduces energy loss (Amadi B, 2008). In this way, nutritional deficiencies can be ameliorated. ART alone, however, may not be sufficient to revive an already compromised nutritional status. Despite current global efforts to increase ART coverage, HIV-infected children remain nutritionally challenged due to socio-economic, disease, and other specific health-related factors (Fergusson P, 2009).

A study done in Tanzania showed higher rates of underweight, wasting, and stunting among ART-treated HIV-positive children relative to HIV-negative children in Dar es Salaam. HIV-positive serostatus remained an independent risk factor for underweight and wasting in the adjusted analyses (Bruno F Sunguya, 2011). Although the association between HIV status and stunting was not statistically significant in the adjusted analysis, a higher proportion of HIV-positive children were stunted than were those in the HIV-negative control group.
The study concluded that HIV/AIDS is more likely to be associated with an increased burden of child underweight and wasting even under ART in Dar es Salaam, Tanzania. Factors associated with underweight among ART treated.

HIV-positive children included low birth weight, lower feeding frequency, household hunger, and low household socio-economic position. Similarly, wasting was associated with diarrhea, lower feeding frequency, and low household socio-economic position.

Introduction of ART is associated with decreases in mortality and hospital admissions rates among HIV-infected children that are similar to corresponding decreases observed in resource-rich countries.

Daily cotrimoxazole prophylaxis has been shown to substantially reduce non- Pneumocystis jiroveci- related deaths and hospital admissions in children after infancy, and it is a recommended standard of care for all HIV-infected children (WHO, 2006).

Statement of the problem:

Elevations in serum liver enzyme levels have been described in relation to all the major classes of antiretroviral drugs. However, there are few data on liver toxicity among the paediatric population receiving antiretroviral treatment in developing countries. Most of the information relating to therapeutic toxicity comes from the settings of developed countries.

The prevalence of base line transaminitis in pre-ART HIV infected children in Zambia is not known and there is no information on rates of hepatotoxicity among HIV–TB co-infected patients.

Although co-administration of ART with ATT is associated with mild elevations of ALT during the early phase of treatment, little is known about the relative rates of hepatotoxicity with either NVP or EFV in the setting of rifampicin-based TB treatment. Therefore, frequent measurement of transaminases may be more important in following up patients on ART and concurrent TB treatment as well as those with jaundice to avoid missing hepatotoxicity.

Study justification:

Elevation of transaminases occurs commonly in HIV infected children but resolves spontaneously despite continuing administration of the same ART, suggesting a clinically driven laboratory monitoring of HIV antiretroviral therapy.

Routine laboratory monitoring, although desirable, may not be essential and it is recognized that it may not be available in all situations for the management of ART drug toxicity in resource limited countries, like Zambia.

The expansion of ART in Zambia has been achieved in urban and rural settings with poor health infrastructure, and often without access to routine laboratory monitoring for toxic effects or efficacy. If routine laboratory tests do not add significant benefit, ART programmes would be open to decentralisation with long-term follow-up in local clinics rather than distant hospitals. Laboratory services could be targeted to assess for ART eligibility and to diagnosis and management of opportunistic infections or clinical toxicity, rather than being done routinely.

If in assessing drug toxicity only mild elevations of serum transaminases are commonly seen, which often improve despite administration of the same drug, the need to provide routine monitoring, particularly for toxicity will be less.

Decisions on the frequency of monitoring may be determined by the patient's individual risk profile and particular drug choice and will be clinically driven. For example, a patient who is Hepatitis B virus (HBV) seropositive with abnormal aminotransferases at baseline requires closer monitoring than one who is HBV seronegative and has normal liver function tests.

Funding could be focused on drug procurement, strengthening of diagnostic laboratory services, and training and supervision for health-care workers to foster quality clinical monitoring, to support scale-up of ART roll-out to rural Zambia.

The study results may have major implications for ART programmes in Zambia at this time when there is uncertainty about long-term funding and sustainability and when most people still cannot access treatment in far to reach areas.

Research Question:

What is the prevalence of base line transaminitis in Pre-HAART HIV infected children? What is the role for routine liver enzyme testing and monitoring in HIV-infected children?

Hypothesis:

The prevalence of baseline transaminitis in HIV infected children is low and therefore, routine laboratory monitoring for toxic effects in HIV children receiving ART has no benefit

Objectives:

Main Objective:

To evaluate the prevalence of transaminitis and role of routine monitoring of LFT's in ART naïve HIV-infected children.

Specific Objectives:

1. Determine the baseline transaminitis in ART naïve in HIV- infected children.
2. Describe the incidence of transaminitis at three and six months after initiation of first line ART.
3. Describe the factors associated with transaminitis.

CHAPTER THREE

Methodology:

Study design:

Cross sectional retrospective study: This was a secondary data analysis of a cohort of patients on first line ART from initiation to more than six months follow-up by reviewing their medical record charts.

Study area:

Lusaka is the administrative capital of Zambia with a population of over 2.8 million inhabitants of whom more than 55% are children. It is centrally located and it is of cosmopolitan nature. The outpatient clinic at the Paediatric Centre of Excellence (PCOE) of the UTH is located in the heart of the city. The PCOE has a huge population of HIV infected children and has well- structured treatment protocols for the management of HIV. The centre carries out routine laboratory monitoring of liver and kidney function as per Zambian Ministry of Health national guidelines.

Study population:

The study included both male and female children and adolescents (0-16 years) who were followed up at the PCOE, Lusaka, from a period between 1st, January 2012 to 31st, December 2012.

Target population:

ART naïve HIV positive children were targeted. Between 500 and 600 children are enrolled onto the PCOE ART program every year. These children come from peri-urban areas and are routinely followed up at the centre.

