ASSOCIATIONS OF SEVERE HYPERLACTATAEMIA AND LACTIC ACIDOSIS IN HIV-INFECTED PATIENTS RECEIVING ANTIRETROVIRAL THERAPY

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DECLARATION

I, Margaret Osler, hereby declare that the work on this dissertation is based on my original research and has not, in whole or in part, been submitted towards another degree, at this university or elsewhere.

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ABSTRACT

Introduction

Severe symptomatic hyperlactataemia and lactic acidosis (SHLA) are life-threatening events that are occurring at increasing incidence levels in South Africa. Globally, the rise in SHLA cases is closely correlated to the increased accessibility of antiretroviral (ARV) medication for human immuno-deficiency virus (HIV)[1]. Although hyperlactataemia and lactic acidosis were once thought of as rare conditions, they are now being recognized as important concerns when administering antiretroviral therapy. A better understanding of the risk factors for SHLA is important in combating the morbidity and mortality associated with such an adverse event.

Methods

This is a matched case-control study with incidence density sampling. Cases were matched to controls according to ARV start date, duration on ARV therapy and facility. The population consists of people accessing ARV therapy in the public health care sector at G.F. Jooste Hospital and the five referral clinics in the G. F. Jooste drainage area between 1 August 2003 and 30 November 2005. Severe symptomatic hyperlactataemia was defined as having a serum lactate ≥5 mmol/L. Lactic acidosis was defined as having a venous standard bicarbonate <20 mmol/L and a pH < 7.35 in addition to a serum lactate ≥5 mmol/L. Cases diagnosed with any other known causes of severe hyperlactataemia including sepsis, a history of alcoholism, thiamine deficiencies, severe hypoxia, dehydration or hepatitis were not included in the study.

Results

There were 71 cases referred to G.F. Jooste Hospital during the 27 month period. Ninety-five percent of the cases were diagnosed after being on ARV therapy between 6 to 18 months (range, 3-30 months). This particular cohort had a referral rate of 32.1 SHLA cases and 16.8 lactic acidosis cases per 1000 patient years at risk (each patient contributes time between their 6-18 month duration of risk on ARV therapy). Female sex (odds ratio [OR] 23.4; 95% confidence interval [CI] 4.0 – 136.6), weighing between 60 kilograms (kg) and 75 kg (OR 4.5; 95% CI 1.4 – 14.1), and especially ≥ to 75 kg (OR 23.39; 95% CI 4.00 – 136.63) at baseline and gaining ≥ 6 kg in the first three months on therapy (OR 3.5; 95% CI 1.3 – 9.5) are independent risk factors identifying patients who may subsequently develop SHLA. Weight loss of ≥ 3 kg (OR 11.53; 95% CI 3.0 – 44.6), experiencing at least one of the three major symptoms (vomiting, nausea or abdominal pains) of SHLA (OR 10.7; 95% CI 2.7 – 42.0), together with gaining ≥ 10 U/L of ALT since baseline (OR 12.4; 95% CI 1.8 – 85.4), are the clinical parameters that are most able to identify patients about to develop SHLA. Biochemical parameters reflecting acidosis at time of diagnosis are the only factors associated with acute mortality, whilst a raised creatinine of ≥ 77 mmol/L (OR 17.8; 95% CI 2.8 – 113.2) at presentation is additionally associated with lactic acidosis.

Conclusion

It is recommended that overweight women not be started on a d4T containing ARV regimen. Early diagnosis is important in order to minimise morbidity and mortality from SHLA. To facilitate early diagnosis, health care providers should be well versed on the risk factors related to this condition while maintaining a high index of suspicion when a patient has been on ARV therapy for between 6-18 months. The World Health Organization's recommendation to decrease the dosage of d4T is expected to decrease incidence, but health care workers should remain alert as 4 cases in this study were on the decreased d4T dosage throughout their duration on ARV therapy.

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ABBREVIATIONS

3TC	lamivudine
ABC	abacavir
ALT	alanine transaminase
ARV	antiretroviral
ATP	adenosine triphosphate
AZT	zidovudine
BMI	body mass index
CHC	commuity health centre
CI	confidence interval
cm	centimetres
d4T	stavudine
ddI	didanosine
DNA	deoxyribonucleic acid
HAART	highly active antiretroviral therapy
HIV	human immunodeficiency virus
kg	kilograms
LA	lactic acidosis
mg	milligrams
mtDNA	mitochondrial DNA
NADH	nicotinamide adenine dinucleotide
NAFLD	non-alcoholic fatty liver disease
NRTI	reverse transcriptase inhibitors
NVP	nevirapine
OR	odds ratio
PMTCT	Prevention of Mother To Child Transmission
SHLA	severe hyperlactataemia and lactic acidosis
TDF	tenofovir
WHO	World Health Organization

1. Introduction

Severe symptomatic hyperlactataemia and lactic acidosis (SHLA) is a life-threatening event that is occurring with increasing frequency in South Africa. Globally, the rise in SHLA cases is closely correlated to the increased accessibility of antiretroviral medication for human immuno-deficiency virus (HIV).[1] Although hyperlactataemia and lactic acidosis were once thought of as rare conditions, they are now being recognized as important concerns when administering antiretroviral therapy.

In April of 2004, South Africa started a free antiretroviral therapy service in the public healthcare sector. By September of 2007, more than 31,000 people in the Western Cape alone had already been placed on antiretroviral therapy, and the numbers are continuously increasing. A better understanding of the risk factors for SHLA is important in combating the morbidity and mortality associated with such an adverse event.

2. Background

Antiretroviral (ARV) therapy has significantly improved the long term prognosis of patients infected with the human immunodeficiency virus (HIV).[2-4] However, the widespread use of ARV therapy has led to an increase in sometimes fatal adverse events.[2,4] SHLA is one of the uncommon, but more serious and often fatal complications related to the wide-spread use of ARV therapy.[2,4-6]

Currently, not enough is known about the risk factors leading to SHLA to accurately identify high risk patients who may potentially progress to such conditions. The onset of SHLA can be sub-acute for months, with or without symptoms similar to other complications related to HIV infection, or it can be acute and fulminant.[4] The latter cases can lead to multi-organ dysfunction and heart failure within hours.[4,7]

The diagnosis of hyperlactataemia depends on serum lactate measurements. Previous studies suggest that many patients receiving highly active antiretroviral therapy (HAART) may reach new higher, but stable lactate levels during treatment.[2,4,6] One prospective study found that many of the patients with higher blood lactate elevations during therapy remained at the higher stable levels for the three year duration of the

study.[4,6] The majority of patients diagnosed with higher lactate levels remain asymptomatic, without progression to a more severe state of SHLA. The homeostatic mechanisms may be preserved enough in these patients to compensate for mild lactate excess.[2,4] Therefore, the use of raised serum lactate levels to predict subsequent severe hyperlactataemia has been shown to have a poor positive predictor value,[2,4,7] with one study reporting this value to be 39%.[8] Routine surveillance of serum lactate levels for the general population receiving ARV therapy is a costly tool, providing limited benefits with the current knowledge available, obviating the need for routine testing in asymptomatic patients.[4,6,7,8,9]

2.1. Classification

Unstressed patients have a normal lactate concentration of 1-1.5 mmol/L.[10] Hyperlactataemia is clinically defined as a serum lactate concentration between 2.5 and 5 mmol/L, while severe symptomatic hyperlactataemia (SH) is denoted as a serum lactate greater than or equal to 5mml/L with accompanying symptoms. Lactic acidosis (LA) is defined as the combination of an elevated lactate level $\geq$ 5 mmol/L and associated metabolic acidosis (pH < 7.35 and bicarbonate concentration < 20 mmol/L).[7,9]

2.2. Prevalence and Incidence

Nucleoside reverse transcriptase inhibitor (NRTI) associated hyperlactataemia can present both with and without symptoms. Previous studies have indicated a prevalence of increased serum levels to be between 10-35%, while the prevalence of SH makes up 0.8-3% of that figure.[2,9] The prevalence of SH was estimated to be 1.3 cases per 1,000 person years in a retrospective study between 1989 and 1994. However, more recent studies have indicated rates up to 20.9 cases per 1000 person years.[1,3] In South Africa, McCord's ARV clinic and the Khayalitsha programme recently reported incidence rates of 17-19 cases per 1,000 person years on d4T .[11] High rates of stavudine usage in resource poor countries, more accessible supplies of ARV therapy, better informed clinicians and patients, and more evidence associating NRTIs with hyperlactataemia may contribute to the recent rise in the number of detected cases.[1] Severe hyperlactataemia with metabolic acidosis (LA) has a reported mortality rate of 40 to 60%, and can rise to greater than 80% in patients with lactate measures in excess of 10mmol/l. [2,4,9,12]

2.3. Clinical Symptoms and associated conditions

A review of studies has shown that symptoms of hyperlactataemia are non-specific and generally similar to the more severe forms of SH and LA.[2,4] Studies of hyperlactataemia have shown symptoms to include malaise, asthenia, hepatomegaly, weight loss and gastrointestinal complaints such as nausea, vomiting and abdominal pain.[2,4,6] LA includes the above symptoms plus tachyponea, dysponea, cardiac arrhythmia, seizures, abnormal mentation and renal or heart failure.[2,4] Many of the milder symptoms are also commonly associated with HIV infection, which may lead to a delayed diagnosis resulting in increased morbidity and mortality. The more severe symptoms, such as dysponea and tachyponea present themselves too late for predictive tools, when effects may not be reversible.[2]

SHLA has been correlated with NRTI use, hepatic macro and microvesicular steatosis, liver dysfunction and fulminant liver failure.[2,4,6,9] More recently, reports have associated LA with the occurrence of myopathy, cardiomyopathy, neuropathy, pancreatitis, lipoatrophy and pancytopaenia .[2,6] It is still unclear whether hyperlactataemia is a cause of or only highly correlated to these conditions.

2.4. Risk factors and Predictors

The rarity of SHLA suggests that many factors must co-exist in order for such conditions to occur.[4] The functioning and number of mitochondria, the level of damage due to co-morbid infections and their medications, metabolic abnormalities and liver damage are likely factors that affect the outcome of SHLA.[4,6,7]

Regimens

At least 43 in vitro and animal studies have shown that all NRTIs promote hyperlactataemia, however the inhibition of mitochondrial deoxyribonucleic acid (mtDNA) polymerase γ varies according to the affinity of the NRTIs' nucleoside analogues for the enzyme.[1,2,4,13] The NRTIs have been ranked according to their ability of reducing mtDNA content during in vitro studies as follows: "zalcitabine (ddC) >didanosine (ddI) >stavudine (d4T) >lamivudine (3TC) > nevirapine (NVP) > zidovudine (AZT) > abacavir (ABC) = tenofovir (TDF)".[2] Controversy in tenofovir's inhibitory potency exists with Day's study reporting greater affinity of TDF in

comparison with AZT and ABC.[4] Another in vitro study reported that the combined effects of d4T/3TC were more pronounced than those of either NRTI alone indicating possible synergistic mitochondrial toxicity.[14] Most studies agree that the prevalence of SHLA is highly correlated to ddI and d4T use.[1-3,6,9,12,13,15] Marceau and colleagues reported statistically significant ($p<0.05$) odds ratios (OR) for d4T and ddI as 2.5 and 3.1 respectively,[9] while a literature review of 75 cases reported 76% of the patients on d4T.[1] The high percentage of reported cases on d4T in comparison to ddI is most likely due to the greater use of d4T in ARV regimens. Most cases of SHLA manifest between 3 to 20 months duration on NRTI's.[4,16] Symptoms of SHLA are reported to recede when NRTIs are discontinued.[3,7,12]

Patient Profile

Female gender, age and obesity have been inconsistently reported as risk factors for SHLA, although the small number of cases during analysis may be a contributing factor. Most bigger studies and reviews report females at a greater risk,[1,2,4,7,9,15,17] older age was reported as being associated to hyperlactataemia in two studies,[9,18] while obesity or high body mass index was suggested in two reviews to have possible correlations with hyperlactataemia.[15,17] A further study in South Africa by Boulle et al. found a baseline weight between 60 and 75 kilograms (kg) to be independently associated with d4T substitutions due to SHLA in comparison to patients on ARV therapy weighing less than 60 kg.[16] Pregnancy was also reported to be a risk factor for SH in one study.[2]

Rapid weight gain and loss, parental malnutrition and current malnutrition all have been reported to be associated with the development of non-alcoholic fatty liver disease.[19]

Clinical signs symptoms and manifestations

A literature review of 75 cases of LA reported gastrointestinal symptoms (abdominal pain, vomiting, nausea and diarrhoea) as the number one complaint affecting over 77% of the patients.[1] Respiratory symptoms (cough and dyspnoea) and muscular symptoms (asthenia and malaise) have also been widely reported .[1,2,7-9,15]

Liver abnormalities are a common feature in patients with hyperlactataemia. A significant difference in transaminase, bilirubin and bicarbonate levels were found in patients with normolactatemia compared to those with hyperlactataemia.[2,7,9,13] While hepatitis B and C viruses have been poorly studied in patients with hyperlactataemia, some correlations have been found.[4,7] One case-control study in France reported 66% of LA patients to be co-infected with hepatitis.[15] Anti-tuberculosis therapy has also been linked to liver damage,[20] and should be considered as HIV and TB co-infection is prevalent in South Africa.

As the kidneys are lactate consumers, kidney impairment may be a contributor to SHLA. One study found a low nadir CD4 count less than 250 cells/mm3 (OR 8.4; 95% confidence interval [CI] 1.2- ∞) and low creatinine clearance (OR 15.8; 95% CI (3.0 – 86.5) at baseline the only significant factors for LA, suggesting a link between impaired kidney functioning and LA.[15] However two other studies reported no significant association between creatinine levels and hyperlactataemia.[7,9] Additionally, studies have reported concurrent infectious illness at presentation of SH.[7]

Metabolic disorders including lipoatrophy, elevated triglycerides, insulin resistance, and C-peptide levels have been shown to be significantly associated to hyperlactataemia in some studies.[7,9,15] Diabetes has been reported in several cases of LA.[2,9]

Peripheral neuropathy and myopathy are only more recently being looked at in relation to hyperlactataemia patients and possibly result from mitochondrial toxicity. Although studies have reported frequencies of such conditions, the degree of association in patients with SHLA has not been reported.[3,7,8]

2.5. Physiology

The mechanisms describing the relationship of known risk factors with SHLA are not fully understood, but there are quite a few possible pathways described below.

Lactate is normally found at low concentrations in the body and is a product of glycolysis under anaerobic conditions. It is produced in most tissues and cleared mainly by the liver and kidneys. Additional uptake by the skeletal muscles and lungs can take place when increased production occurs .[2,4] Decreased mtDNA, disruption of the metabolic metabolism through oxidative phosphorylation, and liver or kidney disease can all disrupt the homeostatic controls that keep lactate at normal levels within the body.[4]

Mitochondrial toxicity

Recent studies have shown that NRTIs can theoretically cause mitochondrial toxicity.[2,4,5,13] NRTIs prevent DNA elongation and viral reproduction of the HIV virus by their affinity to reverse transcriptase.[3] NRTIs are nucleoside analogues that become nucleotides after intercellular triphosphorylation. They are then incorporated via the viral reverse transcriptase enzyme into the viral DNA chain, halting transcription and replication of HIV.[3]

NRTIs also have the potential to act in the same destructive way in the mitochondria, inducing toxicity by inhibiting DNA γ polymerase.[3,7,9,15] The physiological concentrations of NRTIs minimally inhibit nuclear polymerases, but mitochondrial DNA γ polymerase seems more susceptible to such inhibition.[4,7] This is the only enzyme involved in the replication of mtDNA, causing mitochondrial depletion and structural changes to the mitochondrial genome.[4,7,9,10] Alterations in the mitochondrial genome further cause disruptions in the normal functioning of the respiratory chain, which takes place within the mitochondria.[4]

One of the necessary functions of the mitochondria is to convert pyruvate (an end product of glycolysis) into carbon dioxide and the cellular fuel adenosine triphosphate (ATP).[6, 9,10] During this process, most of the pyruvate is converted into acetylcoenzyme A, which enters the tricarboxylic acid cycle to form nicotinamide adenine dinucleotide (NADH). The mitochondria use the NADH through oxidative phosphorylation to produce ATP.[3,21] When the numbers of mitochondria are decreased or not functioning normally due to impaired oxidation, pyruvate is instead converted by lactate dehydrogenase into lactate, outside of the mitochondria. As the deterioration of the mitochondria continues, decreases in ATP production ensue, and

an accumulation of lactic acid transpires .[3,4,15] SHLA results from an increase in serum lactate levels that other organs such as the liver and kidneys cannot compensate for.[4,10,22]

Although many studies are in agreement with the theory of NRTI toxicity leading to depletion in mtDNA, there has been a case report of a patient with a marked reduction in respiratory chain enzymes but not mtDNA depletion.[5] It has also been reported that HIV infection is a risk factor for mitochondrial death. HIV infected patients, whom have never been on NRTIs, have shown significantly lower ratios of mitochondrial to nuclear DNA. The depletion of mitochondria may make HIV infected people more susceptible to further mitochondrial damage when treated with NRTI therapy.[3]

Hepatic Steatosis

Hepatic Steatosis is a common pathological finding thought to occur in 20-30% of the adult population in the United States.[4,19,23,24] Although global prevalence has not yet been determined, it is thought to occur in 10 – 30% of various populations.[19,24] This condition affects all races and ethnic groups and does not have any age or gender prerequisites. However, prevalence figures have been reported to increase significantly up to 57 - 74% in obese individuals.[19] Hepatic steatosis along with LA presents the most fulminant cases of ARV toxicity.[4] If steatosis presumably occurs in 10 – 30% of the general population of South Africa, a closer look is needed into the synergies between mitochondrial toxicity and hepatic steatosis, as well as the affects steatosis may have, on its own, in lactic acid production.

