# Stavudine induced lactic acidosis, risk factors and predictive laboratory markers: A nested case-control study in South Africa

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# ABSTRACT

**Introduction**

The incidence of antiretroviral therapy induced lactic acidosis and its associated mortality may be reduced by appropriate dosing, risk stratification and early detection. This study describes the epidemiology, risk factors and predictive laboratory markers for lactic acidosis in subjects commenced on stavudine containing antiretroviral therapy between 2004 and 2007 at a hospital in KwaZulu- Natal. Persons with body weight above 60kg received 40mg twice daily and those below 30mg.

**Methods**

A nested case-control study design was used. Risk factor analysis was adjusted for the established risk factors of weight and gender.

**Results**

Lactic acidosis occurred in 79 (17 per 1000 person years) of 1 762 persons. Significant baseline risk factors were female gender (Adjusted Odds Ratio (AOR) =5.4) and increased body weight (AOR, compared to persons <60 kg, was 6.6 for persons 60 to 69 kg, 6.9 for persons 71 to 80 kg, and 95.7 for persons >80 kg). Predictors six months into therapy were an alanine transaminase >50 IU /L (AOR=11.1) and triglyceride between 1-1.5 mmol/l (AOR=11.2 compared to persons with triglyceride <0.5 mmol/l). No associations were found with regard to age, CD4 counts, viral loads or creatinine and albumin levels.

**Conclusion**

Obese females are at greatest risk for lactic acidosis with exponential increased in risk at weights above 80kg. The 30mg dose may be preferable, given that a sharp increase in risk occurred at 60kg, and that that the 30mg dose has been shown to have adequate virologic suppression. Additional risk factors for LA include an increase in alanine transaminase and triglyceride at 6 months of treatment.

Number of words = [260]

# DECLARATION

I, Christopher Alan Luke declare that

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II. This dissertation has not been submitted for any degree or examination at any other university.

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# ACRONYMS AND ABBREVIATIONS

ALT: Alananine transaminase

ART: Antiretroviral therapy

HIV: Human immunodeficiency virus

KZN: KwaZulu- Natal

LA: Lactic acidosis

NRTI: Nucleoside reverse transcriptase inhibitors

pyrs: Person years of observation

SA: South Africa

SHL: Symptomatic hyperlacataaemia

TRIG: Triglyceride

WHO: World health organisation

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# CHAPTER 1: LITERATURE REVIEW

## *1.1 INTRODUCTION*

South Africa (SA) has one of the highest HIV prevalence globally (11% in 2008) with KwaZulu-Natal (KZN) the worst affected province (16%)(1). Limiting drug toxicity, in the context of a limited formulary, is a major challenge (2).

A number of reports of from SA in 2006 and 2007 described a higher than expected incidence of ART induced lactic acidosis (LA) (3, 4, 5) attributed to stavudine. This is of particular concern given the associated mortality (6).

Here we describe the epidemiology, clinical presentation and risk factors associated with LA. In addition we explored early warning markers for LA.

## *1.2 BACKGROUND*

### 1.2.1 Incidence of LA

In the developed world LA is reported to occur at an incidence rate of 0.6 to 10 per 1000 person years exposure to ART (6, 7). In developing countries LA has been reported to occur at an incidence rate of 11 to 19/1000pyrs (2, 4, 8) on stavudine based therapy.

### 1.2.2 Mechanism of NRTI induced LA

Nucleoside reverse transcriptase inhibitors (NRTIs), by inhibiting the mitochondrial DNA polymerase γ enzyme cause mitochondrial dysfunction (6, 9, 10). This forms the basis for several of the toxic

effects, namely; LA, asymptomatic and symptomatic hyperlactatemia (SHL), peripheral neuropathy, lipodystrophy, hepatic steatosis and pancreatitis (10, 11, 12, 13).

The risk for LA is not equal across all NRTIs. Effect is greatest with didanosine (DDI) which is > stavudine (D4T) > zidovudine (AZT) > abacavir (ABC) = lamivudine (3TC) = tenofovir (TNF) (14, 15).