Procedure and sample size:

We reviewed all available (294) medical records for children aged between 0 and 16 years who had been on first line ART for more than 6 months, and enrolled between 1st January 2012 and 31st December, 2012. First line ART drugs included any of the first line ART regimens as per Zambian guidelines; Zidovudine (AZT), Lamuvidine (3TC), Abacavir ABC), Nevirapine (NVP), and Efavirenz (EFV).

The review of patients' medical records was done within the confines of data room to maintain maximum confidentiality.

Data were analyzed using the statistical software SPSS version 21. All statistical tests were at 5% significance level. Independent samples T-test, Paired and ANOVA were used to compare mean values between groups accordingly, and the Pearson's chi-squared test was used for comparison of proportions between groups, and Fisher's exact test applied to expected cell counts of less than 5.

There is no uniform and internationally accepted definition of drug hepatotoxicity or drug-induced liver injury. However, aminotransferase elevation reflecting hepatocellular injury is more commonly used as definition of hepatotoxicity. Hepatotoxicity grades were based on ALT levels and defined in accordance with AIDS Clinical Trials Group (ACTG) criteria.

The AIDS Clinical Trials Group criteria grades it according to the following score system: grade 1 (1.25 - 2.5× upper limit of the normal range [ULN]); grade 2 (2.6 - 5×ULN); grade 3 (5.1 - 10×ULN); and grade 4 (>10×ULN) (Rockvill 1996).

Significant hepatotoxicity was defined as an ALT elevation of grade 2-4 in patients who had normal liver enzymes at baseline (using 40 IU of ALT as the upper limit of normal).

Table 4. Grading of Transaminitis according to ACTG criteria (Grade 1 to 1V)

	I	II	III	IV
ALT (ULN)	1.25 – 2.5	2.6 – 5.0	5.1 – 10.0	> 10

Inclusion criteria:

- Documented HIV infection,
- ≤16 years of age
- Children initiated on ART at PCOE between Jan, 2012 and Dec, 2012
- ≥ six months on ART

Exclusion criteria

- Less than six months on ART
- Pre-existing liver disease as evidenced by abnormal ALT levels and jaundice

- Patients on 2nd line ART
- Mortality before six months on ART
- Loss to follow up

CHAPTER FOUR

Results

We abstracted and analyzed patient file data for 294 children aged between 1 year and 16 years enrolled to ART at PCOE between January 2012 and December 2012.

The median age was 4.5 years and mean age was 6 years (SD = 4.2 years). Table 5 shows age distribution summary statistics.

Table 5. Age distribution summary statistics

N	Mean	Median	Minimum	Maximum	Std. Deviation
294	6.0 years	4.5 years	1.0 years	16.0 years	4.20 years

There were more male children 161/294 (54.8%) than female children 133/294 (45.2%). However, this difference in sex proportion was not significantly different (P-value = 0.10). Figure 1 shows a pie chart for the sex distribution frequency.

Figure 1. Sex frequency distribution
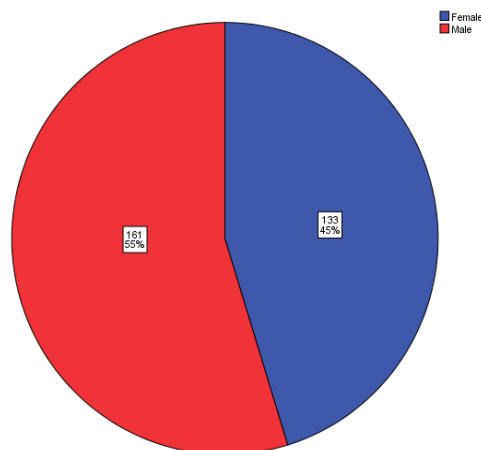
All the 294 had HIV test serology and 290/294 (98.6%) had DNA-PCR results. The minimum age when HIV test was confirmed was 11 weeks and maximum 14 years. The median age when HIV test was confirmed was 2.5 years and mean 4.4 years (SD = 4.16).

Both mean and median CD4 counts increased steadily from baseline through to 6 months. The mean baseline CD4 count was 917, at 3 months CD4 count was 944, and at 6 months mean CD4 count was 1102.

The median CD4 count at baseline, 3 months, and 6 months was 677, 845, and 876, respectively. Table 6 shows the summary descriptive statistics for CD4 counts. The mean CD4 count from baseline to 24 weeks was marginally significant, P-value = 0.06.

Table 6. Summary descriptive statistics for CD4 cell count after ART initiation

|	Baseline CD4 cell/ uL	3 months CD4 cell/ uL	6 months CD4 cells/ uL
Number	268	129	155
Mean	917.8	944.33	1102.48
Median	677.5	845	876
Minimum	3	17	34
Maximum	4346	3756	5270

At baseline there were 77/293 (26.3%) children with HIV clinical stage I, 5/293 (1.7%) with stage II, 60/293 (20.5%) with stage III, and 151/293 (51.5%) with stage IV. At 3 months follow-up there were 72/212 (34%) children with clinical stage I, 9/212 (4.2%) with stage II, 58/212 (27.4%) with stage III, and 73/212 (34.4%) with stage IV. At 6 months follow-up there were 79/174 (45.4%) children with stage I, 3/174 (1.7%) with stage II, 42/174 (24.1%) with stage III, and 50/174 (28.7%) with stage IV.

HIV Clinical stage was significantly associated with review period, P-value <0.001. The proportion of children in clinical stage I increased from 26% at baseline to 34% and 45% at 3 months and 6 months of being on ART respectively. This increase proportional increase was not significant at 3 months P-value = 0.29, but was significant at 6 months, P-value = 0.01.