Steatosis (fatty liver) is often asymptomatic and is characterized by excess fat (> 5%) accumulation in the parenchyma of the liver.[4,19,23] Laboratory results such as increased levels of serum aminotransferases, elevated lipid profiles and glucose concentrations can all give a clinician a high index of suspicion for steatosis,[7,19,25] but only a liver biopsy can confirm such a diagnosis.[19] As effective medical therapy is lacking for steatosis, and the risks high for biopsy, such confirmation is highly debated.[19] Although non-alcoholic fatty liver disease (NAFLD) is thought to be a common component of SHLA, its frequency in HIV infected patients is not known.[2,4] There have been cases of LA without steatosis found during biopsy of the liver

suggesting that LA and hepatic steatosis may occur independently;[2] however it seems likely that ARV therapy plays a role in increasing the likelihood of steatosis.[2-4]

Tumour necrosis factor-alpha (TNF-α), a pro-poptotic cytokine which may act on the mitochondria, has been seen to be elevated in individuals on ARV therapy.[7] Previous studies have also shown that patients treated with NRTIs exhibiting peripheral lipoatrophy have significantly increased numbers of serum triglycerides, insulin, C-peptide levels and hepatic fat content.[2,4,7,14]

There may be multiple pathways in which HIV infection and HAART promote NAFLD. Damage to the liver has been hypothesized as a two level process. The first damage occurs during mitochondrial depletion resulting in an imbalance in lipid homeostasis, which may be aggravated by insulin excess.[4,19] During times of insulin excess, free fatty acids are released through lypolysis in adipose tissue, triggering increased hepatic fatty acid synthesis while oxidation is inhibited.[4,19] Free fatty acids then accumulate, only to be metabolized to triglycerides.[3] Furthermore, export of very low-density lipid proteins from the liver is impaired.[4] This results in accumulation of fat in the liver.[1,4,21] The second level of damage occurs during oxidative stress from mitochondrial toxicity and cytokine mediated hepatocellular damage, causing non-alcoholic steatohepatitis (NASH - a more serious level of NAFLD).[4,19] As LA almost always occurs with hepatic steatosis, the extent of mitochondrial toxicity in the hepatoctyes may be a key factor for progressing into a state of SHLA in NRTI treated patients.[6]

2.6. Previous study designs

The majority of published studies on SHLA describe a case or series of cases within a health facility or larger cohort (cases being described as having raised lactates, SH or LA).[1,8,26-30,31] During a literature search five studies published after the year 2000 were found that compared exposures amongst patients with and without the outcomes of raised lactates, SH and/or LA using various forms of cohort analysis.[9,18,32-34] Only two case-control studies were found, one comparing LA and one comparing raised lactates (>3.5 mmol/l) against controls on ARV therapy.[13,15]

Two of the case series studies were carried out in South Africa and were nested within larger cohorts. Both studies described incidence levels of symptomatic hyperlactataemia and/or LA (Geddes et al reported 19 cases of LA per 1000 patient years of treatment, and Bolhaar et al reported 10.6 cases of LA and 20.2 cases of symptomatic hyperlactataemia (lactate ≥4.5 mmol/L) per 1000 patient years on treatment). These studies further described the clinical and demographic variables of the cases at baseline and at diagnosis, without comparison to controls. Both studies also described symptoms experienced at case presentation.[27,28]

Of the five cohort analyses, four looked at exposures associated with raised serum lactates (two compared exposures of patients with lactates ≥ 2 mmol/L against exposures of patients with lower lactates and two considered lactates ≥2.25 mmol/L as the threshold) [9,18,32,34]. The main objectives of these four studies were to describe the incidence of raised serum lactates in their population, describe risk factors associated with a raised serum lactates and one also assessed the utility of routine lactates for ARV safety monitoring. As it has been shown in these and other studies that raised serum lactate levels are poor predictors of future SHLA,[2,4,7,8,9,18,33] risk factors identified with such an outcome may not be wholly relevant in preventing the morbidity and mortality connected with SHLA.

Only one of the cohort studies looked at exposures associated with a serum lactate > 5 mmol/l. This large study of 1566 participants from the Swiss HIV Cohort reported 49 cases of SH and may have been the only study powerful enough (a large enough sample size) to look at associations between exposures and the rare outcome of SH. Although many baseline characteristics and ARV drugs during follow-up were analysed, only specific ARV drugs were found to be independently associated with SH during multivariate analysis.[33]

Both case-control studies looked at baseline information and last laboratory results before case diagnosis and/or at the end of the study period (for controls in the un-matched case control study). The matched case-control by Datta et al. found d4T to be the only independent variable associated with a lactate ≥ 3.5 mmol/L. Cases in this study were matched according to inclusion criteria (at least two lactate measurements, on ARV therapy for at least 4 months and stable on treatment at the time of the study)

and did not necessarily constitute a true matched case-control in the traditional sense (matching by variable). A small sample size (21 cases and 42 controls), limited variables and a low lactate threshold may have all contributed to having found only one variable associated with SHLA.[13]

The second case-control study presented by Bonnet et al. reported that a low baseline CD4 count and a creatinine clearance <70 mL/min (taken within one month prior to diagnosis) were associated with lactic acidosis during crude analysis (lactate >5 mmol/L and a plasma pH of <7.38). Multivariate analysis was not carried out and may not have been done due to the small sample size (9 cases and 41 controls). This case-control study also described symptoms amongst the cases at the time of diagnosis.[15]

A third case-control study is currently under-way, collecting cases and controls internationally. This case-control study by Arenas-Pinto et al. will have the advantage of many diagnostic tests not available in the South African context (imaging results, biopsies and mitochondrial function tests).[35]

The cohort studies were extremely important in describing the effects of different ARV drugs on serum lactate levels, and were also very important in trying to understand the epidemiology of raised serum lactates in an HIV-infected population on ARV therapy. The case-control studies showed further proof of association between stavudine and SHLA. There are, however, still many gaps left in the knowledge of risk factors contributing towards SHLA.

Further more, none of the studies looking at SHLA considered clinical variables or symptoms during follow-up, with the exception of one study which looked at lactate levels at stated intervals.

3. Aim

The aim of this study was to identify baseline risk factors and possible associations during clinical follow-up for severe symptomatic hyperlactataemia and lactic acidosis in a Southern African public health sector treatment programme.

4. Objectives

1. To describe the study population and differences in characteristics between the cases and matched controls at baseline and during follow-up appointments leading to case diagnosis.
2. To characterise the cases and to look for associations related to the severity of SHLA and mortality within this sub-set of study participants.
3. To describe the number of cases being referred to G.F. Jooste Secondary Referral Clinic from within the provincially demarcated referral area in relation to the patient years of ARV therapy at the referral clinics.
4. To look for associations with SHLA in variables collected at initiation of ARV therapy and the first few months of care before manifestations of signs and symptoms occur in hopes to identify risk factors to be aware of when making decisions on ARV regimen choices.
5. To look for associations between clinical parameters recorded during follow-up consultations and SHLA.

5. Hypotheses

1. Specific patient characteristics may put a person at a greater risk of developing SHLA including baseline weight and gender.
2. Rapid weight gain may further exacerbate lactic build up in patients at risk of SHLA.

6. Methods

6.1. Study Design

This was a matched case-control study with incidence density sampling. As SHLA is rare, a case-control study design proved to be the most efficient way of analysing risk factors. The case-control study is nested within the larger cohort of ARV patients attending public ARV services in the Western Cape Province.

Although matching may not be the first choice when conceiving a study design, logistics made this technique the most feasible. The monitoring programme in the Western Cape, South Africa does not have a central location where details of all patients initiated on ARVs are kept. The information at central level is aggregated, with registers containing patient information kept at facility level. As it was not practical to build a database for purposes of random sampling to incorporate all patients in the Western Cape, or at least patients from the sites cases were referred from, the decision that controls be matched by health care site was made. Duration on ARV therapy also proved a problem, as the literature review and local knowledge showed that the duration on ARV therapy, in particular on d4t and ddI containing regimens was a risk factor for SHLA. As the ARV rollout in the Western Cape was continuously growing, a direct random sample of controls at facility level would have meant the study would sample more controls at the beginning stages of ARV therapy compared to controls at longer durations. In addition, patients were recorded at most facilities using a paper-based register, with names recorded chronologically by ARV start date. For these two reasons, matching by ARV duration and commencement date was the most plausible method. Therefore, the controls were matched to the cases according to ARV commencement month, duration on an ARV regimen and facility. As patients were matched by facility, this had the added benefit that controls may come from relatively the same socio-economic status. Beside the benefits due to practicality, matching also limited possible confounding factors pertaining to different management characteristics between the facilities, drug durations and policies that may have changed over time.

The registers placed at each ARV health facility keep track of all patients receiving care at the facility and is updated on a monthly basis. This allowed for easy

determination of the status of a randomly selected control at the time of the matched cases' month of diagnosis. As incidence density sampling for case-control studies most closely reflects the rate ratio, and sampling in this way was feasible, it was decided to use this type of control sampling method.

6.2. Population

The population consisted of people accessing ARV therapy in the public health care sector with referral routes to the G.F. Jooste Hospital Secondary Referral Clinic. The drainage area for G.F. Jooste hospital includes the infectious disease clinic within the hospital and the five surrounding primary health care facilities with ARV services including Site B and Site C Community Health Centres in Khayalitsha, Gugulethu Community Health Centre, Mitchells Plain Community Health Centre and Crossroads Community Health Centre. All of these facilities are ARV dedicated sites in the Western Cape, South Africa.

Inclusion Criteria:

- Symptomatic cases were referred to the G.F. Jooste Secondary Referral Clinic between 1 August 2003 and 30 November 2005
- Cases needed a confirmed blood lactate level $\geq$ 5 mmol/L
- Patients included in the study needed to be at least 18 years of age

Exclusion Criteria:

- Patients diagnosed with any other known causes of SHLA including sepsis, a history of alcoholism, thiamine deficiencies, severe hypoxia, dehydration or hepatitis.
- Treatment experienced patients whom are defined as interrupting ARV therapy for greater than one month with inaccessible details of their previous ARV treatment history.
- Controls not remaining in care within the ARV services at the date of the matched case's SHLA presentation date.

6.3. Sampling size

All eligible cases diagnosed with SHLA between 1 August 2003 and 30 November 2005 were included in the study.

This study was restricted by the size of the case-series study generated at G.F. Jooste Hospital. At the time the proposal to ethics was written, 50 cases had already been diagnosed. As the determined study period included several more months, we projected that at least 65 patients' folders would be available for review and inclusion to both the case-series and case-control study.

Although the study was restricted by the number of cases that could be included, the following sample size calculations deduced that the sample size would be adequate to answer the hypotheses. Given the small number of cases, two controls were sampled for every case to increase statistical power. With 50 cases and 100 controls, the study would had 0.80% power to detect a 10% difference in the proportion of cases and controls with weights over 80kg, premised on 7.5% of controls being over 80kg (based on existing Khayelitsha cohort data).

6.4. Sampling Strategy

Cases

All cases of SHLA that had a confirmed diagnosis at G. F. Jooste Hospital and met the eligibility criteria were included in the study.

Controls

Two controls for every case were sampled. The controls were systematically selected from the same cohort (the same month commencing ARVs and at the same clinic) as each case. The controls were selected by using the ARV registers held in each of the facilities offering ARV services within the public health sector.

At the facilities where the ARV register was paper-based, each case was located within the register according to the month they started ARV therapy. The first patient listed after each case was counted as number one, with each patient listed thereafter as the next chronological number. The fourth and the tenth person listed after each case, if eligible, became part of the study. If the systematically selected control was not eligible, the next person listed in the register was chosen. All determined cases were skipped in the enumerating process. If, during counting, the end of the cohort of

people was reached, the counting continued at the beginning of the same cohort once again, skipping the cases. Paper-based registers are placed at G.F. Jooste Hospital, Mitchell's Plain Community Health Centre, Crossroads Community Health Center, Robbie Nurrock Community Health Centre.

Médecins Sans Frontières supports an electronic ARV database within the Khayelitsha sub-district, while the Desmond Tutu Foundation supports an electronic database for the Guguletu Community Health Centre. The facilities offering ARV services within the Khayelitsha sub-district include Site B and Nolungile Community Health Centres. Controls were sampled from the electronic register in a similar way to the paper-based registers. Once a case was found within the database, the database was filtered for the same clinic and ARV commencement month. The database was then sorted according to start date and the controls were selected according to the fourth and tenth eligible person listed after the case.

Although the selection of controls was intended to be done without replacement, this was not always the case. Facilities starting up new ARV services often started very small, initiating only a few people in the beginning months. Due to this, four controls had to be re-sampled as all other patients in the same cohort were either a case or ineligible participant.

6.5. Data Collection

Tools

A Microsoft Access™ database was developed to collect all of the information gleaned from the patients' folders.

Variables

All variable data for the study was collected retrospectively, using the ARV registers at the clinics and the selected patients' folders. All information collected from the records of each patient was related to the time period while the patient was receiving care within the ARV services. Data was collected for all cases up to the point of diagnosis, and for the controls up to the last follow-up appointment in the month of the matched case diagnosis date.

The ARV register provided the folder number, name and ARV commencement date of each participant. The patients' folders at the primary health care facility were accessed to collect all further information used for analysis within this study, with the exception of the clinical findings on the date of case diagnosis at the G. F. Jooste Secondary Referral Clinic. Information collected from the patient's folder included demographic information (date of birth and sex), the WHO stage and the WHO stage defining illness at baseline, prior ARV exposure and/or history of tuberculosis or hepatitis, ARV drug information (the type of regimen and any substitutions within the regimen, duration of each ARV drug, time off ARV therapy), and immuno-virologic parameters (baseline and 6 monthly CD4 T-cell counts and HIV viral loads). Baseline and follow-up weights and laboratory results (CD4 counts, viral loads, lactates, pH, standard venous bicarbonate, alanine transaminase (ALT), haemoglobin (Hb), total white cell counts (WCC), absolute neutrophil counts (NCC) and creatinine,) were also collected. Further follow-up assessment variables included any recorded symptoms, concomitant diseases and/or conditions and the medications for treatment of the symptoms and illnesses.

Historically, heights were not measured in adults attending public health care services. As past research reported body mass index (BMI) as possibly being correlated with SHLA, efforts were made to collect this data. After going through each participant's folder to collect data, a request was written on the patient's clinical record to measure the patient's height at the next appointment and report the findings to the authors of the case-series or case-control study. Many of the clinics were visited a second time, with new notes written in the patients' folders requesting height measurements.

Logistics

A clinician from G.F. Jooste ARV referral clinic is completing a case-series on patients with a diagnosis of SHLA. The names of the cases, their primary health care facility and their laboratory results at diagnosis were provided by the author of the case series.

6.6. Data Analysis

Referral Rates

The referral rate was calculated by adding up all of the patient months on ARVs at each of the primary health care facilities in the G.F. Jooste drainage area since the start of ART. The months were then divided by twelve to elicit the total patient years on treatment during the study period at each of the ARV health facilities. The number of cases referred from each site was then multiplied by 1,000, with the result then divided by the number of patient years at each facility, giving a referral rate by each clinic. The same calculation was done on the sum of all cases referred from the ARV services in the G. F. Jooste Hospital drainage area to result in an overall referral rate.

A newly proposed referral rate was also determined, by following the same calculations above, but only counting the months from patients during their 6-18 month duration on ARV therapy.

Inclusion and exclusion of variables

There were many variables that had very small numbers of observations, such as some symptoms, World Health Organization (WHO) stage defining illnesses and occasionally prescribed medications. If the event did not occur within at least 3 participants, it was not analysed.

Defining measurements of non-numerical data

True categorical data (sex, exposure to the Prevention of Mother To Child Transmission [PMTCT] programme and WHO stage) were left in their natural categories during analysis. WHO stage defining illnesses were also naturally grouped as a binary variable, as these illnesses were recorded for eligibility and staging criteria if the patient had ever experienced them during the work-up appointments prior to initiating ARV therapy. All cases had been exposed to d4T for several months, providing no variance in the cases when treating d4T as a binary variable, exposed and unexposed. As duration on ARV therapy was also matched, according to study design, the only measure that would provide variance would be categorizing ARV drugs into a binary variable. A participant that experienced an ARV drug for 100

days or more (the first case presented after 109 days of ARV exposure) during the study period was considered exposed.

Upon looking through the database at individual cases, symptoms which were repeated at adjacent visits prior to case presentation were carefully traced back to determine that a cut-off of 80 days prior to case diagnosis would pick up most if not all symptoms possibly related to SHLA. As different health facilities had different management protocols, including different lengths of time between clinical consultations (from 1 to 4 months), counting the number of complaints per symptom per patient could potentially bias the study (as each clinic contributed a different number of cases). A systematic determination for the duration of a symptom also could not be assessed due to the different lengths of time between scheduled appointments (applying half the time between consultations to a symptom that was not complained about from one appointment to the next would be biased depending on the length of time between appointments). Breaking symptom data into a binary variable, ever having complained of the symptom at an appointment during the 80 days prior to the index date, seemed to be the most judicial way of determining association with SHLA. However, the median time from appearance of each symptom to case diagnosis was further looked into when exploring the data.

The preferable way to categorize the prescribed medications (other than ARV drugs) would have also been according to duration of exposure. Upon closer inspection of the data, many of the prescribed drugs did not have further detail other than the name of the drug and date prescribed. Without stop dates, number of pills, or length of days for the prescription, exposure duration could not be determined. The medications were also grouped into binary categories as well, exposure being determined if the participant ever received the medication within the 80 days prior to the case diagnosis.