### 1.2.3 Clinical features of NRTI induced LA

The majority of patients (75%) experience weight loss (4, 16). Loss of appetite (63%) is also common (4). Other symptoms include fatigue, myalgia, abdominal pain, abdominal distension, nausea, vomiting, weakness or dyspnoea (7, 12, 17). Patients generally present with a partially compensated high anion gap metabolic acidosis with a lactate that is characteristically greater than 5mmol/L (12). The symptoms of LA begin several months before the diagnosis is made (7, 17).

Not surprisingly several studies have found other manifestation of mitochondrial toxicity, such as peripheral neuropathy (4, 16, 17), lipodystrophy (17, 18, 19,) and liver abnormalities to be associated with LA (6, 16, 12, 17). Thus presentation with one form of toxicity should alert the clinician to the possibility of others.

LA is the most serious of the toxic effects with a case fatality of 33% to 60% (6, 7, 20). Serum lactate levels greater than ten are associated with a mortality of around 80% (17, 21). Lower body weight and higher alanine transaminase (ALT) has also been associated with higher mortality (21). The lower mortality reported in more recent studies (21-30% (4, 5, 16)) may be explained by increased clinician awareness resulting in earlier diagnosis (6, 22).

Lactic acidosis has been reported to occur from one to 20 months of treatment (7). The majority of patients present six to 17 months from ART initiation (4, 16). Median time to presentation is eight to ten months (4, 5, 16).

### 1.2.4 The relationship between LA and liver abnormalities

Hepatic abnormalities are a common finding at presentation of LA. These manifest as a tender hepatomegaly and mild elevations in ALT (16) in up to 65% of patients (8). Jaundice is rarely seen. Hepatic steatosis has been documented on imaging studies with histologic finds of necrosis in more fulminant cases (6, 12, 17).

The relationship between the liver abnormalities and LA is poorly understood (6). It is thought that both LA and hepatic steatosis have a common pathogenesis, via mitochondrial toxicity (6, 23). However, hepatic steatosis and steatohepatitis could affect the livers ability to metabolise lactate thus contributing to LA (6, 24, 25). This latter theory is supported by the fact that a strong association has been shown between LA and obesity (4, 5, 16), and that underlying hepatic steatosis is common in obese persons (26). In addition, the mechanism by which hypertrigylcerideamia, hyperglycemia and viral hepatitis may predispose to LA might be hepatic steatosis and steatohepatitis (27).

### 1.2.5 Early warning markers for LA

Elevation of lactate levels has been shown to be common during treatment with NRTIs, and does not reliably predict LA (11, 28, 17). To impact on the high mortality associated with LA it is imperative to find alternate ways to predict or diagnose LA (17).

### 1.2.6 Definition of LA

There is no universally accepted definition of ART induced LA (17). Most definitions include a lactate greater than 5mmol/L with the standard serum bicarbonate less than 20mmol/L (5, 16, 29, 17, 15). Some also include a Ph <7.34 (30) and a high anion gap. A universal requirement is the exclusion of all

other causes of LA. Conditions that should be excluded include; recent exercise, sepsis, diabetic ketoacidosis, biguanide therapy, renal disease, liver disease, malignancy and hypo perfusion states (12).

### 1.2.7 Lactic Acidosis and Symptomatic Hyperlactatemia

Patients with SHL present with symptoms similar to those with LA except that they are not acidotic and generally have lower lactate levels (12). There is no set definition for SHL, which has contributed to the wide variation in incidence reported in the literature (6, 12). SHL and LA are likely to be different manifestations of the same pathogenic process with each being different ends of the same spectrum of illness, with SHL likely preceding LA (12). Risk factors for the development of SHL are therefore likely to be the same as that for the development of LA. The incidence of SHL in developed countries varies from 3,5 to 20/1000 pyrs of ART (6, 11, 22) compared to 17,5 to 36/1000pyrs (4, 8, 31) reported in developing countries, with stavudine the main culprit.

### 1.2.8 Treatment options

Treatment is mainly supportive with discontinuation of the offending agent, and intravenous fluids (12). In patients with decompensated acidosis bicarbonate infusion is often given however, there is no evidence to support its use (12). In severe cases bicarbonate dialysis and respiratory support is often offered. No studies have shown any specific therapy to be effective beyond general supportive care (12). AZT has been shown to be safe when rechallenging patients with NRTIs (4, 15, 17).