At baseline, the majority (52%) of the children were in stage IV improving to 34% and 29% at 3 months and 6 months of being on ART respectively

Figure 2 shows clinical stage frequency distribution at each follow-up review period.

Figure 2. HIV clinical stage distribution at each review period after ART initiation
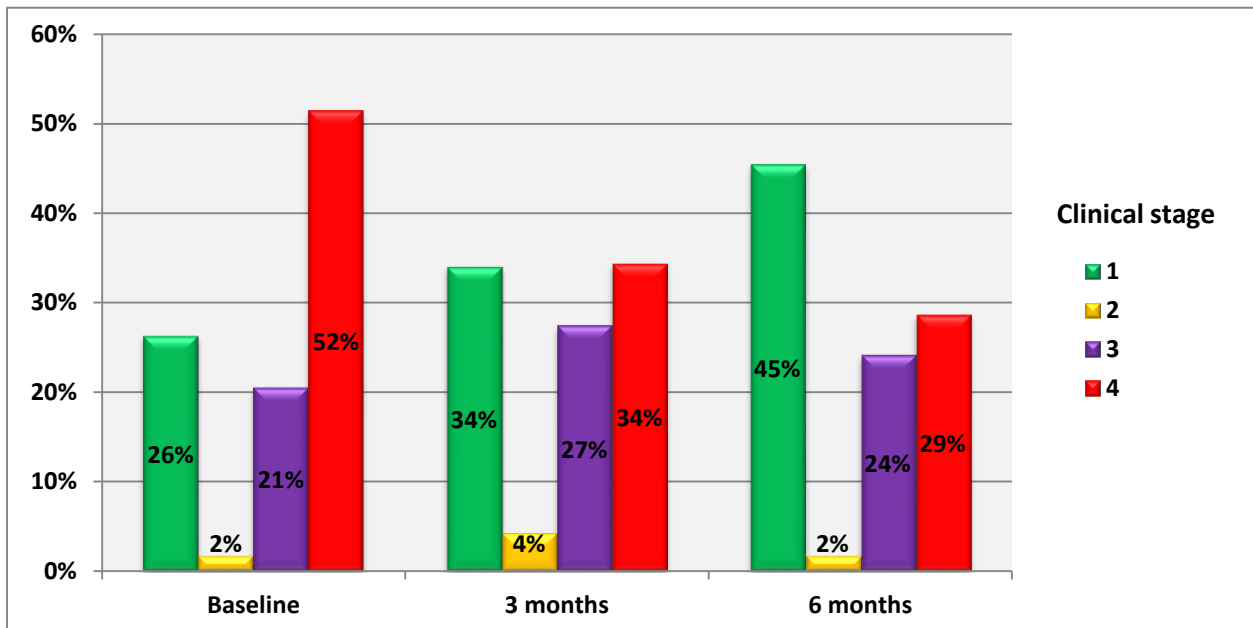
At baseline there were 147/291 (50.5%) children with nutrition z-score -3SD, 46/291 (15.8%) with nutrition z-score -2SD, 56/291 (19.2%) with nutrition z-score -1SD, and 42/291 (14.4%) with median nutrition z-score or better. At 3 months review period there were 72/211 (34.1%) children with nutrition z-score -3SD, 45/211 (21.3%) with nutrition z-score -2SD, 56/211 (26.5%) with nutrition z-score -1SD, and 38/211 (18.0%) with median nutrition z-score or better. At 6 months review period there were 52/174 (29.9%) children with nutrition z-score -3SD, 29/174 (16.7%) with nutrition z-score -2SD, 49/174 (28.2%) with nutrition z-score -1SD, and 44/174 (25.3%) with median nutrition z-score or better.

Figure 3. Nutrition Z-Score distribution at each follow-up review period
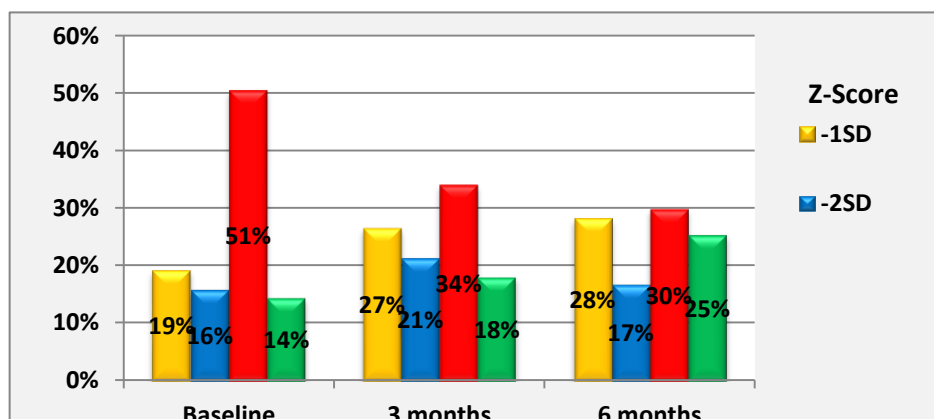
Nutrition z-score was significantly associated with review period (P-value <0.001). The proportion of children with median z-score increased from 14% at baseline to 25% at 6 months.

At 3 months review, the proportion of children with nutrition z-score -3SD decreased from 51% at baseline to 30% at 6 months review.

Figure 3 shows nutrition z-score frequency distribution at each follow-up review period.

Baseline prevalence of transaminitis (Grade $\geq$ 2) in the children was 5.5%. Table 7 shows frequency distribution by grading of transaminitis according to ACTG criteria (Grade 1 to 1V) at baseline. The median AST was 43 and mean AST was 68.97. The median ALT was 23 and mean was 43.01.