Exploratory analysis

The data was analysed using STATA™ (Version 10, College Station, TX, USA). Categorical data was described using frequencies and proportions. The nature of the distribution of the continuous variables was determined using the Shapiro-Wilk test for normality. Normally distributed continuous variables were reported using frequencies and means. Non-normally distributed continuous variables were

described using frequencies and medians. It was expected that some of the variables may be highly correlated with each other. To examine potential multicollinearity, the relationships between variables were examined using correlation coefficients. For normally distributed continuous variables, the Pearson Correlation Coefficient was calculated, while the Spearman Rank Correlation Coefficient was determined for variables with skewed distributions.

Groupings within variables

All symptoms and medications were looked at in univariate analysis to detect any possible new signals. Due to the small numbers of patients experiencing a particular symptom or ingesting a particular prescribed drug, it was decided to categorize within these variables in order to increase statistical power. Symptoms of SHLA were grouped according to literature. The symptoms that were repeatedly described as correlated with SHLA in all articles describing symptoms for this condition formed the group called major symptoms, while symptoms that were sporadically reported in articles formed the minor symptoms category.

Univariate regression models

The variables and their relationship with SHLA were first described univariately in crude form using a conditional logistic regression. All explanatory variables that were found to be associated with SHLA in the univariate analysis were included in the model building steps for the multivariate analysis, with the exception of the occasional medications, height and body mass index.

The occasional medications were found to be highly correlated to the symptoms during exploratory analysis and were therefore not chosen to be in the full models. Measurements for height and body mass index were also dropped from the full models due to the very small numbers of participants having this data.

Multivariate model building procedure

A systematic model building process was used in determining the final full models. First, a priori confounders were entered into the conditional logistic regression model, one at a time. For those confounders that were collinear, the strongest variable was chosen (largest chi^2 ratio). Interactions between the confounders were then looked at.

A newly generated interaction variable was kept in the model if there was enough evidence (the confidence interval did not cross the null) to support the fact that the interaction variable impacted on the model. The next step was to add the variables found during univariate conditional regression analysis to be associated with SHLA, one at a time. The best model (the one with the lowest Akaike's information criterion (AIC) and Bayesian information criterion (BIC) number (if the compared models were close in terms of the AIC and BIC, then the one keeping the most observations)) was chosen each time. Then the process of adding one variable at a time was started again with all of the remaining variables. This systematic process was stopped when the model became unstable due to the small cells, or when the addition of another variable did not seem to make an impact on the model (the AIC or BIC number did not numerically decrease).

Once all variables that impacted the model were built into it, interactions between all variables in the final model were looked at. Interactions between all the variables were tested one at a time within the model to see if any newly generated interactions had enough evidence to support association with SHLA. If there was an association found, the interaction variable was kept in the model. Variables were placed back into the model at this point (if the were removed due to small cells) and interactions variables were generated one at a time with each of the variables left in the model to see if the decision for their exclusion was premature. None of the variables removed contributed to an interaction variable that had an association with SHLA. As there was little impact on other variables with inclusion of the significant interaction variables, and their inclusion highly magnified the odds ratios, the inclusion variables were dropped. Please see appendix I for a close look at the model building process.

Different multivariate models

The above described model building process was used to build 3 multivariate conditional logistic regression models. The first model was built to describe risk factors at baseline and the first few months on ARV therapy. As there are fewer variables involved, the data describing the sex, age and baseline CD4 count could stay in the model without creating too much instability. It was deemed necessary to have a model that would not only describe these important variables that often fell out of the

more complex models, but also to emphasize risk factors that could be managed in a preventative way during the time of initiating patients onto ARV therapy.

The second model, which was the first to include data recorded during follow-up appointments, quickly became very unstable after inclusion of the very strong variable called ALT gain, which described the difference in ALT from baseline to peak values. The numbers of participants having this measurement was small, resulting in the exclusion of over 100 patients whose matched group did not have at least one case and control with this measurement taken. The result of dropping so many patients was an unstable model that could not hold additional variables. This model has been fully reported in the results.

The third model was built for variables explored during follow-up. As described above in the model building process, some of the variables were removed from the model due to small numbers in some cells during regression resulting in a model too complex to compute correctly.

A further two models were built using data from the cases only. As the cases were no longer matched, logistic regression was used instead of a conditional logistic regression. The fourth model attempted to describe variables associated with the severity of this condition, namely an outcome of SH versus an outcome of LA. A fifth and last model was built to describe factors associated with acute death after diagnosis of SH or LA. Both the fourth and fifth models considered variables found upon case diagnosis at G.F. Jooste Secondary Referral clinic (laboratory tests) and variables that were in the above described multivariate models.

After all models were decided upon for their best fit, the numeric variables were broken into categories for easier interpretation when sensible, as long as the model remained stable.

Model Checking

The models were assessed for fit using the diagnostics for a matched one-to-many conditional logistic regression. However, further than fit, there is no definitive way to check the diagnostics of a model in a one-to-many matched case control, so it was

decided to break each pair of controls into two separate groups, and combine each group separately with the data for the cases. This will result in two separate models of one-to-one conditional logistic regressions, allowing for further diagnostics.

7. Ethics:

The protocol was submitted to the Research Ethics Committee of the University of Cape Town Health Sciences Faculty for review. Confidentiality was ensured and practiced at all times.

Although this study was not being funded by the Provincial Department of Health, the author was working with them as the assistant director of monitoring and evaluation of the ARV programme. As per job requirement, and under strict obligations to confidentiality, the author had access to all ARV registers and patients' folders at the public ARV dedicated facilities across the Western Cape. However, information accessed for purposes of this study was with approval from the Ethics Committee, the five named ARV facilities, and under supervision by the sites' clinicians.

All information linked to unique identifiers, such as names and folder numbers was kept solely by the authors of the case series, my supervisor, and the author of this study. Written reports will only contain aggregated numbers.

As this study was a review of records, no invasive techniques or direct contact with the patient was required during collection of the data. Therefore, no foreseeable harm to the individual patient was perceived during data collection. The results of the study may heighten fear of adverse events surrounding ARV therapy, however, emphasis has been placed on the fact that SHLA is a rare condition.

The information elicited from this study should greatly outweigh any harm to the individual or community studied. This study was approved without the requirement of informed consent, as per discussions held with the ethics committee on enhanced routine monitoring.

It is anticipated that results from this study will benefit the studied population, as possible risk factors and clinical features of SHLA in the South African context are more clearly defined.

Reporting of results

The results of the study will be made available in a written report to all clinicians at the ARV dedicated sites, all referral hospitals, the department of health and other interested parties. The study will be forwarded to reputable journals in hope of publication.

8. Results

8.1. Descriptive Statistics

8.1.1. Participants

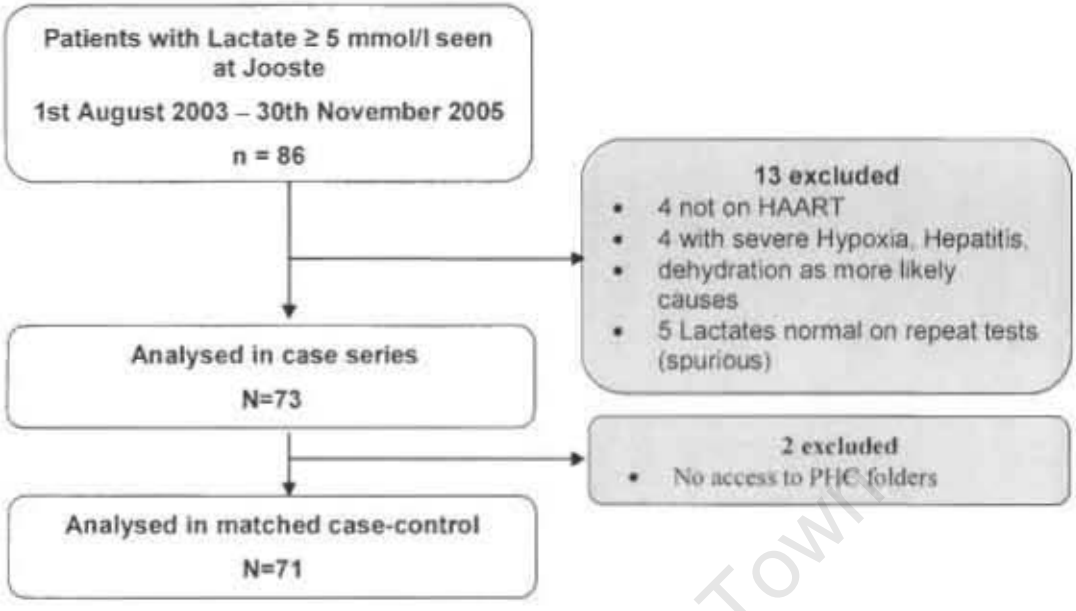
Cases

Eighty-six patients were referred to the G. F. Jooste Secondary Referral Clinic with a lactate ≥ 5 mmol/l between 1 August 2003 and 30 November 2005. Of the 86 patients, 13 were excluded from the study as they had SHLA attributable to causes other than ARV therapy. Of the 13 excluded patients, 4 were not on HAART, 4 had severe hypoxia, hepatitis or dehydration as likely causes and 5 had normal lactates on repeated tests signifying possible spurious results. As access to the primary health care folders of two cases was not obtainable, this study includes 71 cases of SHLA (Figure 1).

Controls

For increased statistical power, two controls were matched to every case by facility and ARV commencement month. Therefore, altogether there are 142 randomly sampled controls consisting of 138 patients of which 4 are sampled twice. As there were few patients commencing ART per clinic when anti-retroviral medication first became publicly available, the number of available patients that fit the inclusion criteria was limited in early cohorts causing 4 repetitions in controls.

Figure 1: Cases included in study



8.1.2. Primary Health Care Clinics

There are 6 primary health care facilities with ARV services in the G. F. Jooste Secondary Referral Clinic area, including Crossroads CHC, Guguletu CHC, G. F. Jooste Hospital, Mitchell's Plain CHC, Nolungile CHC, and Site B CHC. During the study entrance period, 67 eligible cases were referred to the G. F. Jooste Referral Clinic from the above mentioned clinics. G. F. Jooste also accepted 4 SHLA cases from 3 health facilities with ARV services outside of their drainage area during the study entrance period (Table 1). Chapel Street Clinic, which is not in the G.F. Jooste Hospital drainage area, is the primary health care site for the 2 cases not included in the study.

Table 1: Primary Health Care Referral Clinics

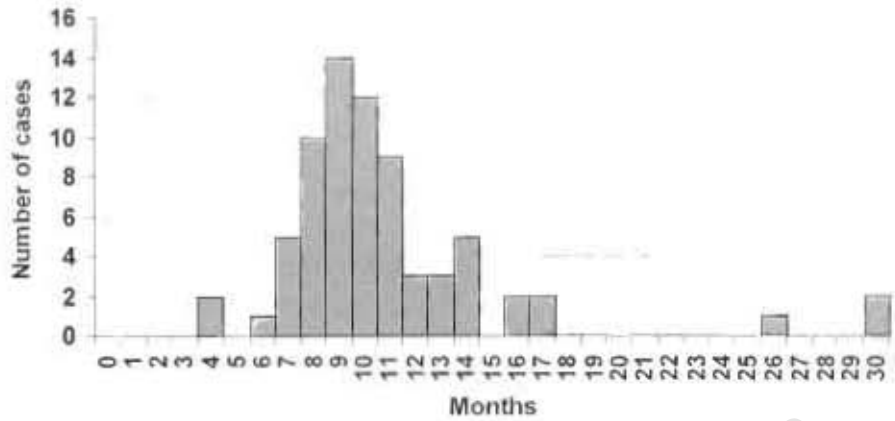
	Clinic	Cases	Controls	Percent
G.F. Jooste Secondary Referral Clinic drainage area	Crossroads CHC	6	12	8.45
	Guguletu CHC	10	20	14.08
	G. F. Jooste Hospital	13	26	18.31
	Mitchell's Plain CHC	13	26	18.31
	Nolungile CHC	10	20	14.08
	Site B, Khayelitsha CHC	15	30	21.13
other referrals	Michael M CHC	1	2	1.41
	Red Cross Children's Hospital	1	2	1.41
	Robbie Nurock CHC	2	4	2.82
	Total	**71**	**142**	**100%**

8.1.3. Duration on ARV therapy prior to case diagnosis

Cases were on ARV therapy for a median duration of 10 months (IQR: 8.5 – 11.8) prior to presenting at the G.F. Jooste Secondary Referral Clinic. All 71 cases presented at some point between 3.5 and 31 months on ARV therapy, with the distribution being slightly skewed to the right. Ninety-five percent of the cases presented between 6.5 months and 17.1 months on ARV therapy.

Some of the outliers presenting after longer durations of ARV therapy are due to the patients being on AZT prior to substitution with d4T. The 2 cases presenting at 30 months both started on AZT, one for 10 months and one for 13 months, prior to AZT being substituted with d4T. The case which presented at 26 months was on d4T 60mg per day for 10 months prior to the dosage being switched to d4T 80mg per day, while the case presenting at 17 months was on d4T 60mg for five months prior to d4T 80mg. The case which presented at 16 months was on AZT for 4 months prior to the NRTI being substituted with d4T 80mg (Figure 2).

Figure 2: Duration on ART prior to case diagnosis (lactate ≥ 5 mmol/L)



8.1.4. Adult case referral rate

There were 73 SHLA case referrals to the G.F. Jooste Secondary Referral Clinic during the study period, with 67 referrals coming from the primary health care centres in their drainage area.

The largest number of referrals (n=15) from a clinic in the referral area came from Site B CHC, in the Khayelitsha sub-district, 13 referrals came from both Mitchell's Plain CHC and G.F. Jooste Infectious Disease Clinic, 10 from the Gugulethu CHC and 6 from Crossroads CHC. However, Crossroads had the highest overall referral rate (36 per 1,000 patient years) and Gugulethu CHC, Nolungile CHC and Site B CHC had the lowest referral rates (9.1, 9.1 and 9.5 cases per 1,000 patient years on treatment, respectively). With the exception of G.F. Jooste Hospital, the referral rates tended to increase in accordance to the start date of the ARV service at each of the health facilities, with more mature services referring more cases per patient load. The overall referral rate, when considering all patients on ARV therapy during the study period, was 13.5 cases of SHLA per 1000 patient years on treatment (Table 2).

Ninety-five percent of the cases were diagnosed between 6-18 months on ARV therapy. The newly proposed referral rate only counts the months contributed by patients during their 6 to 18 month duration on ARV therapy during the study period.

The overall newly calculated referral rate was 32.1 SHLA cases and 16.8 LA cases per 1000 patient years at risk. Although there was still a lot of variation across the clinics, the referral rate for years at risk was similar among the facilities with longer-standing ARV services.

Table 2: Adult case referral rate from ARV services in the G.F. Jooste referral area

Referral clinics	ARV service Start Date	Total patient months	Total patient years	cases	SHLA Referral Rate (per 1,000 patient years)	Newly proposed SHLA referral rate*
Crossroads CHC	Sep. 2004	2,221	166.6	6	36	99.4
Mitchell's Plain CHC	Mar. 2004	7,751	581.3	13	22.4	41.7
G. F. Jooste Hospital	Oct. 2003	5,529	414.7	13	31.3	60.0
Guguletu CHC	Sep. 2002	14,662	1099.7	10	9.1	20.9
Nolungile CHC	May 2001	14,702	1102.7	10	9.1	27.0
Site B, Khayelitsha CHC	May 2001	21,085	1581.4	15	9.5	23.2
Total		**65,950**	**4946.3**	**67**	**13.5**	**32.1**
			Lactic acidosis referral rate =	**35**	**7.1**	**16.8**

**based on all patients with 6-18 months duration on ART during study period*

8.1.5. Baseline Characteristics

Demographic and clinical characteristics

The age of the participants did not vary greatly between the studied groups, with the median age for cases being 36 years old and 34 for controls (OR 0.9; 95% CI 0.8 – 1.1). Ninety four percent of the cases and 96% of the controls had a baseline weight that was measured and recorded in their primary health care folder. Almost 75% of these cases had a baseline weight of 65 kg or more, while only 30% of the controls had baseline weights ≥ than 65 kg. The median baseline weight for cases was 73 kg and 60 kg for controls (OR 1.1; 95% CI 1.0 – 1.1). Seventy-three percent of the cases' heights and only 35% of the controls' heights were reported to the author. Interestingly, there was a large difference in the height of the cases and controls, with the mean for cases being almost 5 centimetres (cm) shorter then the controls (OR 0.6 per 5 centimetres (cm); 95% CI 0.4 – 0.9). The mean BMI for cases was 29.9 (IQR: 28.3 – 31.5) and 24.4 for controls (IQR: 22.6 – 26.3).

Over 94% of the cases were female, where only 66 % of the controls were female. In the ARV roll-out in the public health sector of the Western Cape, 65.9% of the adult population is female, suggesting that the controls represent the Western Cape population well, at least when considering gender. None of the cases were pregnant when starting ARVs, but 3 of the selected controls had been referred to the ARV services while pregnant. Fourteen percent of the cases and 11% of the controls were exposed to ARVs in the past as attendees in the Prevention of Mother To Child Transmission (PMTCT) Programme during pregnancy (Table 3).