## *1.3 RISK FACTORS FOR LA*

Many possible risk factors for LA (discussed further and referenced below) have been identified including; female gender, pregnancy, advanced age, impaired renal or liver function, regimens containing efavirenz, co-infection with hepatitis B, increased weight or BMI, diabetes, low CD4 counts, good adherence to ART, low serum albumin, dyslipidaemias and hyperglycemia.

Risk factors will be considered in two groups; baseline risk factors (factors that predict increased an risk of LA at commencement of ART) and evolving risk factors (factors that emerge while on treatment).

### 1.3.1 Baseline risk factors

**Female gender** has been well established as a strong risk factor for LA (2, 3, 4, 5, 6, 8, 16, 29, 32). LA is estimated to occur up to 10 times more commonly in females than males (3, 4, 5, 8, 31).

Excess **weight** (or high body mass index) has also been convincingly shown to be a risk factor for LA (2, 3, 4, 5, 8, 16, 32, 36). The risk continues to increase as the weight increases (2, 16). Osler *et al* showed an adjusted odds ratio of five for weight > 60kg compared to 19 for weight > 75kg (16). Mathews *et al* showed an additional three fold increase in risk for every 30% increase in BMI (2).

**Stavudine** induced LA appears to be dose dependent. This is supported by the fact that higher doses have been associated with greater mitochondrial toxicity in vivo (33). Higher doses have also been shown to cause an increased incidence of other toxicities such as of peripheral neuropathy and lipoatrophy (34). However, there are currently no control studies that explored dose as a risk factor for LA. It has been suggested that 30mg or even 20mg may be more appropriate since they have equal antiviral efficacy to the 40mg dose (34).

There is conflicting evidence regarding **advanced age** as a risk factor. Age greater then 40yrs. was found to be a risk factor for hyperlacateamia in two small cases series (18, 28) and in a case control study (30). Age was however not found to be a risk factor in other studies from both high and low income settings (2, 3, 4, 5, 8, 9, 16, 29).

**Diabetes** has been suggested as a risk factor based on small studies in the developed world (19, 35). There are no controlled studies assessing this as a risk factor.

**Pregnancy** has been suggested to be a risk factor for LA, based on a number of case reports (6), but has not been evaluated in control studies.

There is conflicting evidence regarding **low CD4 counts** as a risk factor for LA. Small cohorts (18, 36) in studies from the developed world demonstrated an association with hyperlactataemia. This was supported by two controlled studies in the developing world (2, 30) but not by others (2, 16, 19, 29).

There is also conflicting evidence on **impaired renal function** as a risk factor for LA. Two studies (2, 18) found high creatinine levels associated with LA, as opposed to a large control study from the developing world (16).

**Liver abnormalities** have been suggested as risk factors for LA (16). This might be due to the fact that stavudine is commonly associated with steatohepatitis (12).

The association between hyperlactatemia and **raised cholesterol and triglyceride** suggests a possible association between lipid abnormalities and LA (18, 19, 35, 37). Lipid levels have not yet been assessed for an association with LA in controlled studies.

**Efavirenz** has been suggested as a risk factor for LA in small case series (38) and a small prospective study (29), but not confirmed in a large controlled study (16).

### 1.3.2 Early warning signs of LA after commencing HAART

**Early rapid weight gain** in the first 3 months (16) and an **increase in ALT** (6, 16) while on ART have all been associated with the subsequent development of LA.

Based on small cohort studies in the developed world hyperglycemia and hyperlipidaemia have been associated with hyperlactatemia (18, 19). These remain to be evaluated in LA.

## *1.4 AIMS OF OUR STUDY*

To determine the incidence of stavudine associated adverse events in a local cohort of patients in a South African setting with a special focus on risk factors associated with the development of LA.

### 1.4.1 Objectives

1. Determine the incidence of specific stavudine related toxicities that necessitate a drug switch in a large antiretroviral rollout programme.
2. Describe the demographic, clinical and biochemical features of a group of patients that develop LA.
3. Determine the factors that might predict the development of LA prior to commencement of ART and while on ART (early warning signs) and therefore define a category of patients at particular risk for the development of LA.