Table 7. Baseline AST and ALT Grade

	Normal	I	II	III	IV
AST	121/206 (58.7%)	69/206 (33.5%)	7/206 (3.4%)	5/206 (2.4%)	4/206 (1.9%)
ALT	178/219 (81.3%)	29/219 (13.2%)	5/219 (2.3%)	4/219 (1.8%)	3/219 (1.4%)

At 3 months review incidence of transaminitis in the children was 2.9%. Table 8 shows grading of transaminitis frequency distribution at 3 months review. The median AST was 40 and mean AST was 45.7. The median ALT was 22 and mean was 29.1.

Table 8. AST and ALT Grade at 3 months review

	Normal	I	II	III	IV
AST	77/105 (73.3%)	25/105 (23.8%)	2/105 (1.9%)	1/105 (1.0%)	0/105 (0%)
ALT	95/105 (90.5%)	7/105 (6.7%)	2/105 (1.9%)	1/105 (1.0%)	0/105 (0%)

At 6 months review incidence of transaminitis in the children was 1.4%. Table 9 shows grading of transaminitis frequency distribution at 6 months review. The median AST was 31 and mean AST was 43.33. The median ALT was 19.5 and mean was 25.86.

Table 9. AST and ALT Grade at 6 months review

	Normal	I	II	III	IV
AST	127/139 (91.4%)	10/139 (7.2%)	1/139 (0.7%)	1/139 (0.7%)	0/139 (0%)
ALT	132/139 (95.0%)	5/139 (3.6%)	1/139 (0.7%)	1/139 (0.7%)	0/139 (0%)

Figure 4 and 5 show the frequency distribution of the AST and ALT grades by review period.

A One-Way Analysis of Variance (ANOVA) did not show significant difference in means between review period for AST, P-value = 0.10. However, there was significant difference for means for ALT, P-value = 0.025. Particularly, there was significant difference in means at baseline and 6 months review period, P-value = 0.037. There was no significant difference between means at baseline and 3 months review, P-value = 0.19.

A paired T-test at baseline and 3 months was significant, P-value = 0.031, and at baseline and 6 months P-value = 0.038.

Figure 4. Bar chart of AST grade by review period
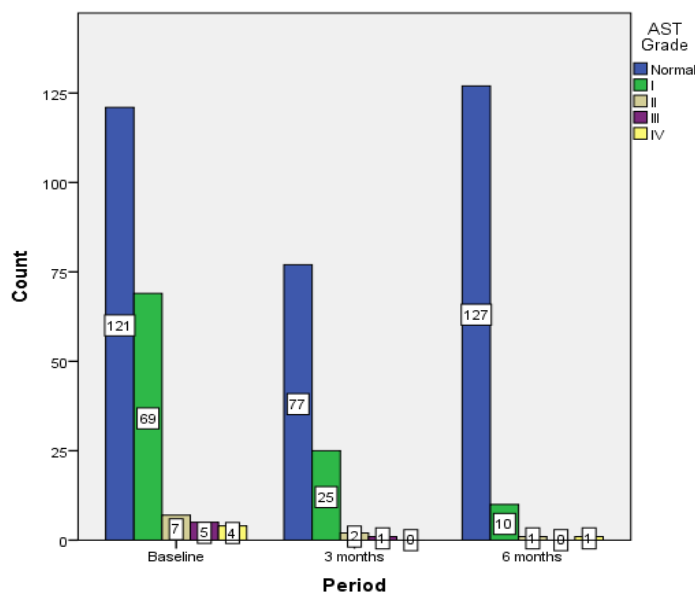
Figure 5. Bar chart of ALT grade by review period

Table 10 shows a cross-tabulation of ALT at baseline and at 3 months. There were no children with normal liver enzymes at baseline that had ALT elevation of grade 2-4 at 3 months.

Table 10. ALT cross-tabulation at baseline and 3 months

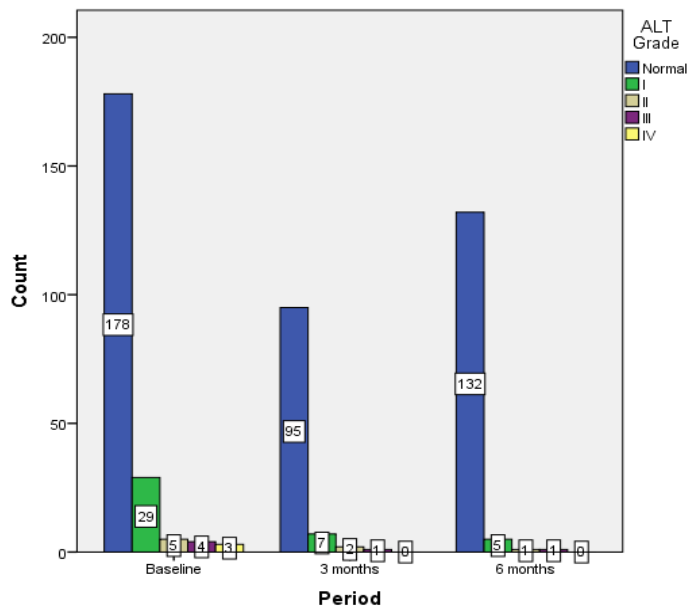|		3 Month ALT				Total (baseline)
		Normal	I	II	III	
Baseline	Normal	56	2	0	0	58
ALT	I	5	2	0	0	7
	II	1	0	0	0	1
	III	1	1	0	0	2
	IV	0	0	1	1	2
Total		63	5	1	1	70

Table 11 shows a cross-tabulation of ALT at baseline and at 6 months. There was only one child with normal liver enzymes at baseline that had ALT elevation of grade 2-4 at 6 months.