Table 3: Baseline demographic and clinical characteristics of the cases and controls

		Cases (n = 71)		Controls (n = 142)				
		Pts.	Median	Pts.	Median	Crude OR	p-value	95% CI
Age, per 5 year interval		69	34.0	142	36.3	0.9	0.277	(0.8 - 1.1)
Weight, kg (cont)		67	73.0	136	60.0	1.1	<0.001	(1.0 - 1.1)
Weight, kg (cat)		Pts.	Percent	Pts.	Percent			
	<55	6	9.4	43	32.3	reference category		
	55 to <60	6	9.4	23	17.3	2.1	0.246	(0.6 - 7.4)
	60 to <65	5	7.8	26	19.6	1.3	0.659	(0.4 - 4.7)
	65 to <75	20	31.3	23	17.3	5.8	0.002	(1.9 - 17.6)
	≥75	27	42.2	18	13.5	8.1	<0.001	(2.8 - 23.8)
		Pts.	Mean	Pts.	Mean			
BMI (cont)		49	29.9	49	24.42	1.2	0.001	(1.1 - 1.4)
BMI (cat)		Pts.	Percent	Pts.	Percent			
	<25	9	18.4	31	63.3	reference category		
	25 to <30	15	30.6	12	24.5	3.6	0.067	(0.9 - 14.2)
	≥30	25	51.0	6	12.2	28.5	0.002	(3.3 - 242.7)
		Pts.	Mean	Pts.	Mean			
Height, per 5 cm (cont)		52	157.2	49	161.94	0.6	0.012	(0.4 - 0.9)
Height, cm (cat)		Pts.	Percent	Pts.	Percent			
	<160	36	69.2	19	38.8	reference category		
	160 to <165	9	17.3	11	22.5	0.9	0.841	(0.2 - 3.2)
	≥165	7	13.5	19	38.8	0.1	0.014	(<0.1 - 0.7)
Sex		Pts.	Percent	Pts.	Percent			
	Female	67	94.4	94	66.2	10.0	<0.001	(3.0 - 33.2)
	Male	4	5.6	48	33.8	reference category		
Prior ARVs								
	PMTCT	10	14.1	16	11.3	1.3	0.572	(0.6 - 2.8)
Pregnant								
	Yes	0	0.0	3	2.1	can't compute (paucity of cases)		

Note: Patients (Pts); continuous (cont); categories (cat)

Baseline laboratory results

There were not any baseline laboratory tests measured in this study which were found to be associated with SHLA during crude analysis. The median CD4 nadir cell count for cases was 90 cells/mm^3, while for controls the median was just over 100 cells/mm^3 (OR 0.8 per 100 cells/mm^3; 95% CI 0.5 – 1.2). The median alanine transaminase (ALT) levels (median = 24), haemoglobin levels (median = 11) and neutrophil levels (median= 2) were the same for cases and controls. Baseline creatinine levels (OR 0.8 per 10 mmol/L increase; 95% CI 0.7 – 1.3) and total white cell counts (OR = 0.7 per 10 cells/mm^3; 95% CI 0.1 – 7.8) were also comparable between cases and controls (Table 4).

Table 4: Baseline laboratory results

	Cases (n = 71)		Controls (n = 142)				
	Pts	Median	Pts	Median	Crude OR	p-value	95% CI
CD4 count, per 100 cells/mm3	65	90	129	101	0.8	0.298	(0.5 - 1.2)
Viral load count, per 100,000 copies/mL	60	110,000	114	97,519	1.0	0.340	(1.0 - 1.1)
ALT, per 10 U/L	52	24	93	24	0.8	0.298	(0.8 - 1.1)
Hemoglobin, per 10 g/dL	49	11	94	11	0.9	0.855	(0.3 - 2.6)
Creatinine, per 10 mmol/L	10	67	21	61	0.8	0.548	(0.6 - 1.2)
Nueutrophil count, cells/mm3	34	2	58	2	0.9	0.715	(0.7 - 1.3)
Total white cell count, per 10 cells/mm3	48	4	83	5	0.7	0.775	(0.1 - 7.8)

Note: The ORs refer to the odds of being a case per unit increase

WHO stages and the stage defining illnesses

Seventy of the 71 cases and 141 of 142 controls had a stage defining illness or WHO stage recorded in their primary health folder at baseline. The percentage of cases and controls were comparable for all stages. About 35% of the participants had a stage IV defining illness, 50% had a stage III defining illness, and 15% had a stage I or II defining illness upon commencement of ARV therapy. There are relatively the same percentages of cases and controls if one compares the WHO stages to the CD4 count categories, with about 30% of cases and controls beginning ARV therapy with CD4 counts less than 50 and about 45% with CD4 counts less than 150 cells/mm^3. Cases with SHLA were not found to be at greater odds of having a chronic medical condition prior to commencing ARV therapy (Table 5).

Table 5: WHO stage defining illnesses, WHO stages, CD4 count categories and medical conditions

	Cases (n = 71)		Controls (n = 142)				
	Pts	Percent	Pts	Percent	Crude OR	p-value	95% CI
WHO stage defining illness							
Diarrhoea >1 month	2	2.8	10	7.0	0.3	0.196	(0.1 - 1.7)
Oral Candidiasis	30	42.3	50	35.2	1.4	0.312	(0.7 - 2.5)
Oral hairy leukoplakia	7	9.9	8	5.6	1.8	0.264	(0.6 - 5.3)
Tuberculosis	30	42.2	58	40.1	1.1	0.850	(0.6 - 1.8)
Oesophageal candidiasis	8	11.3	10	7.0	1.7	0.288	(0.6 - 4.8)
Pneumocystis carinii pneumonia	4	5.6	5	3.5	1.7	0.458	(0.4 - 7.0)
HIV wasting syndrome	1	1.4	4	2.8	0.4	0.489	(0.0 - 4.6)
HIV encephalopathy	1	1.4	2	1.4	1.0	1.000	(0.1 - 11.0)
Cryptococcal Meningitis	4	5.6	1	0.7	8.0	0.063	(0.9 - 71.6)
Cervical cancer	3	4.2	0	0.0	can't compute (paucity of controls)		
Kaposis sarcoma	0	0.0	5	3.5	can't compute (paucity of cases)		
Immune thrombocytopenia purpura	1	1.4	4	2.8	0.5	0.535	(0.1 - 4.5)
WHO stage							
I	3	4.3	7	5.0	reference category		
II	8	11.4	14	9.9	1.2	0.852	(0.3 - 4.9)
III	34	48.6	71	50.4	1.0	0.949	(0.3 - 3.3)
IV	25	35.7	49	34.8	1.0	0.974	(0.3 - 3.7)
CD4 count, cells/mm3	Pts	Percent	Pts	Percent			
<25	13	20.3	20	15.6	reference category		
25 to 49	6	9.4	17	13.3	0.4	0.189	(0.1 - 1.5)
50 to 99	16	25.0	29	22.7	0.8	0.565	(0.3 - 2.0)
100 to 149	13	20.3	29	22.7	0.6	0.340	(0.2 - 1.7)
≥150	16	25.0	33	25.8	0.7	0.496	(0.3 - 1.8)
Medical conditions							
Diabetes	3	4.2	2	1.4	3.0	0.229	(0.5 - 18.0)
Hypertension	4	5.6	1	0.7	8.0	0.063	(0.9 - 71.6)
Pregnant	2	2.8	9	6.3	0.4	0.277	(0.1 - 2.0)
Epilepsy	2	2.8	2	1.4	2.7	0.429	(0.2 - 33.0)

Note: Patients (Pts)

8.1.6. Characteristics of PHC follow-up period

Anti-retroviral drugs

One hundred percent of the cases were exposed to d4T for ≥ 100 days during therapy, while not one d4T naïve patient presented with SHLA to G.F. Jooste Hospital during the 27 month study period. The few cases that were not on d4T at the time of presentation had recently been switched from d4T. Eighty-seven percent (n = 124) of the controls had experienced d4T for ≥ 100 days during the study period. Just over 11% (n = 8) of the cases and 39% (n = 56) of the controls experienced ≥ 100 days of d4T at a dosage of 30 milligrams (mg) twice a day (crude OR 0.1; 95% CI 0.0 – 0.4). Ninety four percent of the controls (n = 67) and 57% (n = 81) of the cases experienced d4T at a dosage of 40 mg twice a day (OR 10.8; 95% CI 3.8 – 30.8). By adding the percentage of patients whom experienced the two different dosages of d4T together, by group (cases and controls separately), one can see that they each add up to more

than 100%. According to the Western Cape protocol, a patient below 60 kg is given a dosage of 60 mg of d4T per day, while those over 60 kg are given 80 mg of d4T per day. Seven of the cases, and 31 of the controls had weights that crossed over the 60 kg threshold during the study period resulting in the patients experiencing both dosages of d4T. Only 4 cases and 13 controls received both doses for ≥ 100 days. Just over 4% (n = 3) of the cases received ≥ 100 days of AZT before being exposed to d4T (please see Figures 3 and 4 for reasons leading to drug substitutions). Seventy percent of the cases and the controls (n = 50, n = 98 respectively) were on EFV for ≥ 100 days (OR 1.1; 95% CI 0.5 – 2.2). Thirty one percent (n = 22) of the cases and almost 27% (n = 38) of the controls experienced ≥ 100 days exposure to NVP (OR 1.3; 95% CI 0.6 – 2.8). As all patients experienced 3TC, this drug did not enter into analyses. There were no other ARV drugs that were experienced by a patient for ≥ 100 days during the study (Table 6).

Table 6: Exposure to ARV drugs for 100 days or greater

	Cases (n = 71)		Controls (n = 142)				
	Pts	Percent	Pts	Percent	Crude OR	Median	95% CI
d4T (60 and 80 mg) per day	71	100.0	124	87.3	can't compute (all cases on d4t)		
d4T 60 mg per day	8	11.3	56	39.4	0.1	<0.001	(0.0 - 0.4)
d4T 80 mg per day	67	94.4	81	57.0	10.8	<0.001	(3.8 - 30.8)
AZT	3	4.2	17	12.0	0.1	0.052	(0.0 - 1.0)
EFV	50	70.4	98	69.0	1.1	0.808	(0.5 - 2.2)
NVP	22	31.0	38	26.8	1.3	0.454	(0.6 - 2.8)

Note: Patients (Pts); all drugs are calculated as binary variables

Weight gain and loss during follow-up appointments

According to exploratory analysis, cases gained ≥ 6 kg more rapidly compared to controls. The difference in weight gain during the first three months on ARV therapy ranged from a loss of 9 kg to a gain of 22 kg amongst both the cases and controls. Almost 40% of the cases, compared with just over 25% of the controls, gained more than 6 kg in the first 3 months on ARV therapy (OR 3.5; 95% CI 1.0 – 12.0). Almost 75% of the cases and 57% of the controls gained 3 or more kg during the same time period.

Cases also experienced a greater loss of weight during the last 3 months prior to the matched case presentation date (OR 1.4; 95%CI 1.2 – 1.6). The median weight lost

for cases, during this time period, was 5 kg (IQR: 2 to 9), and for controls the median was 0 kg (IQR: -2 to 2). Over 70% of the cases (n = 45) and 17% of the controls (n = 22) lost more than 3 kg in the 3 months prior to case diagnosis (OR 9.7; 95% CI 4.27 – 21.85). Thirty-six percent of the cases compared to 4% of the controls lost more than 6 kg during this same time period (OR 52.52; 95% CI 9.83 – 280.65). Please see Table 7.

Table 7: Weight gain during the first three months on ARV therapy, and weight lost during the 3 months prior to case diagnosis

		Cases (n = 71)		Controls (n = 142)				
		Pts	Median	Pts	Median	Crude OR	p-value	95% CI
Weight gain (cont)		65	4	131	3	1.1	0.074	(0.9* - 1.1)
		Pts	Percent	Pts	Percent			
≥6 kg weight gain	no	40	61.54	98	74.81	reference category		
	yes	25	38.46	33	25.19	1.8	0.067	(0.9* - 3.5)
Weight gain (cat)	<0	6	9.23	24	18.32	reference category		
	0 to <3	11	16.92	32	24.43	1.6	0.504	(0.4 - 6.2)
	3 to <6	23	35.38	42	32.06	2.6	0.119	(0.8 - 8.4)
	≥6	25	38.46	33	25.19	3.5	0.045	(1.0 - 12.0)
		Pts	Median	Pts	Median			
Weight loss (cont)		64	5	126	0	1.4	<0.001	(1.2 - 1.6)
		Pts	Percent	Pts	Percent			
≥3 kg weight loss	no	19	29.7	104	82.5	reference category		
	yes	45	70.3	22	17.5	9.7	<0.001	(4.3 - 21.8)
Weight loss	<0	10	16.4	44	43.6	reference category		
	0 to<3	12	19.7	39	38.6	2.7	0.160	(0.7 - 10.8)
	3 to <6	17	27.9	14	13.9	9.2	0.003	(2.2 - 38.6)
	≥6	22	36.1	4	4.0	52.5	<0.001	(9.8 - 280.6)

*Note: Patients (Pts); the * stands for >0.9, not rounded to show presence of overlap with the null; weight gain data is from baseline to 3 months on therapy; weight loss data is from last three months prior to case diagnosis*

Laboratory Tests during follow up appointments

The median CD4 count at 6 months was 195 cells/mm^3 for cases, with the median gain from baseline to 6 months of 107 cells/mm^3 in the same group. Controls had a median CD4 count of 122 cells/mm^3 at 6 months, with a median 126 cells/mm^3 gain between baseline and six months. During crude analysis, it was found that cases tended to have lower CD4 counts at 6 months when compared to controls (OR 0.9 per 50 cell/mm3 increase; 95% CI 0.8 – 0.9). However, the gain in CD4 count from baseline to 6 months was not found to be associated with case status (OR 0.9 per 50 cell/mm3 increase; 95% CI 0.9 - 1.1). The Spearman Correlation Coefficient showed

that the CD4 count at baseline and 6 months is highly correlated with one another. Six cases and 7 controls had not suppressed their viral load by 6 months on therapy (OR 1.1; 95% CI 0.9 – 1.2). The difference in median ALT units gained from baseline to peak values between the cases (median = 20 U/L) and controls (median = 5.5 U/L) was significant (OR 1.1 per 10 U/L increase; 95% CI 1.0 – 1.3). Over 70% (n = 35) of the cases and 35% (n = 30) of the controls gained at least 10 U/L of ALT from baseline to peak values reached during the study period. The difference (or gain) between baseline and peak values for hemoglobin, absolute neutrophil count, and total white cell count were comparable between cases and controls (Table 8).

Table 8: Comparisons of the follow-up laboratory results between cases and controls

	Cases (n = 71)		Controls (n = 142)				
Clinical parameters during follow-up	Pts	Median	Pts	Median	crude OR	p-value	95% CI
CD4 count at 6 months,per 50 cells/mm3	65	195.0	122	239	0.9	0.049	(0.8 - 0.9*)
CD4 gain (nadir to 6 months), per 50 cells/mm3	61	107.0	117	126.0	0.9	0.171	(0.9 - 1.1)
Viral load > 400 at 6 months, pr 50 copies/mL	6	9.1	7	5.6	1.1	0.366	(0.9 - 1.2)
Hemoglobin gain (baseline and peak), per 5 g/dL	41	2.0	79	1.0	1.2	0.717	(0.4 - 3.4)
NCC gain (baseline and peak), per 10 cells/mm3	27	1.0	48	0.0	0.9	0.872	(0.3 - 2.5)
WCC gain (baseline and peak), cells/mm3	40	1.0	75	1.0	1.1	0.584	(0.9 - 1.3)
ALT gain (baseline and peak), per 10 U/L	47	20.0	84	5.5	1.1	0.019	(1.0 - 1.3)
ALT gain, U/L (baseline and peak, cat)	Pts	Percent	Pts	Percent			
<0	3	6.4	18	21.4	reference category		
0 to <10	9	19.2	36	42.9	1.2	0.856	(0.3 - 5.3)
10 to <20	11	23.4	12	14.3	6.1	0.024	(1.3 - 28.9)
≥20	24	51.1	18	21.4	6.7	0.010	(1.6 - 28.5)
	Pts	Median	Pts	Median			
ALT gain, U/L (baseline and last 80 days)	39	51.0	39	51.0	1.1	0.118	(0.9* - 1.2)
ALT gain, U/L (baseline and last 80 days, cat)	Pts	Percent	Pts	Percent			
<0	3	11.5	12	41.38	reference category		
0 to <10	3	11.5	7	24.14	1.4	0.830	(0.1 - 28.6)
10 to <20	6	23.1	5	17.24	5.0	0.227	(0.4 - 68.7)
>20	14	53.8	5	17.24	10.6	0.144	(0.4 - 252.4)

*Note: the * means > 0.9, but did not round to show the presence or absence of crossing the null; categories (cat); patients (pts)*

Symptoms reported during 80 days prior to case diagnosis

Table 9 describes the number of patients ever reporting a particular symptom during the 80 days prior to case presentation. Only those symptoms that had a frequency of >3 are reported below. The symptoms most strongly associated (OR ≥ 8) with SHLA include chest pain (OR 8.3; 95% CI 1.8 – 38.7), abdominal pain (OR 13.5; 95% CI 4.7 – 38.9), nausea (OR 8.0; 95% CI 2.3 – 28.3), poor appetite (OR 19.3; 95% CI 4.5 – 82.6) and vomiting (OR 30.0; 95% CI 7.0 – 128.1). However, back pain, general body pain, weakness, facial and limb swelling, flu-like symptoms, itching eyes and cough were also positively associated with SHLA. Conclusive evidence to support the association between diarrhoea and SHLA were borderline (OR 3.1; 95% CI 0.9 –

9.7). There was no association found between SHLA and symptoms of rash, headaches, insomnia or sexually transmitted diseases. Odds ratios cannot be reported for those complaining of being tired or experiencing heartburn due to a lack of controls reporting the same symptoms.

Symptoms related to the lipodystrophy and peripheral neuropathy were also strongly associated with SHLA. Patients with SHLA were found to have a 6-fold increased odds for symptoms of lipoatrophy or lipohypertrophy (95% CI 1.6 – 22.2), while those with SHLA were 6.4 times (95% CI 2.9 – 14.3) more likely to suffer from symptoms related to peripheral neuropathy. Concomitant pulmonary tuberculosis during ARV treatment was not shown to be correlated with SHLA.