## 1.5 *OPERATIONAL DEFINITIONS USED IN OUR STUDY*

**Lactic acidosis (LA)** for the purposes of this study was defined as a patient with symptoms consistent with LA (weight loss, abdominal pain or distension, dyspnoea, fatigue, nausea, vomiting), a venous lactate $\geq 5$ AND a serum standard bicarbonate or $CO_2$ level <20 mmol/L (5, 15, 16, 29). Blood for lactate levels had to have been taken without a tourniquet in a tube containing potassium citrate and sodium fluoride and kept on ice until processed by the laboratory within 4 hours (12).

**LA Exclusion criteria** (12). Patients with a history of chronic persistent diarrhoea, disseminated TB, renal failure, liver failure or malignancy or examination findings suggestive of ketoacidosis (HGT >15 and ketones on urine analysis), dehydration (defined as BP<110/60 with rapid normalization of lactate with fluids or, evidence of systemic sepsis (increased WCC, clinical evidence of organ specific infection, or fever) or evidence of malignancy will be excluded (12).

To be considered to have **symptomatic hyperlactatemia** patients had to have a serum lactate ≥ 2.5 with symptoms but not meeting the criteria for LA.

**Lipodystrophy**: asymmetric weight loss or severe breast hypertrophy.

# CHAPTER 2: METHODS

## *2.1 STUDY DESIGN*

A retrospective cohort is described, followed by a nested LA case-control analysis.

## *2.2 STUDY POPULATION AND SETTING*

This study was conducted at Port Shepstone hospital in KwaZulu- Natal, serving a semi-rural population. The study population consisted of adult persons (>18yrs) living with HIV with a CD4 count less than 200 cells/µl or Stage 4 illness (World Health Organization criteria (39)) commenced on ART between July 2004 and April 2007, and followed until November 2008. Patients routinely received twice-daily stavudine 40 mg if over 60 kg, or 30 mg if less than 60 kg, combined with lamivudine and either nevirapine or efavirenz, according to the South African National guidelines (40). Zidovudine was substituted for stavudine when severe toxicity occurred.

Port Shepstone hospital has a dedicated onsite ART clinic and pharmacy. All ART patient data were routinely captured on a Microsoft access electronic register. Routine blood tests were performed on all patients at baseline and after six months of follow-up. All serum analysis were performed at an onsite laboratory (utilizing the DXI 800 machine for chemistry and DXI 600 machine for haematology investigations), except for viral load estimations, which were sent conducted at a central virology laboratory (utilizing the Abbott m2000sprt and Abbot m2000rt machines) Pharmacy routinely captured a record of all ART regimen changes due to side effects.

At each visit the ART nurse recorded the patient symptoms and weight. If the patient had signs or symptoms of hyperlactateamia (weight loss or any abdominal symptoms) a bedside venous lactate level (using the Synchron systems quantitative reagent method) was recorded. This was followed by a formal lactate if the bedside lactate reading exceeded 3 mmol/L. Patients with a formal lactate greater than four were referred to a doctor for assessment. Patients were assessed by the one of a team of five doctors and managed using standard guidelines (12, 19).

## *2.3 DESCRIPTION OF THE COHORT*

The relevant cohort for the study was determined using the ART clinic register by selecting patients that initiated ART between dates of interest. Data was obtained from the pre-existing ART clinic Microsoft access electronic database. Baseline data that had routinely captured on the electronic

register included the patient name, age, gender, ART initiation date, weight, CD4 count, haemoglobin level, treatment regimen and follow-up dates. Relevant information was obtained using the Microsoft access database applying Microsoft software functions.

### 2.3.1 Identifying stavudine toxicity cases

Pharmacy Regimen change records consisted of the motivation forms submitted by the clinician who captured the date, the side effect that prompted the regime change, and if the toxicity was related to lactate then the lactate level was recorded. The number (n) of patients experiencing a particular side effect was determined by counting the number of motivations submitted for regimen change to pharmacy for that side effect.

In the case of **LA case identification** clinical notes were then retrieved to verify the diagnosis and collect further data. In addition mortuary records were interrogated to capture additional cases of LA that would not have been captured by pharmacy records because a regimen change would not have been motivated for, for obvious reasons. All cases of LA identified were subjected to the case definition (see section 1.5) by two experienced clinicians to generate a final list of LA cases.