Table 11. ALT cross-tabulation at baseline and 6 months

		6 Month ALT			Total (baseline)
		Normal	I	III	
Baseline ALT	Normal	86	3	1	90
	I	6	1	0	7
	II	1	0	0	1
	III	3	0	0	3
	IV	3	0	0	3
Total		99	4	1	104

There were 30/294 (10.2%) children on ART type 3TC, AZT, NVP, 17/294 (5.8%) on 3TC, AZT, EFV, 79/294 (26.9%) on 3TC, ABC, NVP, 42/294 (14.3%) on 3TC, ABC, EFV, and 58/294 (19.7%) on 2nd Line/Other. 68/294 (23.1%) had missing ART type information on patient file. Figure 6 shows ART type frequency distribution for all children with regimen information.

Figure 6: Bar chart for ART type frequency distribution.

28
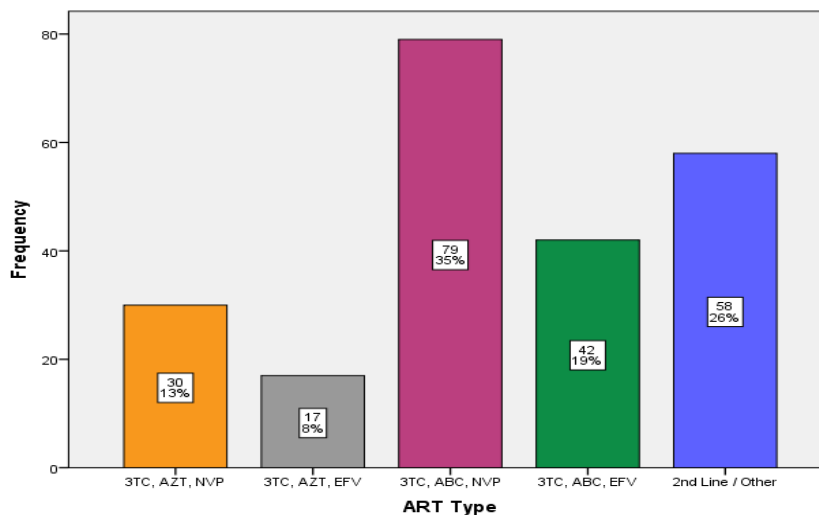
There were 279/294 (94.9%) children on cotrimoxazole and 15/294 (5.1%) not on cotrimoxazole. Cotrimoxazole treatment was not associated with gender, P-value = 0.14, and there was further no significant association between cotrimoxazole treatment and hepatotoxicity at baseline, 3 months, and 6 months review; Fisher's exact P-values = 0.99 apiece.

Figure 7: Pie chart of cotrimoxazole treatment distribution
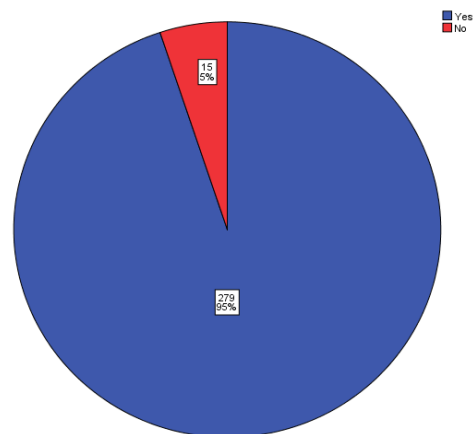
There were 72/294 (24.5%) children on intensive phase ATT whereas 222/294 (75.5%) were not on ATT. This proportional difference was significant with P-value <0.01. There was no significant association between ATT and hepatotoxicity at baseline, 3 months, and 6 months; Fisher's exact P-values = 0.19, 0.12, and 0.46, respectively. None of the children were reportedly taking herbal medicine or illicit drugs.

Figure 8. Pie chart of intensive phase ATT distribution
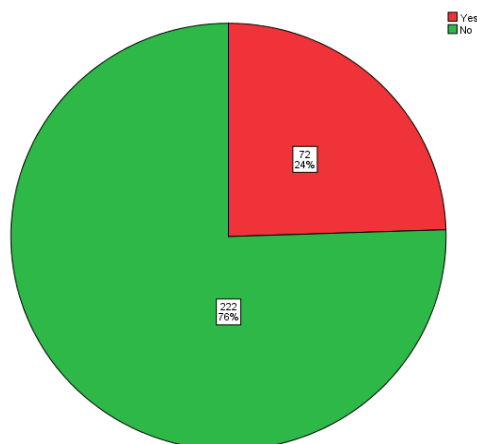
Neither age nor CD4 count were significantly correlated with ALT at baseline, 3 months or 6 months, P-values = 0.16, 0.59, 0.33 for age and 0.64, 0.20, 0.43 for CD4 respective with ALT measurement period.

T-tests for the difference in ALT levels by sex were not statistically different at each review period (Figure 9).

Figure 9: Group statistics for equality of means

	Sex	**N**	**Mean**	**P-value**
Baseline ALT	Female	99	34.72	0.16
	Male	120	49.73	
3 Month ALT	Female	45	28.74	0.92
	Male	59	29.32	
6 Month ALT	Female	63	27.62	0.56
	Male	75	24.3	

Whereas there was significant association between baseline clinical stage and baseline ALT (P < 0.01), there was, however, no significant association between baseline clinical stage and ALT at 3 months (P = 0.14) and ALT at 6 months (P = 0.31).

There was no significant association between baseline nutritional z-score and ALT at baseline (P = 0.18), ALT at 6 months (0.15). However, there was a significant association between baseline nutritional score and ALT at 3 months (P = 0.04).