The symptoms affecting the most people, a combination of cases and controls, were those related to upper respiratory tract infections. Forty-six of the 213 patients (24 cases and 22 controls) complained of having flu-like symptoms during the 80 days prior to diagnosis. Peripheral neuropathy affected the most cases, with 29 people (41%) complaining of pins and needles, cramping and pain in their hands and feet. Twenty-eight cases (39%) suffered from abdominal pain and 27 cases (38%) suffered from vomiting.

According to the unadjusted odds ratio, cases with SHLA were 20 times more likely (95% CI 7.1 – 56.6) to experience at least one of the three major SHLA symptoms (abdominal pain, nausea, vomiting) compared to controls during the 80 days prior to case presentation at G.F. Jooste Hospital. Patients with SHLA were 6.2 times more likely (95% CI 3.0 – 12.5) to have suffered from at least one of the minor symptoms related to SHLA in the 80 day time period prior to case diagnosis. Minor SHL symptoms include chest pain, cough, diarrhoea, generalised body pain, shortness of breath and fatigue. Having ever had an HIV correlated infectious disease in the same time period was not found to be associated with SHLA, nor was ever having a concomitant non-SHLA associated co-morbidity (arthritis, cancer, hypertension, epilepsy, diabetes, guilliane barre or Steven-Johnsons syndrome).

Cases were more likely to experience a combination of symptoms when compared to controls. Cases had a median of 4 symptoms (IQR 1-6) associated with SHLA in the

80 days prior to case diagnosis, while controls had a median of 0 symptoms (IQR 0-1) during that same time period (OR 2.0 per each additional symptom; 95% CI 1.5 – 2.6).

Table 9: Number of patients reporting symptoms in 80 days prior to case diagnosis

	Cases (n = 71)		Controls (n = 142)				
Body as a whole	Pts	Percent	Pts	Percent	Crude OR	p-value	95% CI
back pain	8	11.3	3	2.1	5.3	0.013	(1.4 - 20.1)
chest pain	9	12.7	3	2.1	8.3	0.007	(1.8 - 38.7)
general body pain	10	14.1	3	2.1	7.2	0.003	(1.9 - 26.8)
tired	8	11.3	0	0.0	can't compute (lack of controls)		
weakness	6	8.5	3	2.1	4.0	0.050	(1.0 - 16.0)
facial or limb swelling	12	16.9	5	3.5	4.8	0.003	(1.7 - 13.6)
flu-like symptoms	24	33.8	22	15.5	2.8	0.003	(1.3 - 5.4)
itching eyes	8	11.3	5	3.5	4.4	0.030	(1.2 - 17.1)
rash	11	15.5	20	14.1	1.1	0.777	(0.5 - 2.6)
headache	9	12.7	13	9.2	1.5	0.420	(0.6 - 3.7)
insomnia	1	1.4	3	2.1	0.7	0.725	(0.1 - 6.4)
Digestive							
abdominal pain	28	39.4	7	4.9	13.5	<0.001	(4.7 - 38.9)
nausea	12	16.9	3	2.1	8.0	0.001	(2.3 - 28.3)
poor appetite	21	29.6	5	3.5	19.3	<0.001	(4.5 - 82.6)
vomiting	27	38.0	2	1.4	30.0	<0.001	(7.0 - 128.1)
heartburn	10	14.1	1	0.7	can't compute (lack of controls)		
diarrhoea	8	11.3	6	4.2	3.1	0.057	(0.9* - 9.7)
Respiratory							
cough	21	29.6	19	13.4	2.8	0.006	(1.4 - 5.8)
shortness of breath	8	11.3	2	1.4	8.0	0.009	(1.7 - 37.67)
Genital Tract							
sexually transmitted disease	4	5.6	4	2.8	1.7	0.384	(0.5 - 6.1)
Comorbidities							
lipodystrophy	9	12.7	3	2.1	6.0	0.007	(1.6 - 22.2)
peripheral neuropathy	29	40.9	14	9.9	6.4	<0.001	(2.9 - 14.3)
tuberculosis - pulmonary	3	4.2	4	2.8	1.5	0.596	(0.3 - 6.7)
Categories							
Major SHLA symptoms	41	57.8	9	6.3	20.0	<0.001	(7.1 - 56.6)
Minor SHLA symptoms	42	59.2	28	19.7	6.2	<0.001	(3.0 - 12.5)
HIV related illness	12	16.9	16	11.3	1.6	0.243	(0.7 - 3.8)
Comorbidities	15	21.1	13	9.2	1.5	0.436	(0.5 - 4.7)
	Median	IQR	Median	IQR	crude OR	p-value	95% CI
Combination of symptoms							
Number of symptoms	**4**	**(1-6)**	**0**	**0-1**	2.0	<0.001	(1.5 - 2.6)

*Note: Patients (Pts); the * stands for >0.9, not rounded to show presence of overlap with the null;*
OR for 'number of symptoms' is per each additional symptom

In an attempt to better describe symptoms experienced by patients before diagnosis with SHLA, the median delay, in days, between the symptom first appearing and case diagnosis was determined. The time period to look at symptoms was restricted to the last 80 days prior to case diagnosis, as this interval was felt wide enough to be sensitive, yet short enough to exclude as many of the non-SHL symptoms as possible. This exploration was also limited to cases.

The analysis showed that back pain (median delay =47 days; IQR=33-67), chest pain (median delay =44 days; IQR 8-57) and itching or red eyes (median delay =37 days; IQR=16-66) had onsets that were most distant from case presentation. Fatigue (median duration =8 days), shortness of breath (median duration =11 days), nausea (median duration =12 days), generalized body pain (median duration =12 days), and poor appetite (median duration =14 days) seem to manifest closer to the time of case presentation (Table 10).

Table 10: Median delay from first presentation of symptom to case diagnosis (in 80 days prior to case presentation)

		Cases (n = 71)		
Body as a whole		Obs	median duration	IQR
	back pain	8	47	(33-67)
	chest pain	9	44	(8-57)
	general body pain	10	12	(1-30)
	tired	8	8	(6-23)
	weakness	6	21	(3-40)
	facial or limb swelling	12	23	(11-50)
	itching eyes	8	37	(16-66)
Digestive				
	abdominal pain	28	22	(6-38)
	nausea	12	12	(4-21)
	poor appetite	21	14	(1-44)
	vomiting	27	21	(3-42)
	heartburn	10	21	(7-25)
Respiratory				
	shortness of breath	8	11	(3-25)
Comorbidities				
	lipodystrophy	9	17	(4-24)
	peripheral neuropathy	29	27	(11-50)

Prescribed medications during 80 days prior to case diagnosis

Drugs prescribed more than 3 times by study participants in the 80 days prior to case diagnosis are listed in Table 11. Hyoscine butylbromide (OR 4.3; 95% CI 1.1 – 16.5), ibuprofen (OR 6.1; 95% CI 1.9 – 19.4), loperamide (OR 4.0; 95% CI 1.0 – 16.) and metoclopramide (OR 10.0; 95% CI 2.9 – 34.5) were all found to be correlated with SHLA. As there were few control patients taking seizure medications (controls = 1) and sennosides (controls = 0) it was not possible to present odds ratios for associations between prescribing these drugs and case status.

Some of the drugs were grouped into the following categories: known drugs that can aggravate the liver, non-steroidal inflammatory medications, antibiotics in pill form and vitamins. Patients with SHLA were 60 percent less likely to be prescribed vitamins (multi-vitamins, B-complex, or pyridoxine), even once, during the 80 days prior to case diagnosis (OR 0.4; 95% CI 0.2 – 0.8). The three other drug categories were not associated with SHLA.

Table 11: Medication prescribed within the 80 days prior to case diagnosis

	Cases (n = 71)		Controls (n = 142)				
	Pts	Percent	Pts	Percent	Crude OR	p-value	95% CI
occasional drugs							
amitriptyline	9	12.7	8	5.6	2.4	0.086	(0.9 - 6.5)
hyoscine butylromide	7	9.9	4	2.8	4.3	0.039	(1.1 - 16.5)
ibuprofen	13	18.3	6	4.2	6.1	0.002	(1.9 - 19.4)
fluconazole	3	4.2	3	2.1	2.0	0.396	(0.4 - 9.9)
loperamide	6	8.5	3	2.1	4.0	0.050	(1.0 - 16.0)
metoclopramide	15	21.1	3	2.1	10.0	<0.001	(2.9 - 34.5)
panadiene	3	4.2	2	1.4	3.0	0.229	(0.5 - 17.9)
panado	14	19.7	19	13.4	1.7	0.199	(0.8 - 3.9)
paracetanol	5	7.0	4	2.8	4.0	0.109	(0.7 - 21.8)
promethazine	2	2.8	3	2.1	1.3	0.753	(0.2 - 8.0)
pyridoxine	3	4.2	2	1.4	4.6	0.193	(0.5 - 46.9)
seizure medication	2	2.8	1	0.7	can't compute (lack of controls)		
sennosides	5	7.0	0	0.0	can't compute (lack of controls)		
tuberculosis regimen	3	4.2	2	1.4	3.0	0.229	(0.5 - 18.0)
diclofenec	2	2.8	2	1.4	2.0	0.488	(0.3 - 14.2)
Categories of occasional drugs							
drugs aggravating the liver	14	19.7	15	10.6	2.2	0.064	(0.9* - 5.2)
non-steroidal anit-inflamatories	15	21.1	42	29.6	0.6	0.194	(0.3 - 1.3)
antibiotics (excluding injected)	15	21.1	18	12.7	1.9	0.105	(0.9 - 4.3)
vitamins**	42	59.2	104	73.2	0.4	0.016	(0.2 - 0.8)

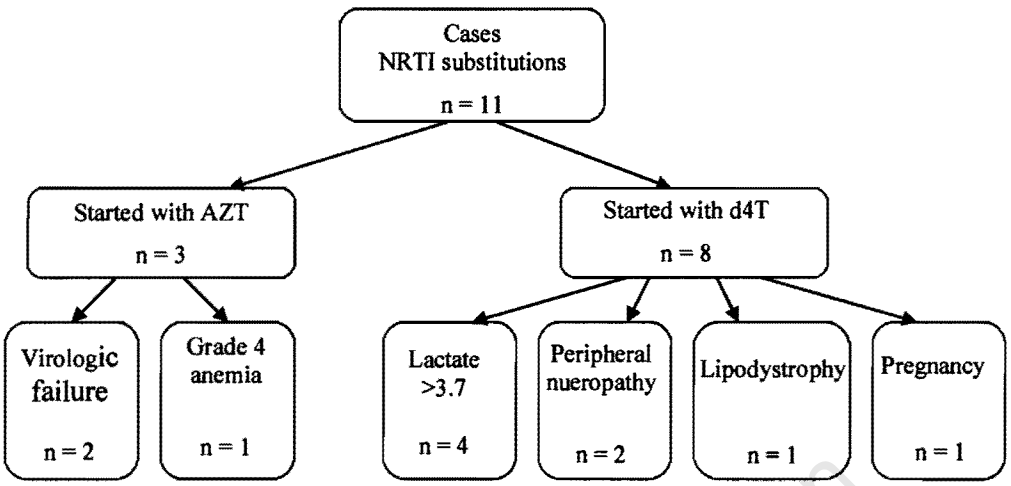
*Note: * greater than 0.9, not rounded to show presence or absence of overlap with null;*
*** vitamins category contains pyrodoxine, multi vitamins, and B complex vitamins*

ARV Drug substitutions prior to case diagnosis

Prior to the case presentation date, which also provided the end of follow-up for the matched controls, 11 cases and 14 controls had NRTI drugs substituted (Figure 3).

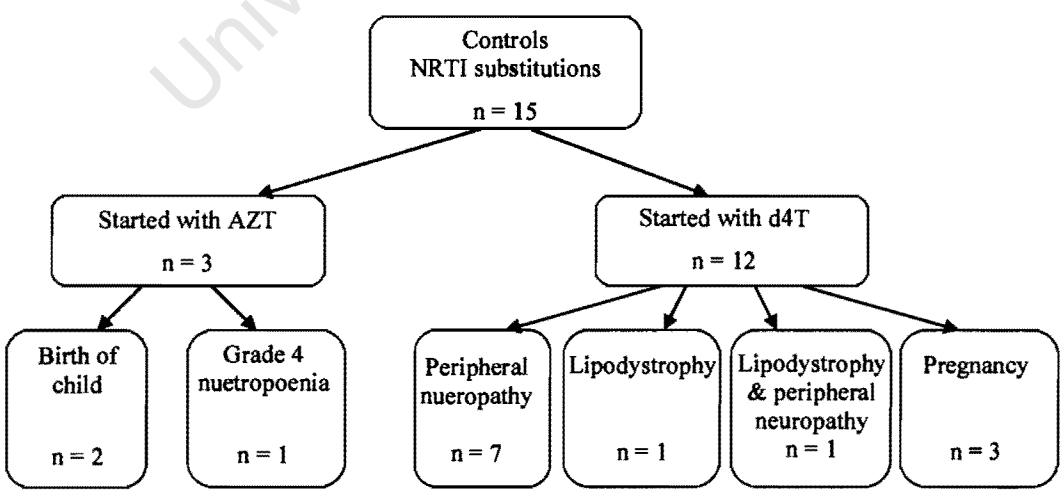
Three of the 11 cases started on AZT and later changed to d4T with the remaining 8 starting first on d4T. Two of the cases starting on AZT changed drugs due to virologic failure, and one due to an adverse event of a grade 4 anemia. Of the 8 patients starting on d4T, 4 patients had their d4T substituted with AZT due to a raised lactate greater than 3.5 mmol/L, 2 were changed to AZT due to a diagnosis of peripheral neuropathy, 1 due to a diagnosis of lipodystrophy and 1 due to pregnancy. All cases that had drug substitutions from d4T to AZT remained on AZT for a short period of time prior to case presentation (mean 10.6 days; 95% CI 4.1-17.0).

Figure 3: NRTI drug substitutions among the cases prior to case presentation date



Three of the 14 controls started on AZT which was later substituted to d4T with the remaining 11 starting first on d4T. Of the 3 starting on AZT, 2 patients were changed to d4T after giving birth to their child and one patients AZT was substituted with d4T due to a grade 4 nuetropoenia. Of the 11 starting on d4T first, 6 were changed to AZT due to peripheral neuropathy, 1 due to lipodystrophy, 1 due to both symptoms of peripheral neuropathy and lipodystrophy, and two were changed to AZT due to contraindication of d4T drugs with pregnancy (Figure 4).

Figure 4: NRTI drub substitutions among the controls prior to the matched case presentation date



8.1.7. Characteristics of cases at time of diagnosis

Crude analysis of associations with acidosis

Sixty of the 71 cases had both a pH and standard bicarbonate reading done at the time of diagnosis. Eleven cases did not have one or either of these results recorded in their folder, which did not allow for them to be grouped according to the severity of their condition (SH or lactic acidosis). Analysis of the laboratory results from the day of case diagnosis revealed creatinine and lactate levels to be associated with lactic acidosis. Patients with LA were more likely to have higher lactate levels (OR 1.4 for each mmol/L increase; 95% CI 1.1-1.8) and raised creatinine levels (OR 1.5 per 10 mg/L increase; 95% CI 1.00 – 2.4). Those patients diagnosed with lactic acidosis had 3.6 times increased odds of gaining more than 6 kg during the first 3 months on ARV therapy in comparison to controls (95% CI 1.1 – 12.1). ALT, lipase and resting glucose levels taken at case diagnosis do not appear to be associated with the severity of SHLA in this study population.

Twelve of the 71 cases (16.9%) passed away after diagnosis with SHLA. Cases with lactic acidosis were 8.3 times more likely to pass away (95% CI 0.9 – 70.6) when compared to cases that didn't have as severe a condition (Table 12).

Table 12: Univariate analysis of lactic acidosis, cases only

	Lactic acidosis (n = 35)		SHLA (n = 25)				
Laboratory results at diagnosis	pts	median	pts	median	crude OR	p-value	95% CI
pH	35	7.25	25	7.40	**part of LA definition		
standard venous bicarbonate, mmol/L	35	14.0	25	20.0	**part of LA definition		
base excess, mEq/L	31	-13.0	23	-5.0	**part of LA definition		
lactate at presentation, mmol/L	35	9.1	25	6.3	1.4	0.010	(1.1 - 1.8)
ALT, per 30 U/L	21	48.0	15	48.0	1.1	0.631	(0.8 - 1.4)
lipase, per 20 U/L	27	48.0	17	38.0	1.1	0.152	(0.9* - 1.2)
resting glucose mg/L	11	7.9	4	6.5	1.3	0.283	(0.8 - 1.9)
creatinine, per 10 mg/L	23	80.0	16	64.5	1.5	0.049	(1.0 - 2.4)
Parameters measured during follow-up follow-up							
CD4 count at baseline, per 100 cells/mm3	35	87.0	23	101.0	1.1	0.806	(0.5 - 2.4)
CD4 count at 6 months, per 100 cells/mm3	30	175.0	24	245.5	0.7	0.173	(0.4 - 1.2)
ALT gain, per 10 U/L	22	14.0	20	28.0	1.1	0.263	(0.8 - 1.1)
weight at baseline, per 10 kg	34	71.5	23	78.0	0.8	0.250	(0.6 - 1.2)
weight loss, kg (last 3 months in study)	31	5.0	23	7.0	0.9	0.287	(0.9 - 1.0)
weight gain, kg (first 3 months on ARVs)	32	5.5	23	3.0	1.2	0.036	(1.0 - 1.3)
age, per 10 years	34	35.9	25	33.7	0.9	0.594	(0.5 - 1.5)
	pts	percent	pts	percent			
female	33	94.3	24	96.0	0.7	0.765	(0.1 - 8.0)
major SHLA symptoms	18	51.4	17	68.0	0.5	0.202	(0.2 - 1.4)
peripheral neuropathy	13	37.1	9	36.0	1.1	0.928	(0.4 - 3.1)
numerical variables split into categories							
creatinine <77mmol/L	6	26.1	13	81.3	reference category		
creatinine >77mmol/L	17	73.9	3	18.8	1.0	0.002	(1.0 - 1.1)
weight gain <6kg (first 3 months on ARVs)	16	50.0	18	78.3	reference category		
weight gain ≥6kg (first 3 months on ARVs)	26	50.0	5	21.7	3.6	0.038	(1.1 - 12.1)
Status of recovery after SHLA							
died***	9	25.7	1	4.0	8.3	0.052	(0.9* - 70.6)
Mortality	obs	median	IQR	range			
duration in days from diagnosis to death	12	2.5	.5 - 18.5	0 - 172			

*Note: * greater than 0.9, not rounded to show presence or absence of overlap with null;*
*** these variables make up the definition of LA, so were not regressed*
****2 patients died with unknown LA status*

Crude analysis of associations with mortality amongst the cases

All together there were 12 cases that died after diagnosis with SHLA. The median time of death after diagnosis was 2.5 days with an inter-quartile range of 0.5 to 18.5 days. There was one case that died 172 days after diagnosis. Unfortunately, this was due to post-obstetric sepsis, and most likely was not affected by the SHLA diagnosis. Only cases were looked at during this crude analysis of mortality. As the number of cases that died was relatively small, this particular analysis had limited power to demonstrate associations with mortality in univariate models. Variables found in univariate analysis to be associated with death include pH (OR <0.01; 95% CI <0.1 – 0.1), standard venous bicarbonate (OR 0.8 per unit increase of mmol/L; 95% CI 0.7 – 0.9) and base excess (OR 0.9 per unit increase of mEq/L; 95% CI 0.8 – 0.9). Analysis of long term plus recently diagnosed diabetes in cases with SHLA and lactic acidosis did not appear as a risk factor for death amongst the cases (OR 2.7; 95% CI 0.2 –

323.6), however the confidence interval is wide and warrants further research (Table 13).