Table 12. Cross-tabulation of Nutritional Z-score and ALT at 3 months (P=0.04)

			Transaminases at 3 mths		Total
			No	Yes	
Z Score Baseline	-1	Count	26	30	56
		% within Month3ALT1	25.7%	15.8%	19.2%
	-2	Count	12	34	46
		% within Month3ALT1	11.9%	17.9%	15.8%
	-3	Count	44	103	147
		% within Month3ALT1	43.6%	54.2%	50.5%
	M	Count	19	23	42
		% within Month3ALT1	18.8%	12.1%	14.4%
Total		Count	101	190	291
		% within Month3ALT1	100.0%	100.0%	100.0%

CHAPTER FIVE

Discussion

Introduction of potent antiretroviral therapy (ART) in the mid-1990s has been regarded as a major breakthrough in HIV-related care. Antiretroviral drugs (ARV) have succeeded in delaying the onset of illness, decreasing mortality from AIDS, and improving the quality of life for children living with HIV/AIDS (PLHA) (WHO, 2010).

However, antiretroviral agents can be responsible for a wide range of toxicities, from low-grade intolerance that may be self-limiting, to life-threatening side-effects. The majority of these adverse drug-induced hepatic events are unpredictable and are either immune-mediated hypersensitivity reactions or are idiosyncratic. Regardless of the severity of the hepatotoxicity, the interplay of the effects of the ART drugs and of associated risk factors, such as underlying diseases and concomitant drugs influences the patient's susceptibility to the hepatotoxic effects of all drugs (DeLeve 2004)

Because of the complex nature of HIV and AIDS, and the complexity associated with the interaction of the drugs with the virus, the causal relationship between therapy and toxicity is often difficult to determine definitively.

Liver enzyme elevations are common in HIV-infected patients, especially those on ART. However, analysis of the events surrounding liver enzyme elevations is limited, because HIV infected patients present several risk factors for biochemical abnormalities, and a precise aetiology is rarely defined clearly. Other than ART-derived hepatotoxicity, some liver diseases are often associated with HIV infection and should also be ruled out (Pol 2004).

In most cases of drug-related hepatitis, apart from fulminant presentations, liver enzyme abnormalities resolve after discontinuation of the drug and do not relapse, for example, after changing the offending drug (Ana Cecilia, 2007). However, the extent and risk factors for HAART related liver disease and the role of liver enzymes in monitoring this event have not been well studied in RLS.

In this study, patient file data for 294 children aged between 1 year and 16 years enrolled to ART at PCOE between January 2012 and December 2012 were reviewed. There were more male children 161/294 (54.8%) than female children 133/294 (45.2%). The median age was

4.5 years and mean age was 6 years (SD = 4.2 years). All the 294 had HIV test serology and 290/294 (98.6%) had DNA-PCR results. The minimum age when HIV test was confirmed was 11 weeks and maximum 14 years. The median age when HIV test was confirmed was 2.5 years and mean 4.4 years (SD = 4.16).

The study evaluated the role of routine monitoring of liver function tests in HIV-infected children on ART. Based on clinical and laboratory findings, we determined the prevalence of baseline transaminitis in Pre-ART HIV-infected children, as well as its related risk factors following HAART initiation and described the incidence of transaminitis at 3 months and at 6 months after initiation of ART.

Levels of transaminitis or hepatotoxicity grades were based on ALT levels and defined in accordance with AIDS Clinical Trials Group (ACTG) criteria. The ACTG criteria compares well with other toxicity scales as shown above in table 1. Significant hepatotoxicity was defined as an ALT elevation of grade 2-4 in patients who had normal liver enzymes at baseline (using 40 IU of ALT as the upper limit of normal). Few studies have described baseline liver enzyme elevations as a risk factor for severe hepatotoxicity in patients on ART.

In our study, the baseline prevalence of significant transaminitis (grade 2-4) in the HIV-infected children was 5.5%. This does not differ from similar studies that have reported baseline (grade 2-4) transaminitis or rates of hepatotoxicity from various registration trials that ranged from 1% to 9.5% (Kiragga A, 2011).

The incidence of significant (grade 2-4) transaminitis among the patients on ARV's at 3 months was 2.9% and the incidence of significant transaminitis in the HIV-infected children at 6 months review was 1.4%. The low incidence could be due to the low prevalence of other risk factors for hepatotoxicity in our study population, such as low prevalence of HBV co-infection, older age, high alcohol intake and use of illicit drugs as reported in the Sao Paulo, Brazil study (Ana Cecilia, 2007). None of the children in our study were reportedly taking herbal medicine or illicit drugs.

The diagnosis of hepatotoxicity was mainly based on chronological and semiological criteria. Chronological criteria include the recent introduction of a potential hepatotoxic drug (within the preceding 3 months), biochemical improvement after the discontinuation of the drug and relapse of liver abnormalities after rechallenge.

Semiological factors are mainly the exclusion of other causes of liver disease: alcoholism, viral infections (e.g., hepatitis A virus, HBV, HCV, hepatitis D virus, CMV, Epstein-Barr virus, and herpes simplex virus), and/or concomitant medications (Aithal GP, 2000).