Table 13: Univariate analysis of mortality, cases only

	Died (n = 12)		Survived event (n = 59)				
Laboratory results at diagnosis	pts	median	pts	median	crude OR	p-value	95% CI
lactate at presentation, mmol/L	12	9.3	59	6.8	1.1	0.109	(0.9* - 1.3)
pH	10	7.2	51	7.3	<0.1	0.004	(0.0 - 0.1)
venous standard bicarbonate, mmol/L	10	10.4	50	18.0	0.8	0.003	(0.7 - 0.9)
base excess, mEq/L	9	-18.0	46	-7.5	0.9	0.013	(0.8 - 0.9*)
ALT, per 20 U/L	8	37.5	32	53.0	0.9	0.521	(0.7 - 1.2)
lipase, per 100 U/L	9	69.0	39	38.0	1.1	0.243	(0.9 - 1.2)
resting glucose, mg/L	3	12.9	12	6.9	1.2	0.214	(0.9 - 1.7)
creatinine, per 30 mg/L	8	102.5	35	75.0	1.1	0.475	(0.9 - 1.3)
Parameters measured during follow-up follow-up							
CD4 count at baseline, per 25 cells/mm3	11	21.0	56	98.0	0.8	0.173	(0.6 - 1.1)
CD4 count at 6 months, per 25 cells/mm3	9	168.0	56	211.0	0.9	0.382	(0.8 - 1.1)
age, per 10 years	11	37.4	58	33.8	1.5	0.207	(0.8 - 3.0)
ALT gain, per 10 U/L	8	23.0	44	28.0	0.9	0.182	(0.7 - 1.1)
weight at baseline, per 10 kg	12	71.0	55	74.0	0.8	0.390	(0.5 - 1.3)
weight loss, per 20 kg (last 3 months)	12	6.0	52	5.0	1.0	0.982	(0.1 - 10.1)
weight gain, kg (first 3 months on ARVs)	11	7.0	54	4.0	1.1	0.206	(0.9* - 1.2)
	pts	percent	pts	percent			
female	10	83.3	57	96.6	0.2	0.100	(0.0 - 1.4)
major SHLA symptoms	6	50.0	35	59.3	0.7	0.553	(0.2 - 2.4)
symptoms of peripheral neuropathy	3	25.0	26	44.1	0.4	0.230	(0.1 - 1.7)
diabetes	1	10.0	2	4.0	2.7	0.443	(0.2 - 323.6)

*Note: * greater than 0.9, not rounded to show presence or absence of overlap with null; patients (pts)*

8.2. Multivariate Models

Three multivariate conditional regression models were built to describe relationships between SHLA and variables collected at baseline and over time prior to case diagnosis. A further 2 multivariate models consider cases only and were performed using logistic regression analysis. The first of these looks at associations with the severity of SHLA and the second looks at variables associated with mortality among the cases.

8.2.1. Risk factors during the beginning months on ARV therapy

This first model contains data from the study participants which describes the time period before manifestation of signs or symptoms related to SHLA, identifying characteristics of patients who may at the outset be at a greatest risk of developing SHLA. According to this model (Table 14), cases were 23.4 times (95% CI 4.0 – 136.6) more likely to be female than controls. Patients with SHLA were 4.5 times more likely (95% CI 1.4 – 14.1) to have a baseline weight of 60 – 74.9 kg, and had a 19.4 increased likelihood (95% CI 4.6 – 82.5) of weighing ≥ 75 kg in comparison to people weighing less than 60 kg. During the first three months on ARV therapy, cases were 3.5 times more likely (95% CI 1.3 – 9.5) to have gained at least 6 kg in comparison to controls. This conditional regression model was adjusted for nadir CD4 count and age.

Table 14: Multivariate analysis of risk factors for SHLA at baseline and first 3 months on ART

Dependent variables	OR	p-value	95% CI
CD4 count nadir, per 50 cells/mm3	0.8	0.305	(0.6 - 1.2)
Age, per 10 years	1.6	0.117	(0.9 - 2.9)
Female	23.4	<0.001	(4.0 - 136.6)
Baseline weight <60 kg	ref. cat.		
Baseline weight 60 to 75 kgs	4.5	0.009	(1.4 - 14.1)
Baseline weight ≥75 kg	19.4	<0.001	(4.6 - 82.5)
≥ 6 kg weight gain in first 3 months on ART	3.5	0.013	(1.3 - 9.5)

8.2.2. Risk factors during follow-up consultations

This second model (Table 15) considers all variables collected at baseline and during follow-up consultations at the primary health care clinics, and intended to identify additional potential risk factors related to interventions, as well as the signs and symptoms most closely associated with case status. Cases with SHLA were 12.4 times more likely (95% CI 1.8 – 85.4) than controls to have had a gain in ALT of at least 10 U/L (from baseline to peak values reached prior to diagnosis). Cases with SHLA were at 23.9 greater odds (95% CI 2.8 – 204.9) of losing at least 3 kg during the last 3 months prior to diagnosis in comparison to the controls. ALT gain and weight loss was adjusted for age and the patient's CD4 count at 6 months.

An ALT threshold of ≥40 U/L was also substituted for ALT gain in the below model. This substitution did not change the direction of the odds ratios or significance of the CIs for the other variables; however it did not prove to be a better fit. Patients with SHLA were 4.5 times more likely to have had an ALT level of ≥ 40 U/L during the study period (95% CI 1.26 – 16.31).

Table 15: Multivariate analysis of risk factors for SHLA during follow-up (Model 1)

Dependent variables	OR	p-value	95% CI
CD4 count at six months, per 50 cells/mm3	0.8	0.227	(0.5 - 1.2)
Age, per 10 years	0.8	0.712	(0.3 - 2.1)
ALT gain (from baseline to peak) <10 U/L	reference category		
ALT gain (from baseline to peak) ≥10 U/L	12.4	0.010	(1.8 - 85.4)
≥ 3kg weight loss (last 3 months in study)	23.9	0.004	(2.8 - 204.9)

This following model (Table 16) also characterizes associations with SHLA amongst data collected at baseline and during follow-up consultations; however it excludes the variable measuring the gain in ALT, as small numbers were complicating the model. According to this model fit, cases were at 10.7 greater odds (95% CI 2.7 – 42.0) of having experienced at least one of the three major symptoms related to SHLA (abdominal pain, vomiting and/or nausea) during the 80 days prior to case diagnosis. Patients diagnosed with SHLA were 11.5 times more likely (95% CI 3.0 – 44.6) to experience weight loss of at least 3 kg in the 3 months prior to case presentation in

comparison to the controls. In this regression, rapid weight gain became less significant as the major symptoms, including weight loss, dominate the model. Weight gain and loss, and major symptoms were all adjusted for the baseline CD4 count. Age was dropped from the model, due to complexity, and was not associated with the outcome.

Table 16: Multivariate analysis of risk factors for SHLA during follow-up (Model 2)

Dependent variable	Odds Ratio	P> value	95% CI
CD4 count nadir, per 50 cells/mm3	0.8	0.265	(0.5 - 1.2)
Major SHLA symptoms (in 80 days prior to study end)	10.7	0.001	(2.7 - 42.0)
≥ 6 kg weight gain (first 3 months on ART)	2.6	0.108	(0.8 - 8.3)
≥ 3 kg weight loss (last 3 months of study)	11.5	<0.001	(3.0 - 44.6)

8.2.3. Risk factors associated with lactic acidosis amongst the cases

In order to consider the severity of the diagnosis, those patient with both a standard venous bicarbonate and a pH laboratory result at diagnosis were split into two groups, cases that met the definition of having lactic acidosis (standard bicarbonate <20 and ph<7.35) and cases that had the less severe condition, namely SH. As the cases were no longer matched to controls, a logistic regression was performed (Table 17). Patients with lactic acidosis were 17.8 times more likely to have a creatinine reading of ≥ 77 mmol/L compared to patients with creatinine levels lower than 77 mmol/L in their blood at the time of diagnosis. The odds ratio of 5.2 suggests that patients who gained greater than 6 kg of weight during their first 3 months on therapy were at greater odds of experiencing lactic acidosis, however as the confidence interval crosses the null the ratio could be due to chance alone (95% CI 0.8 – 34.0).

Table 17: Multivariate logistic regression of risk factors for lactic acidosis amongst cases

Dependent variables	OR	p-value	95% CI
creatinine<77, µmol/L	reference category		
creatinie>77, µmol/L	17.8	0.002	(2.8 - 113.2)
≥ 6 kg weight gain in first 3 months on ART	5.2	0.084	(0.8 - 34.0)

* base excess not fit into model as it measures acidosis

8.2.4. Risk factors associated with mortality amongst the cases

Attempts were made to find associations between variables collected in the study and mortality amongst cases. The venous standard bicarbonate level at case diagnosis was the only variable that stayed in the model when considering associations between cases and mortality. Patients who died tended to have lower venous standard bicarbonate levels in comparison to those who recovered from SHLA (OR 0.8 per mmol/L increase in venous bicarbonate; 95% CI 0.7 – 0.9; p=0.008).

9. Discussion

9.1. Key Findings:

This study capitalises on the cohort monitoring system in the Western Cape and one of the largest case series of SHLA in order to provide a comprehensive analysis of the risk factors, signs and symptoms of patients suffering from SHLA. It is also one of only three SHLA studies undertaken in the context of wide-scale up in South Africa using WHO recommended regimens, and the only in-depth SHLA study using controls to quantify associations. The study further presents a first effort to include longitudinal follow-up variables, above and beyond laboratory findings, in a case-control study on SHLA in the South African setting.

9.1.1. Duration on ARV Therapy

Ninety five percent of all SHLA cases occurred between 6.5 and 17.1 months duration in the South African setting. All cases were on d4T prior to case presentation or had recently been switched off d4T due to severe symptoms. These two findings suggest that HIV health care workers should be especially vigilant to signs and symptoms of SHLA in patients on d4T from 6 months to 18 months duration on ART.

Other studies have also reported a similar window of risk for SHLA (<20 moths) in patients on ARV therapy.[27,28,30,33,36,37] A study by Imhof and the Swiss HIV Cohort Study plotted the probability of an elevated lactate level (>2.4 mmol/L) during the first four years of ARV therapy. The slope was the greatest between baseline and two years on therapy.[33] A further two studies, one by Boffito et al. and one by John et al, found that patients with NRTI exposure are at higher risks of elevated serum lactate levels within their first year of therapy.[6,38] It appears that the majority of patients adapt to their elevated lactate levels, which tend to reach a plateau and then stabalise over time via compensatory mechanisms of the body. However, those few patients that continue to develop SHLA are in severely deteriorated metabolic states in which homeostasis is not re-gained until ARV therapy is discontinued.[6,38]

9.1.2. Adult case referral rate

Overall, there were 67 patients referred from the G.F. Jooste primary health care drainage sites, giving a total of 5,989 patient years and a referral rate of 13.5 SHLA cases per 1000 patients. Thirty-five of the 67 cases had confirmed LA, with a corresponding referral rate of 7.1 cases per 1000 patient years. These referral rates may underestimate the incidence of SHLA due to the possibility of patients passing away at home without diagnosis. More interestingly, it is important to note that ARV services that have been open longer have lower referral rates. This makes sense when one considers the short window of risk from 6 to 18 months on ARVs for 95% of the cases. As a clinic's population of patients receiving ARVs for longer than 20 months increases, their referral rate will decrease proportionally.

Discrepancies in reported incidence rates of SHLA are not only due to the variety of case definitions and first line regimen choice, but also due to the maturity of the ARV cohorts being studied. Literature shows a case rate for SHLA between 1.1 and 30.8 cases per 1,000 patient years.[2,5,7,12,15,27,28,37] One study in France by Bonnet et al. reported 1.1 LA cases per 1000 years, while another study in South Africa by Geddes and colleagues reported 14 cases of lactic acidosis per 1000 patient years. The cohort in Bonnet's study covered patients on ARV therapy over four years, while Geddes's study looked at patients on ARV therapy over a one and a half year period. The case referral rate in Bonnet's study included many additive months of patient time when they were not at risk of SHLA in comparison to the Geddes study, which may partially explain the lower incidence rate.[15,28]

In attempts to propose a standardized way to compare referral or incidence rates of adverse events with discreet windows of risk amongst different cohorts across the world, a new calculation for incidence rates was considered. The new calculation considers only the months a patient contributes when they are at risk for the adverse event, for SHLA this translates into only counting up the months between 6-18 months on ARV therapy. Using these parameters, the overall referral rate for SHLA was 32.1 cases per 1000 patient years and 16.8 cases of lactic acidosis per 1000 patient years in the G.F. Jooste referral cohort. This new calculation was used to look at the referral rates of each different referral clinic as a way to validate the tool. After

stratifying by referral clinic, it was revealed that there was a wide range of referral rates, with the older ARV services referring fewer cases then the newer sites. There may have been more knowledge and shared experience resulting in higher vigilance amongst the older clinics, as they have been seeing such cases for longer periods of time and were more likely to be doing point of care testing. For these reasons, there may have been more drug substitutions from d4T to AZT at the primary health care level in patients whose lactates were not sufficiently raised to require referral. As the setting is reasonably uniform, these differences suggest that local clinical practices can dramatically impact on the referral rates to hospitals for SHLA, and warrants further exploration.

9.1.3. Factors associated with case status

ARV drugs

All cases had been exposed to d4T during their ARV therapy, and not one d4T naive participant during the 27 months of this study suffered from SHLA. Although the case-control study was matched by duration on ARV therapy, many patients (n=26) experienced NRTI drug substitutions during the study period. In an effort to look at ARV drugs as risk factors, any patient receiving 100 days or more of a particular ARV drug was recorded as positive for that drug (the first case presented 108 days after commencing ARV therapy).

Only d4T at a dosage of 40 mg twice a day for ≥ 100 days showed as a strong risk factor during univariate analysis for SHLA in comparison to other ARV drugs. All patients above 60 kg were automatically put on 40 mg of d4T twice a day according to protocol. Therefore, in the South African context, a heavy baseline weight above 60 kg and d4T 80mg per day are directly correlated. Untangling the two risk factors, in this type of non-experimental study, cannot be done and any associations between either of the risk factors would need to be interpreted with consideration to the other risk factor.

During the 14th Conference on Retroviruses and Opportunistic Infections (CROI) the WHO decided to change their protocol to recommend that no patients receive d4T at a dose greater than 30 mg twice a day. This is expected to lower the incidence of

SHLA, however health care workers and patients alike will still need to remain vigilant as 4 cases in this study were on 30 mg of d4T twice a day for the entire duration of the study.

All cases were on d4T prior to diagnosis and the duration on therapy was matched in this case-control. The lack of variance in d4T with regards to case status did not allow for measuring the odds of d4T being associated with SHLA. Therefore, it was not possible to adjust for d4T in the multivariate regressions.

It is possible however, that given the known strong associations between SHLA and d4T exposure that clinicians may have been more likely to look for and ascertain SHLA in patients on d4T compared to other patients.

Gender

According to the multivariate regression, female patients on d4T are at a much greater risk of developing SHLA when compared to males. This finding has also been reported in many international studies.[2,5,28,29,39] The different multivariate models presented in this case-control show that patients with SHLA are 23.4 times more likely to be female; however the magnitude of the odds ratio may be misleading. There were only four male cases and 67 female cases. During regression in STATA, the variables are adjusted for each other and stratified into cells. If there is a lack of variance among a variable (for example, few males and many females), some cells will have very low numbers of observations in them. This can lead to a distortion of the odds ratios. As females are over-represented in this study, the odds ratio may be biased away from the null, but this does not negate the finding that being female remains a strong risk factor for SHLA.

Many studies have reported that females are overrepresented amongst the cases in comparison to the proportion of females on ARV therapy in the source populations, [2,5,28,29] however the only other published study the author found that quantified the association was by Boulle et al. In this South African study, female gender was reported as 10.7 times more likely to have d4T substituted with another drug due to symptomatic hyperlactataemia or LA (hazard ratio, 10.7; 95% CI 1.5 – 79.0).[16]

Baseline weight and initial rapid weight gain

There have been very few studies that have looked at baseline weight as a risk factor for SHLA. Falco et al. reported in their review of cases in the literature, that cases tended to have higher body mass indexes, although this risk factor was not analysed in multivariate analysis.[37] In the multivariate regression analysis of factors at baseline, the current study showed that cases with SHLA were more likely to have a baseline weight of ≥ 60 kilograms (60 – 75 kg OR 4.54; 95% CI 1.45 – 14.11; ≥75kg OR 23.39; 95% CI 4.00 – 136.63). The rapid increase in risk with increasing weight cannot be explained by dose escalation at 60 kg, and suggests a biological phenomenon peculiar to women with high BMI's.

Patients that experienced rapid weight gain of 6 kg or more in the first 3 months of therapy were 3 times more likely to suffer from SHLA when compared to patients who did not gain at least 6 kilograms during the same period.

The literature review described a putative biological mechanism espoused by some clinicians that could link both baseline weight and rapid initial weight gain with the risk of NAFLD, which in turn could predispose to SHLA. As a fatty liver is less able to metabolize lactate, it may become a net producer, contributing to the build-up of lactate in the blood causing metabolic complications including SHLA.[4]

David Sass reported many conditions associated with fatty liver disease including obesity, diabetes mellitus, total parental nutrition, rapid weight loss and nucleoside analogs. Interestingly, Sass also reported a connection between patients with hypothalamic/pituitary dysfunction, whom were at risk of excessive weight gain, as being found to develop progressive NAFLD.[19]

The diagnosis of non-alchoholic fatty liver (NAFLD) and non-alcoholic steatohepatitis (NASH) cannot be confirmed unless a biopsy of the liver is taken. Liver biopsies are hotly debated due to the risk, cost and lack of effective medical therapy for these conditions. For this reason, the prevalence of fatty liver has not been reported in the South African setting as far as the author is aware of. However, prevalence rates reported worldwide have been shown to be between 10-24% in various settings. The prevalence figure increased to 57.5 – 74% amongst obese

individuals.[19,23] Another study in Italy on NAFLD, using ultrasound techniques, found that all 187 young patients with a BMI of ≥30 had some degree of liver steatosis.[17] In this case-control study, 50% of the cases with heights measurements were considered obese (BMI>30), and another 30% had BMIs > 25. If the prevalence of fatty liver is comparable to that in the states, a large proportion of the cases had NAFLD before ever starting ARVs. Rapid weight gain may further aggravate an asymptomatic patient with progressive or potential NAFLD, causing a build-up of lactate in the body.

Laboratory Results

Not one of the clinical parameters at baseline indicated any association with an outcome of SHLA during univariate analysis. Both the cases and controls in this study had very low nadir CD4 counts not allowing for an association to be seen. However, a study by Bonnet et al reported an association between low nadir CD4 counts (<250 cells/mm^3) and SHLA.[18] Assessing measurements during ARV follow-up appointments revealed that an ALT gain of ten or more U/L between baseline and the peak was strongly and independently associated with case status (OR 12.4; 95% CI 1.8 – 85.4). Over 50% of the cases gained at least 10 U/L of ALT during the study time period. An ALT result of greater than 40 U/L during the study period was also found to be significant, but not as strongly associated (OR 4.54; 95% CI 1.3 – 16.3). Literature has shown that raised aminotransferase levels were predictive of NAFLD in 42-90% of cases, after excluding other causes of liver disease.[17]

The gain in ALT was also looked at in the last 80 days prior to diagnosis, but only 26 cases and 29 controls had measurements at both baseline and during the 80 days prior to case presentation. There was not enough power to show any association in univariate analysis with SHLA, however the magnitude of the odds ratios were big and increased with the increased gain in the ALT measurement. This suggests the possibility that had there been a larger number of ALT measurements taken during this period, an association may have been seen.

Weight loss in the last 3 months prior to case presentation

Patients developing SHLA clinically deteriorate over time. One of the early signs of SHLA includes weight loss. Fifty-nine of the 71 (90.8%) cases experienced weight

loss prior to diagnosis of SHLA, and 34% of the cases suffered weight loss of 3 kg or more during this time period. During multivariate analysis patients with SHLA were more likely to experience weight loss of ≥ 3 kilograms prior to the case presentation date. This finding could have important clinical significance, as any patient on d4T who begins to lose weight should be assessed for other signs or symptoms of SHLA, especially if the weight loss happens during the 6-18 month time period on ARV therapy.

WHO stage defining illnesses

The different WHO clinical stages were not associated with case status in multivariate analysis due to the power of this study. However the odds ratios increased per WHO stage, suggesting a dose effect, with patients more severely immuno-compromised potentially being at greater risk for SHLA.

The number of patients experiencing a particular WHO stage defining illness prior to commencing ARV therapy were small (≤10 cases and/or 10 controls), except for tuberculosis and oral candidiasis. Even though the analysis lacked power, it is important to point out that cryptococcal meningitis did reveal an odds ratio of 8 (95% CI .9 – 71.6). This considerable odds ratio warrants further research into a possible association. Both prior tuberculosis and oral candidiasis were not found to be associated with SHLA.

Symptoms during the 80 days prior to case presentation

Various exploratory analyses were performed to try and capture preceding symptoms and present them in a quantifiable way. Exploratory analysis was performed for symptoms as ever having had the symptom, as a median time period for when the symptom first started to case presentation and the combination of symptoms cases and controls experienced.

The final model captured symptoms regressed as binary variables, with ever having the symptom in the stated time period as being indicative of exposure. By assessing symptoms amongst the cases and controls during the 80 days prior to case presentation, one would expect many of symptoms to show association with SHLA, as one is comparing an increasingly sick population (cases) to a relatively healthy

population (controls). Although this confined time period of symptom analysis will push the odds ratio away from the null in comparison to measuring the same symptoms during the entire study period, it an important analysis to describe exactly how the cases presented in contrast to controls in the weeks prior to diagnosis.

Symptoms were categorized as ever having been recorded in the patients' folder in the 80 days prior to the case diagnosis. The symptoms, during univariate analysis, found to be associated with SHLA are not unique to this syndrome, but also tend to describe many HIV-related illnesses. The strongest associations, with odds ratios > 10, include abdominal pain, poor appetite and vomiting which were also closely linked in timing of onset to case presentation. Associations between SHLA and facial and limb swelling, puritic or red eyes and heartburn (lack of controls makes OR impossible to estimate) have not previously been described, but were also temporally more distal to the event. Symptoms of sexually transmitted diseases, headaches and rashes were not found to be associated with SHLA.

The concurrent clinical manifestations of lipodystrophy and peripheral neuropathy, which have overlapping pathophysiological mechanisms, were also both found to be correlated with SHLA during univariate analysis, but did not stay in the models during multivariate regressions.

Perhaps, more importantly, from a health provider's perspective, it is the combination of the above symptoms that should alert one to suspect the possibility of SHLA. The cases had a median of four different symptoms (IQR; 1-6) prior to case diagnosis, while controls had a median of 0 symptoms (IQR; 0-1).

Additional medications other then ARVs

Associations in univariate analysis between SHLA and hyoscine butylbromide, ibuprofen, loperamide and metoclopramide were found. Although there were some associations between occasional medicines and case status, the compounds were most likely prescribed due to symptoms of the developing condition rather than being causes of the condition, and these associations should be treated with extreme caution. In addition, there were no biological mechanisms that could be identified linking these compounds to the outcome.

9.1.4. Variables associated with LA amongst the cases

Cases diagnosed with LA were more likely to have raised creatinine levels in their blood compared to cases presenting with the milder form of this condition, SH. LA cases also appeared to gain weight more rapidly then patients diagnosed with SH, although this finding was not significant, containing wide confidence intervals that crossed over the null.

Creatinine levels are taken to evaluate kidney function; when the kidneys are functioning abnormally creatinine levels in the blood will increase. Mitochondria play a crucial role in cellular metabolism and energy metabolism. When the numbers of mitochondria decrease or they function irregularly it is not surprising to see effects that involve many organ systems, including the kidneys.[2] As kidneys are lactate consumers, any damage may further increase the levels of lactate in the body.

9.1.5. Variables associated with mortality amongst the cases

The only variables, during multivariate logistic regression, found associated with death amongst the cases were measures of acidosis, of which standard venous bicarbonate was the measure most closely associated with mortality. Cases that passed away were more likely to have decreased venous standard bicarbonate levels in comparison to the milder form of the syndrome (SH). Although associations were found with the level of lactate elevation during crude analysis, this fell away when adjusted for acidosis by venous standard bicarbonate.

As the standard bicarbonate levels reflect the degree of metabolic acidosis, clinical manifestation is more severe and rapidly worsening in patients with low standard bicarbonates.[2] The only study found that spoke about bicarbonate levels, was the study by Geddes and colleagues. Although their study did not assess associations with mortality, 3 of the 4 patients that died had the three lowest bicarbonate values (range, 6.3 – 8.3 mmol/L).[28]

9.2. Strengths and Weaknesses

This is the largest case-control study on SHLA that the author is aware of, with 71 cases and 142 controls. It is also the first case-control that has looked at longitudinal variables including clinical parameters and symptoms. It is one of three studies conducted in Southern Africa and in the context of rapid scale up of ARV therapy according to WHO recommended regimens. It is the only case-control study of SHLA in this setting. As part of the Western Cape Cohort, all clinics are public health sector facilities and therefore use the same regimens, laboratory tests and provincial protocols for managing an ARV service.

Study design

SHLA is a rare outcome of patients on ARV therapy. The case-control study is the most efficient sampling technique to reach statistical power for determining risk factors and possible diagnostic tools of rare outcomes, making it the most appropriate design for this study.

Matching

Matching limited the ability to look at drug durations as a risk factor. However, universal use of d4T and matching by duration has taken out two of the strongest associations with SHLA, allowing us to look at other risk factors in more detail.

Incidence density sampling

In this study, incidence density sampling was used while selecting the controls for the study. With incidence density sampling the controls represent the exposure odds which equates to the ratio of person-years of observation of exposed and unexposed. Therefore, the odds ratio from this case control study equals the rate ratio in the underlying cohort study. Ten controls later came down with SHLA after their matched-case diagnosis date, and many more had stavudine substituted to another drug due to peripheral neuropathy or lipodystrophy.

Categorizing exposure variables for analysis

As briefly mentioned in the methods section 6.6, information related to each individual symptom and drug were grouped into binary variables, with exposure being positive if they ever experienced the variable within the stated time period that the variable was being considered. Duration of a symptom is often very difficult to ascertain. However, poor record keeping limited the analysis of duration of drug exposure (other than ARVs). Analysis would have been more robust had duration of exposure to a medication been recorded, but due to many of the patient's folders lacking a stop date, this type of analysis could not be made, weakening the overall results of the study pertaining to occasional drugs.

As symptoms were sometimes poorly recorded, grouping variables into binary exposures meant that patients with an unrecorded symptom had that information aggregated in the data set as not having had the exposure. The same problems existed with analysis of the occasional medications, with poorly recorded data making the duration of the medications unattainable.

9.3. Potential Bias

Recall bias

This is a nested case-control study, with the cohort being all patients on ARV therapy within the public health sector of the Western Cape Province. Collection of patient information (later used as variables in this study) at facility level was part of routine assessment during follow-up appointments in the health facilities; therefore potential exposures were recorded prior to case status. Recall bias was thus avoided by the design of this study.

Selection bias

Case-control study results are heavily influenced by selection bias. With this knowledge careful attention was paid to ensure that both cases and controls would represent the probability of exposure in the source population.

All cases reported from the G.F. Jooste Secondary Referral Clinic during the time of the study period, were included in this study with the exception of two patients. The two patients not included were referred from a site that does not partake in the public health sector roll-out, making their folders difficult to access. As the two patients were not excluded due to any knowledge of treatment or exposure information, selection bias of the cases was not influenced from their exclusion.

Controls were matched to cases according to ARV commencement month, which due to incidence density sampling meant ARV duration as well, and facility. The controls were randomly selected from the group of eligible patients that also met the above stated matching criteria. The matching technique was not chosen intentionally to ensure that possible confounders were equally stratified in analyses, but due to practical reasons (as explained in the methods section 6.1). However, matching by facility had the additional benefit of ensuring that confounding due to resources or different management protocols of patients at the primary ARV sites would not have a big impact on the results (for example, health staff to patient ratios, doctor heavy compared to nurse heavy sites, site differences pertaining to amount of time between patient clinical visits, etc.). Matching by ARV start date allowed potential confounding due to provincial policy change over time to be stratified equally across cases and controls (for example, the policy of taking baseline viral loads, and the use of nevirapine versus efavirenz changed during the study period, as did the availability of lactate meters at each ARV facility). Matching by ARV duration unfortunately meant that this variable could not be analysed overall in the study. As shown in this study, cases presented during a window of 3 to 30 months on ARV therapy. As new patients were being initiated on ARVs at relatively fast rates during the early stages of the public roll-out, direct random selection of controls would have resulted in more controls with shorter ARV duration periods compared to the cases.

In addition, controls were selected if they met the eligibility criteria, regardless of exposure information and whether they had manifestations of the studied condition after the matched cases diagnosis date. As a result, controls were free of the disease at the matched case diagnosis date, but represented those at risk in the population of becoming cases.

Referral bias

As G.F. Jooste Hospital is the referral route for serious adverse events of patients on ARV therapy in their area, and also well-known to have expertise in the area of SHLA, it is unlikely that cases from the source population would have been referred elsewhere. It is therefore unlikely to have a group of cases with different rates of exposure not being measured (unless cases passed away before being referred).

Investigator bias

Every attempt was made to ensure that investigator bias did not enter into the study. Per study design, all symptoms, laboratory results, clinical diagnoses, medications and weights recorded in patient folders during the study period were collected in the database for potential analysis. This excluded any bias in regards to decision making of whether or not to collect a recorded piece of information regarding the above variables during the collection period.

Ascertainment bias and misclassification

As patients at the primary health site started showing signs of clinical deterioration, many symptoms recorded may have been elicited by the health care providers initiative to ask sick patients whether they had been experiencing such symptoms, where as relatively healthy patients probably were not asked as many direct questions relating to symptoms (in relatively healthy patients recorded symptoms were more likely due to complaints initiated by the patient). This probably resulted in symptoms that were not severe being more frequently recorded in patients experiencing clinical deterioration, resulting in differential misclassification, biasing the odds ratio away from the null.

More investigative techniques, such as laboratory tests and more in-depth physical examination could have occurred in sicker patients as well, together with more frequent consultations, resulting in ascertainment bias.

The level of clinical record keeping varied at the different primary care facilities and most likely also varied between the different health-workers within each facility performing the clinical follow-up visit. Poor record keeping resulted in non-differential misclassification biasing results away from the null.

Differential misclassification during analysis

In order to limit differential misclassification of exposure status during analysis, symptoms and laboratory results recorded at the case diagnosis site (G.F. Jooste Secondary Referral Hospital) were not included in the analysis comparing case and control variables in association to SHLA. Clinicians at the G.F. Jooste Secondary Referral Clinic have more training in ARV complications and making deductions during a diagnosis of complex diseases. As they are the referral centre for complex ARV patients in the area, they also have more practice than a clinician or health care worker at a primary site. By trying to ascertain what condition a patient is suffering from, more questions regarding symptoms and exposure would be recorded. It was assumed that cases sent to this referral site would have extensive information recorded during their diagnosis visit/s. As controls did not get this same 'more intensive' follow-up session at their primary site, notes from the case folders at G.F. Jooste were not included in the analysis of associations with SHLA.

Information collected during the referral visit/s was/were used in the analysis of associations with LA in comparison to SHLA though, and in the analysis of mortality in the cases. The inclusion of the referral information during these analyses did not create additional bias, as all controls were dropped from this analysis.

10. Future Studies

According to the old South African protocol, all patients over 60 kg were automatically started on or changed to a 40 mg dose of d4T twice a day. As the WHO has recommended all patients be given a twice daily 30 mg dose of d4T, regardless of weight, risk factors relating to weight would need to be re-established in patients reaching weights of greater than 60 kg while on a d4T containing ARV regimen.

There is very little information about how a BMI relates to SHLA and lactic acidosis. As the BMI is a much better measure of obesity than absolute weight, this variable should be looked into more extensively.

When larger case series are available, findings related to weight should also be confirmed in men. If increased baseline weights or BMIs are not found to be associated with the male gender, interactions between female gender and weight should be analysed.

Genetic studies into patients at a higher disposition of SHLA should also be considered. The patient population in this study was relatively uniform; therefore, risk factors relating to different populations could not be assessed. The paucity of research on this subject warrants further studies.

A further look into different clinical practices that may reduce the incidence of SHLA should also be considered.

11. Conclusion

As d4T remains the preferred choice making up part of the NRTI backbone of ARV therapy in developing countries, the morbidity and mortality associated with d4T use should be minimised as much as possible. As cases of SHLA continue to rise in Southern Africa in correlation to increased accessibility of ARV therapy, preventative measures should be put in place to decrease such illnesses and death in instances where the risk factors are clearly identified.

The majority of cases presented in a narrow window of time between 6 and 18 months duration on ARV therapy. Treatment with stavudine, female gender, initial weight (≥ 60 kg, but especially ≥ 75 kg), and rapid early weight gain (≥ 6 kg) are confirmed as the dominant factors identifying patients who may subsequently develop SHLA. Weight loss (≥3 kg), the major known symptoms of SHLA (abdominal pain, nausea, and vomiting), together with ALT gains (≥10 U/L) during follow-up are the clinical parameters that are most able to identify patients about to develop SHLA. Biochemical parameters reflecting acidosis are the only factors associated with acute mortality amongst the cases, whilst a raised creatinine at case presentation is additionally associated with severity of disease.