The low incidence of transaminitis in our study is similar to that in a cohort study of HIV-infected children who were initiated on a nevirapine-based regimen in Kampala (2.2%) (Kalyesubula R, 2011) and in a 5-year cohort study in Botswana (1.1%)
In the Kampala study the only sign that was statistically significantly related to transaminitis was jaundice. This observation suggests that clinicians in resource limited settings should routinely look for jaundice as a marker of significant transaminitis in patients on ART. Once jaundice is detected, the measurement of transaminases should be done to assess the severity of hepatotoxicity. This is supported by studies that have found jaundice to be a good predictor of severity of liver disease (Navarro VJ, 2006)

Our study also evaluated the association between CD4 count, the clinical stage of the disease and the nutritional status of the patient. The CD4 count increased steadily from the baseline through to 6 months. The mean CD4 count from baseline to 24 weeks was marginally significant (P-value =0.06), while Clinical stage was significantly associated with the review period. At baseline, the majority of the children (52%) were in stage IV improving to 34% and 29% at 3 months and 6 months respectively. These changes were not significant at 3 months P-value = 0.29, but were significant at 6 months, P-value = 0.01.

Studies have suggested a greater risk of the development of hepatotoxicity among patients with low CD4 cell counts, high viral loads, and older age. Children with advanced immunosuppression may have modestly elevated amino-transferases at baseline (Manosuthi W, 2008). It is observed that the development of hepatotoxicity has a significant association with immunosuppression of the patients as measured by CD4 cell counts. One reason could be that patients with low CD4 cell counts are more prone to acquire opportunistic infections, necessitating consumption of different drugs, leading to subclinical liver damage and thereby increasing susceptibility to hepatotoxicity (Towner WJ 2012).

The evidence base of the interaction between HIV and nutrition, which is specifically derived from observations or studies in HIV-infected children, is limited. However, some general points have been extrapolated from research findings related to the nutritional status of HIV-

infected adults and additional points drawn from children who are malnourished but not HIV-infected (WHO, 2009).

In a cross-sectional retrospective study done at Ambo Zonal Hospital in Ethiopia, which was performed to determine the common adverse drug reactions in patients taking ART medications, it was concluded that among the factors that were related to the adverse effects, BMI which reflects the nutritional status of the patient, the presence of other diseases, types of regimen used, duration of therapy and CD4+ lymphocyte less than 400cell/mm3; are strongly associated with the occurrence of adverse drug effects (Stewart R, 2004).

This is in line with the of evidence that suggest differences between children and adults in the level of CD4 and T-cell repopulation, its speed and kinetics, and the relative proportions of naive and memory T cells that return to circulation. Children are known to have a more active thymus than adults and the rates at which cells enter and leave the naive T-cell pool are faster during development than in adulthood. Consistent with this, younger age has been associated with greater increases in CD4 count following ART (Bains I, 2009).
T-cell recovery in adults is biphasic, with a fast initial return of memory cells to circulation followed by a slower repopulation of the naive pool. This biphasic character is less pronounced in children, (van Rossum AMC, 2001) and their T-cell reconstitution is mainly through growth of the naive subpopulation a contrast which may be a further result of age-related changes in thymic activity (De Rossi A, 2002).

Undernutrition increases the probability of developing hepatic toxicity to antiretroviral drugs, especially Nevirapine. A lack of access to appropriate food and the direct effect that HIV has on impaired metabolic functions in absorption, storage, and utilization of nutrients can translate into compromised immunity, nutrient deficiencies, and increased vulnerability to infectious diseases.

In our study, nutritional z-score was significantly associated with review period with the median z-score significantly improving at each review stage (P-value=<0.001). At baseline there were 51% children with -3SD z-score, 16% with -2SD, 19% with -1SD and 14% at median z-score. This significantly improved at 3 months to 34% z-score at -3SD, 21% at -2SD, 27% at -1SD and 18% at median z-score. A steady improvement was also note at 6 months review period.

Z-Score is a statistical measure that reflects the relative deviance form the median value/standard. It is a pure number and is measured as SD (Standard Deviation) in statistical terms. Z-Scores are used in a variety of fields and not just in measuring the nutritional status of children. The association between HIV infection and low weight-for-age or growth faltering in HIV-infected children has been reported in both resource-rich and resource-poor settings.

The ability of HIV to cause profound anorexia and wasting further complicates the situation, especially where resources are not available to thoroughly investigate children to determine whether the primary cause of wasting is HIV infection or food insufficiency or other infections (WHO, 2003).

During and following periods of severe malnutrition, energy requirements may increase by 50% to 100% in order to recover weight. Evidence from children who are severely malnourished and who are not HIV infected indicates that energy intake needs to increase by 50 – 100% in order for children to recover lean body mass and achieve normal weight-for-age (WHO, 2003)

Our study showed that HIV-infected infants and children initially undergo a complete nutritional assessment and thereafter be weighed and have height measured and recorded at each scheduled visit and more often if weight gain is inadequate. Weight and height gain are evaluated with reference to the WHO or national reference growth curves. If growth faltering is identified, then further assessment is made to determine the cause, and to plan appropriate clinical responses with appropriate nutritional counselling and referral as needed.

At clinic visits, the nutritional needs of children who are initiated on ART are assessed and a nutritional care plan agreed upon with the mother or caregiver. The assessment considers the child's growth pattern, appetite, presence of OIs and any clinical signs of malnutrition.

The study demonstrated that the majority of children (95%) were on cotrimoxazole prophylaxis. However, there was no significant association between cotrimoxazole treatment and hepatotoxicity at baseline, 3 months, and 6 months review periods; Fisher's exact P-values = 0.99 apiece.

However, a study done in Lusaka, Zambia concluded that there are clear benefits with the once-daily cotrimoxazole prophylaxis as reflected by the reduction in mortality and hospital admissions (by ~6-fold and ~3-fold, respectively) following ART availability, similar to

findings observed in resource-rich countries (Mulenga V, 2007). Although 24.5% of the children were on intensive phase ATT, the study showed that there was no significant association between ATT and hepatotoxicity at baseline, 3 months, and 6 months; Fisher's exact P-values = 0.19, 0.12, and 0.46, respectively.