According to these results, it is recommended that overweight women not be started on d4T containing regimens. Any patient on d4T that gains greater than 6 kilograms

during their first three months of ARV therapy or any patient losing weight should be monitored carefully for symptoms of SHLA. The use of ALT in identifying patients at risk requires further exploration.

Strategies to prevent morbidity and mortality on a programme level include considering other first line ARV drug choices such as tenofovir, and clinical interventions that promote the identification of patients at risk both when initiating therapy (when alternatives could be used in these patients), and during follow-up (where early substitution of stavudine could prevent subsequent disease). Even in countries that adopt the WHO recommended dose decrease in d4T, vigilance needs to remain high as four cases in this study were on the decreased d4T dosage throughout their entire duration on ARV therapy.

Table 1: Model building for risk factors at the start of ART

	Step	Model	Variables	obs	Log likelihood	chi2	prob >chi2	AIC	BIC
	constant	a	case	213	-79.02				
confounders		b	cd4_nadir	196	-68.20	0.96	0.3265	138.39	141.05
		c	WHO stage	209	-77.52	0.00	0.9501	157.03	160.38
		d	age	207	-76.22	1.21	0.2711	154.44	157.77
		e	(cd4_nadir) (age)	177	-65.85	1.27	0.5304	135.69	142.09
	interactions	f	(cd4_nadir) (age) (cd4_nadir*age)	177	-65.76	1.43	0.6975	137.53	147.12
Risk factors		g	(cd4_nadir) (age) (wt_base)	178	-53.66	24.46	<0.0001	113.33	122.87
		h	(cd4_nadir) (age) (female)	190	-54.91	30.98	<0.0001	115.83	125.57
		i	(cd4_nadir) (age) (wt_3mg6)	169	-59.37	6.21	0.1019	124.75	134.14
		j	(cd4_nadir) (age) (bpcrypto)	190	-67.63	5.55	0.1356	141.26	151.00
		k	(cd4_nadir) (age) (wt_base) (female)	178	-39.89	52.01	<0.0001	87.78	100.51
		l	(cd4_nadir) (age) (wt_base) (wt_3mg6)	169	-48.84	27.27	<0.0001	105.68	118.20
		m	(cd4_nadir) (age) (wt_base) (bpcrypto)	178	-52.80	26.19	<0.0001	113.60	126.32
		n	(cd4_nadir) (age) (wt_base) (female) (wt_3mg6)	169	-33.07	59.18	<0.0001	76.14	91.85
		o	(cd4_nadir) (age) (wt_base) (female) (bcrypto)	178	-39.22	53.34	<0.0001	88.44	104.35
		p	(cd4_nadir) (age) (wt_base) (female) (wt_3mg6) (bpcrypto)	169	-36.37	52.21	<0.0001	84.74	103.52
	interactions	q	(cd4_nadir) (age) (wt_base) (female) (wt_3mg6) (wt_base*female)	169	-36.67	51.62	<0.0001	85.33	104.11
		r	(cd4_nadir) (age) (wt_base) (female) (wt_3mg6) (wt_base*wt_3mg6)	169	-35.86	53.23	<0.0001	83.73	102.51
		s	(cd4_nadir) (age) (wt_base) (female) (wt_3mg6) (female*wt_3mg6)	169	-36.80	51.40	<0.0001	85.56	104.34
		t	(cd4_nadir) (age) (wt_base) (female) (wt_3mg6) (cd4_nadir*wt_base)	169	-36.78	51.39	<0.0001	85.56	104.34
		u	(cd4_nadir) (age) (wt_base) (female) (wt_3mg6) (cd4_nadir*female)	169	-32.59	59.77	<0.0001	79.19	101.10
		v	(cd4_nadir) (age) (wt_base) (female) (wt_3mg6) (cd4_nadir*wt_3mg6)	169	-34.59	55.78	<0.0001	83.17	105.08
		w	(cd4_nadir) (age) (wt_base) (female) (wt_3mg3) (age*wt_base)	169	-35.00	54.95	<0.0001	84.00	105.91
		x	(cd4_nadir) (age) (wt_base) (female) (wt_3mg6) (age*female)	169	-31.85	61.26	<0.0001	84.00	99.48
		y	(cd4_nadir) (age) (wt_base) (female) (wt_3mg6) (age*wt_3mg6)	169	-36.64	51.67	<0.0001	85.29	104.07

***none of the interaction variables showed significance during regression

Table 2: Model building with ALT

	Step	Model	Variables	obs	Log likelihood	chi2	prob >chi2	AIC	BIC
	constant	a	case	213	-79.02				
confounders		z	cd4_nadir	196	-68.20	0.96	0.3265	138.39	141.05
		c	WHO stage	209	-77.52	0.00	0.9501	157.03	160.38
		b	cd4_06	182	-64.35	4.36	0.0369	130.71	133.91
		d	age	207	-76.22	1.21	0.2711	154.44	157.77
		e	(cd4_06) (age)	177	-61.79	5.91	0.0520	127.57	133.92
	interactions	h	(cd4_06) (age) (cd4_06*age)	177	61.43	6.62	0.0851	128.86	138.39
Risk factors		i	(cd4_06) (age) (wt_base)	162	46.96	24.58	<0.0001	99.92	109.18
		j	(cd4_06) (age) (female)	177	-49.90	29.69	<0.0001	105.79	115.32
		k	(cd4_06) (age) (wt_3mg3)	156	-52.72	8.66	0.0342	111.44	120.59
		l	(cd4_06) (age) (wt3m_presloss3)	146	-37.29	30.50	<0.0001	80.57	89.52
		m	(cd4_06) (age) (altg)	106	-32.43	11.68	0.0086	70.86	78.85
		n	(cd4_06) (age) (bi_lamaj)	177	-31.92	65.64	<0.0001	69.84	79.36
		p	(cd4_06) (age) (bvomit)	177	-39.84	49.80	<0.0001	85.68	95.21
			(cd4_06) (age) (bod_vitamins)	177	-57.86	13.76	0.0033	121.72	131.25
		q	(cd4_06) (age) (altg) (wt_base)	99	-18.32	34.93	<0.0001	44.64	55.02
		r	(cd4_06) (age) (altg) (female)	106	(**) -18.82	38.89	<0.0001	45.64	56.29
		s	(cd4_06) (age) (altg) (wt_3mg6)	98	-29.72	11.31	0.0230	67.45	77.79
		t	(cd4_06) (age) (altg) (wt3m_presloss3)	85	-16.96	27.48	0.0011	41.91	51.68
		u	(cd4_06) (age) (altg) (bi_lamaj)	106	(**) -13.32	49.89	<0.0001	34.65	45.30
		v	(cd4_06) (age) (altg) (bod_vitamins)	106	-30.52	15.49	0.0038	69.04	79.70
		w	(cd4_06) (age) (altg) (d4t_80)	106	-24.77	27.00	<0.0001	57.53	68.19
		x	(cd4_06) (age) (altg) (wt3m_presloss3) (wt_base)	80	-12.47	32.86	<0.0001	34.94	46.85*
		y	(cd4_06) (age) (altg) (wt3m_presloss3) (wt_3mg6)	80	-14.20	29.40	<0.0001	38.41	50.32
		z	(cd4_06) (age) (altg) (wt3m_presloss3) (bod_vitamins)	85	-15.67	30.06	<0.0001	41.33	53.55
			(cd4_06) (age) (altg) (wt3m_presloss3) (d4t_80)	85	-13.89	33.61	<0.0001	37.78	50.00

**model starts becoming unstable (big standard deviations); ** Model instability; none of the interactions were significant*

Table 3: Model building with clinical parameters during follow-up assessments

	Step	Model	Variables	obs	Log likelihood	chi2	prob >chi2	AIC	BIC
	constant	a	case	213	-79.02				
confounders		b	cd4_nadir*	196	-68.20	0.96	0.3265	138.39	141.05
		c	WHO stage*	209	-77.52	0.00	0.9501	157.03	160.38
		e	Age	207	-76.22	1.21	0.2711	154.44	157.77
		f	(cd4_n) (age)	177	-61.79	5.91	0.0520	127.57	133.92
	interactions	g	(cd4_n) (age) (cd4_n*age)	177	61.43	6.62	0.0851	128.86	138.39
risk factrs		h	(cd4_n) (age) (wt_base)	162	46.96	24.58	<0.0001	99.92	109.18
		i	(cd4_n) (age) (female)	177	-49.90	29.69	<0.0001	105.79	115.32
		j	(cd4_n) (age) (wt_3mg6)	156	-52.72	8.66	0.0342	111.44	120.59
		k	(cd4_n) (age) (wt3m_presloss3)	146	-37.29	30.50	<0.0001	80.57	89.52
		l	(cd4_n) (age) (bi_lamaj)	177	-31.92	65.64	<0.0001	69.84	79.36
		m	(cd4_n) (age) (bi_hiv)	177	-61.55	6.38	0.0946	129.10	138.63
		n	(cd4_n) (age) (bi_laminor)	177	-51.96	25.56	<0.0001	109.92	119.45
		o	(cd4_n) (age) (bi_comorb)	177	-61.69	6.10	0.1068	129.38	138.91
		p	(cd4_n) (age) (bpnp)	177	-49.98	29.52	<0.0001	105.96	115.48
		q	(cd4_n) (age) (blipo)	177	-60.83	7.82	0.0499	127.66	137.19
		r	(cd4_n) (age) (b_vitamins)	177	-57.68	13.76	0.0033	121.72	131.25
		s	(cd4_n) (age) (d4t_60)	177	-48.41	32.66	<0.0001	102.82	112.35
		t	(cd4_n) (age) (d4t_80)	177	-43.89	41.71	<0.0001	93.77	103.30
		u	(cd4_n) (age) (d4t_80) (AZT)	177	-58.08	13.32	0.0040	122.16	131.69
		v	(cd4_n) (age) (bi_lamaj) (wt_base)	162	-26.75	65.00	<0.0001	61.50	73.85
		w	(cd4_n) (age) (bi_lamaj) (female)	177	-24.84	79.80	<0.0001	57.68	70.38
		x	(cd4_n) (age) (bi_lamaj) (wt_3mg6)	156	-28.81	56.49	<0.0001	65.61	77.81
		y	(cd4_n) (age) (bi_lamaj) (wt3m_presloss3)	122	-23.81	57.46	<0.0001	55.62	67.55
		z	(cd4_n) (age) (bi_lamaj) (bi_hiv)	177	-31.71	66.05	<0.0001	71.43	84.13
		aa	(cd4_n) (age) (bi_lamaj) (bi_laminor)	177	-31.71	66.07	<0.0001	71.41	84.12
		bb	(cd4_n) (age) (bi_lamaj) (bi_comorb)	177	-30.06	69.36	<0.0001	68.12	80.82
		cc	(cd4_n) (age) (bi_lamaj) (bpnp)	177	-28.78	71.92	<0.0001	65.56	78.27
		dd	(cd4_n) (age) (bi_lamaj) (blipo)	177	-30.97	67.54	<0.0001	69.94	82.64
		ee	(cd4_n) (age) (bi_lamaj) (b_vitamins)	177	-30.55	68.38	<0.0001	69.10	81.80
		ff	(cd4_n) (age) (bi_lamaj) (d4t_60)	177	-29.51	70.46	<0.0001	67.02	79.73
		gg	(cd4_n) (age) (bi_lamaj) (d4t_80)	177	-28.72	72.05	<0.0001	65.43	78.14
		hh	(cd4_n) (age) (bi_lamaj) (azt)	177	-29.80	69.88	<0.0001	67.60	80.30
		jj	(cd4_n) (age) (bi_lamaj) (wt_base) (wt3m_presloss3)	132	-20.80	53.30	<0.0001	51.60	66.01
		ll	(cd4_n) (age) (bi_lamaj) (female) (wt3m_presloss3)	146	-18.58	67.92	<0.0001	47.19	62.08
		nn	(cd4_n) (age) (bi_lamaj) (wt_3mg6) (wt3m_presloss3)	127	-20.10	51.48	<0.0001	50.21	64.43
		pp	(cd4_n) (age) (bi_lamaj) (bi_hiv) (wt3m_presloss3)	146	-23.52	58.04	<0.0001	57.04	71.96
		rr	(cd4_n) (age) (bi_lamaj) (bi_laminor) (wt3m_presloss3)	146	-23.81	57.46	<0.0001	57.61	72.53
		tt	(cd4_n) (age) (bi_lamaj) (bi_comorb) (wt3m_presloss3)	146	-22.51	60.05	<0.0001	55.03	69.95
		vv	(cd4_n) (age) (bi_lamaj) (bpnp) (wt3m_presloss3)	146	-21.95	61.18	<0.0001	53.90	68.81
		xx	(cd4_n) (age) (bi_lamaj) (blipo) (wt3m_presloss3)	146	-22.66	59.76	<0.0001	55.32	70.24
		zz	(cd4_n) (age) (bi_lamaj) (b_vitamins) (wt3m_presloss3)	146	-22.92	59.24	<0.0001	55.84	70.75

*continued on next page

*Table continued from previous page

	Step	Model	Variables	obs	Log likelihood	chi2	prob >chi2	AIC	BIC
risk factors		bbb	(cd4_n) (age) (bi_lamaj) (female) (wt_base) (wt3m_presloss3)	132	-16.48	61.94	<0.0001	44.96	62.26
		ddd	(cd4_n) (age) (bi_lamaj) (female) (wt_3mg6) (wt3m_presloss3)	127	-14.89	61.89	<0.0001	41.79	58.85
		fff	(cd4_n) (age) (bi_lamaj) (female) \(bi_hiv) (wt3m_presloss3)	146	-18.53	68.01	<0.0001	49.06	66.97
		hhh	(cd4_n) (age) (bi_lamaj) (female) (bi_laminor) (wt3m_presloss3)	146	-18.47	68.13	<0.0001	48.94	66.84
		jjj	(cd4_n) (age) (bi_lamaj) (female) (bi_comorb) (wt3m_presloss3)	146	-16.95	71.18	<0.0001	45.90	63.80
		lll	(cd4_n) (age) (bi_lamaj) (female) (bpnp) (wt3m_presloss3)	146	-17.11	70.85	<0.0001	46.23	64.13
		nnn	(cd4_n) (age) (bi_lamaj) (female) (blipo) (wt3m_presloss3)	146	-17.14	70.80	<0.0001	46.28	64.18
		ppp	(cd4_n) (age) (bi_lamaj) (female) (b_vitamins) (wt3m_presloss3)	146	-17.59	69.90	<0.0001	47.17	65.07
		rrr	(cd4_n) (age) (bi_lamaj) (female) (wt_3mg6) (wt3m_presloss3) (wt_base)	127	-14.39	62.90	<0.0001	42.78	62.69
		ttt	(cd4_n) (age) (bi_lamaj) (female) (wt_3mg6) (wt3m_presloss3) (b_hiv)	127	-14.68	62.32	<0.0001	43.36	63.27
		vvv	(cd4_n) (age) (bi_lamaj) (female) (wt_3mg6) (wt3m_presloss3) (b_laminor)	127	-14.55	62.58	<0.0001	43.10	63.01
		xxx	(cd4_n) (age) (bi_lamaj) (female) (wt_3mg6) (wt3m_presloss3) (b_comorb)	127	-13.36	64.96	<0.0001	43.33	63.23
		zzz	(cd4_n) (age) (bi_lamaj) (female) (wt_3mg6) (wt3m_presloss3) (bpnp)	127	-14.45	62.77	<0.0001	42.91	62.82
		bbbb	(cd4_n) (age) (bi_lamaj) (female) (wt_3mg6) (wt3m_presloss3) (blipo)	127	-14.04	63.60	<0.0001	42.08	62.00
		dddd	(cd4_n) (age) (bi_lamaj) (female) (wt_3mg6) (wt3m_presloss3) (b_vitamins)	127	-14.36	62.96	<0.0001	42.72	62.63
		eeee	(cd4_n) (bi_lamaj) (wt_3mg6) (wt3m_presloss3)**	127	-19.10	53.46	<0.0001	50.20	67.26
		hhhh	(cd4_n) (bi_lamaj) (wt_3mg6) (wt3m_presloss3) (b_hiv)	127	-19.97	51.74	<0.0001	51.94	69.00
		jjjj	(cd4_n) (bi_lamaj) (wt_3mg6) (wt3m_presloss3) (b_laminor)	127	-20.05	51.95	<0.0001	52.09	69.17
		llll	(cd4_n) (bi_lamaj) (wt_3mg6) (wt3m_presloss3) (b_comorb)	127	-19.90	51.89	<0.0001	51.79	68.86
		nnnn	(cd4_n) (bi_lamaj) (wt_3mg6) (wt3m_presloss3) (bpnp)	127	-19.15	53.38	<0.0001	50.30	67.36
		pppp	(cd4_n) (bi_lamaj) (wt_3mg6) (wt3m_presloss3) (blipo)	127	-19.25	53.18	<0.0001	50.50	67.57
		rrrr	(cd4_n) (bi_lamaj) (wt_3mg6) (wt3m_presloss3) (b_vitamins)	127	-19.71	52.27	<0.0001	51.41	68.48
	interactions	kkkkk	(cd4_n) (bi_lamaj) (wt_3mg6) (wt_base) (wt3m_presloss3) (wt_base*wt3m_presloss3)	127	-9.76	72.16	<0.0001	37.52	63.12

*Note: * cd4_nadir, whostage were collinear, so the strongest variable, by comparing AIC and BIC was chosen;*
*** female and age dropped from the model due to instability, BIC and AIC increased due to less variables*
****only the above listed interaction variable significantly had an impact when placed within the model, however it did not have a big enough impact to warrant it's inclusion. It slightly lowered odds ratios that were extremely inflated when including the interaction*

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