Based on the WHO toxicity scale, the patients who presented with elevated levels of transaminases were found to present with first degree hepatotoxicity which corresponds to low level liver toxicity

A recent study by the DART Trial 1 has shown similar findings; the results clearly show that first-line ART can be delivered safely without routine biochemistry monitoring for toxic effects, but that routine CD4-cell count monitoring has a small but significant benefit in terms of disease progression and mortality ((Sayana 2010).

CHAPTER SIX

<u>**Conclusions**</u>

Our study concluded that the prevalence of baseline transaminitis in HIV infected children is low and therefore, routine laboratory monitoring for toxic effects in HIV children receiving ART has no benefit.

The normalization of initial liver enzyme elevations despite continued therapy suggests that transaminase measurements should not be done frequently because, in most cases, the additional tests are unlikely to influence subsequent patient management.

Moreover, indiscriminate use of these tests confers additional costs to already constrained health systems in RLS.

The incidence of significant hepatotoxicity within six months of first line antiretroviral therapy was low, suggesting that frequent measurement of transaminases in the first six months may not be necessary in all patients initiating ART in RLS. However bigger and longer follow up studies are needed to verify this finding.

Although ATT in patients on ART did not show significant changes in liver enzymes, frequent measurement of transaminases may still be important in following patients on ART and concurrent TB treatment as well as those with jaundice to avoid missing hepatotoxicity.

Decisions on the frequency of monitoring may be determined by the patient's individual risk profile and particular drug choice and should be clinically driven.

We therefore, support the recommendation not to monitor transaminase levels and we recommend not delaying to start ART on the basis of raised ALT.

Study limitations

The limited period of follow up, the use of ALT as the only transaminitis marker and the small number of patients recruited in our study may have underestimated the incidence of possible HAART–associated hepatotoxicity.

The study only provided data on possible hepatotoxicity within the first six months of HAART.

In addition, only a few patients had grade 2-4 transaminitis which limited our analysis for risk factors.

CHAPTER SEVEN

<u>Recommendations</u>

The study results clearly show that first-line ART can be delivered safely without routine biochemistry monitoring for toxic effects, but that routine CD4-cell count monitoring has a small but significant benefit in terms of disease progression and mortality, probably owing to slightly earlier switching to second-line ART.

We have shown that routine laboratory monitoring for toxic effects in HIV patients receiving ART has no benefit. ART can be delivered safely with good quality clinical care, allowing treatment delivery to be decentralised.

Small differences in disease progression suggest a role for CD4-cell testing from the second year on ART to guide the switch to second-line ART and should encourage accelerated development of simpler, cheaper, point-of-care CD4 tests.

Although ATT in patients on ART did not show significant changes in liver enzymes, frequent measurement of transaminases may still be important in following patients on HAART and concurrent TB treatment as well as those with jaundice to avoid missing hepatotoxicity.

This observation suggests that clinicians in RLS should routinely look for jaundice as a marker of significant transaminitis in patients on ART. Once jaundice is detected, the measurement of transaminases should be done to assess the severity of hepatotoxicity, as clinically- driven testing.

With less need to provide routine monitoring, particularly for toxicity, funding can be focused on drug procurement, strengthening of diagnostic laboratory services, and training and supervision for health-care workers to foster quality clinical monitoring, to support scale-up of ART roll-out to rural Zambia.

Laboratories will remain important for assessment of eligibility for ART, in terms of CD4-cell count and contraindications for specific drugs, and for diagnosis and management of opportunistic infections and clinical toxicity.

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<u>**Appendices**</u>

Appendix 1

<u>**Data Capture Sheet**</u>

To study the prevalence of baseline Transaminitis in a cohort of children on first line ART at the University Teaching Hospital, Lusaka, Zambia

1. Study number ………………………………………..☐☐☐☐☐
2. Date of file review ………………………………………..☐☐☐☐☐☐☐
3. Participants ID code ………………………………………..☐☐☐☐☐
4. Age ………………………………………..☐☐
5. Sex   M/F ………………………………………..☐
6. Date when tested for HIV ………………………………………..☐☐☐☐☐☐☐
7. HIV test serology   (Y/N) …………………………………☐
8. HIV test DNA-PCR   (Y/N) …………………………....☐
9. Age when HIV test was confirmed …………………………………☐☐☐☐☐☐☐
10. Immune status:
    a. CD4 cell count …………………………………☐☐☐☐
    b. Clinical stage …………………………………☐☐☐
11. Nutritional status (Z-score) …………………………………☐☐
12. Baseline Transaminitis
    a. AST …………………………………..☐☐☐
    b. ALT …………………………………..☐☐☐
13. Transaminitis   at:
    a. 3 months …………………………………☐☐☐
    b. 6 months …….………………………....☐☐☐
14. Date commenced ART ………………………………☐☐☐☐☐☐
15. ART type (√)
    a. 3TC, AZT, NVP …………………………….☐
    b. 3TC, AZT, EFV …………………………….☐
    c. 3TC, ABC, NVP …………………………….☐
    d. 3TC, ABC, EFV …………………………….☐
16. Cotrimoxazole prophylaxis   (Y/N) ………………………….☐
17. On intensive phase ATT (Y/N) ………………………….☐
18. Use of herbal medicine   (Y/N) ………………………….☐
19. Use of illicit drugs   (Y/N) ………………………….☐

Appendix 2

Budget:

Item	Detail	Estimate in ZMK
Stationery and printing	Data collection and entry tools	3000
Data Assistant	Data retraction and entry	4000
Biostatistician		6000
Research and ethics fee		1000
Total		14000