## *2.4 DATA CAPTURING FOR LA CASES*

All relevant data from the clinical charts was captured onto a Microsoft Excel spread sheet (for a list the of variables collected refer to addenda, section 6.1). All data entry was entered was verified by the author.

## *2.5 IDENTIFYING CONTROLS*

A list of potential controls was generated using the ART clinic Microsoft access electronic register. For each case of LA a control was randomly chosen from a list that matched the case for month of ART initiating and duration of treatment with stavudine. Data was collected for controls in the identical manner as for LA cases (above).

## *2.6 STATISTICAL ANALYSIS: NESTED CASE CONTROL STUDY*

Conditional logistic regression modelling for matched pairs was used to compare the variables at baseline and at six months follow up. STATA version 10 software was used by the study biostatistician.

In the analysis of risk factors, weight and gender as the well established risk factors for LA, were controlled for by using statistical methods. The risk factors, duration of therapy and duration of exposure were controlled for by the matching of cases and controls. Any additional factors found to be significant were controlled for in the assessment of other potential risk factors. A p value of $< 0.05$ was considered significant.

### *2.7 EXTERNAL VALIDITY*

Port Shepstone is within the province of greatest HIV prevalence and serves a mix of urban and rural indigenous communities, similar to the rest of SA. Thus, the study findings are likely relevant and applicable to most ART treatment sites in SA.

### *2.8 CONSIDERATION OF BIAS*

Case detection bias was minimized by retrospective evaluation of objective markers (laboratory results). Information bias was minimized by use of the same laboratory facilities in the same time period for cases and controls. Control selection bias was minimized in the random selection process described.

### *2.9 ETHICS*

The ethical approval was obtained from the hospital ethics committee and the Biomedical Research Ethics Committee of the Nelson R Mandela School of Medicine South Africa.

This was a retrospective chart review, thus Biomedical Research Ethics Committee did not expect us to obtain consent from individual patients. The findings would be beneficial to the community as a whole and not, in any way, detrimental to the study subjects.

## CHAPTER 3: RESULTS

### *3.1 DESCRIPTION OF THE COHORT*

A total of 1 762 adult patients (mean age 36.1 years, mean baseline CD4 count 113 cells/µl) were initiated on stavudine containing ART from August 2004 to April 2007. Follow up continued until November 2008 resulted in observation of 4 566person years. The cohort was followed for a mean of 2.5 and a median 1.5 years.

Baseline patient characteristics categorized by gender and weight are shown in Table 1. The majority of patients were female. Just under half of the cohort weighed <60 kg.

**Table 1. Baseline characteristics of adults commenced on stavudine containing ART, between 2004 and 2007 at Port Shepstone hospital, categorized by gender and weight (N=1 762)**

| | (n) | % | Mean age (yrs) | Mean weight (kg) | Mean CD4 (cells/ul) | Mean Hb (g/dl) |
|---|---|---|---|---|---|---|
| **Females** | **1226** | **69.7%** | 35.4 | 60.6 | 113 | 11.2 |
| <60 kg | 583 | 33.7% | 35.1 | 51.2 | 96 | 10.8 |
| 60-69 kg | 359 | 20.8% | 35.2 | 64.0 | 128 | 11.5 |
| 70-79 kg | 117 | 6.8% | 36.2 | 74.0 | 125 | 11.7 |
| >80 kg | 99 | 5.7% | 36.1 | 88.5 | 144 | 12.1 |
| Weight unknown | 68 | 3.9% | | | | |
| **Males** | **536** | **30.4%** | 37.8 | 60.9 | 114 | 11.3 |
| <60 kg | 244 | 14.1% | 37.2 | 51.5 | 101 | 11.0 |
| 60-69 kg | 164 | 9.5% | 37.9 | 63.9 | 113 | 11.4 |
| 70-79 kg | 56 | 3.2% | 38.1 | 74.0 | 123 | 11.5 |
| >80 kg | 40 | 2.3% | 39.6 | 87.2 | 154 | 12.3 |
| Weight unknown | 32 | 2.0% | | | | |
| **Total** | **1762** | **100%** | **36.1** | **60.7** | **113** | **11.2** |

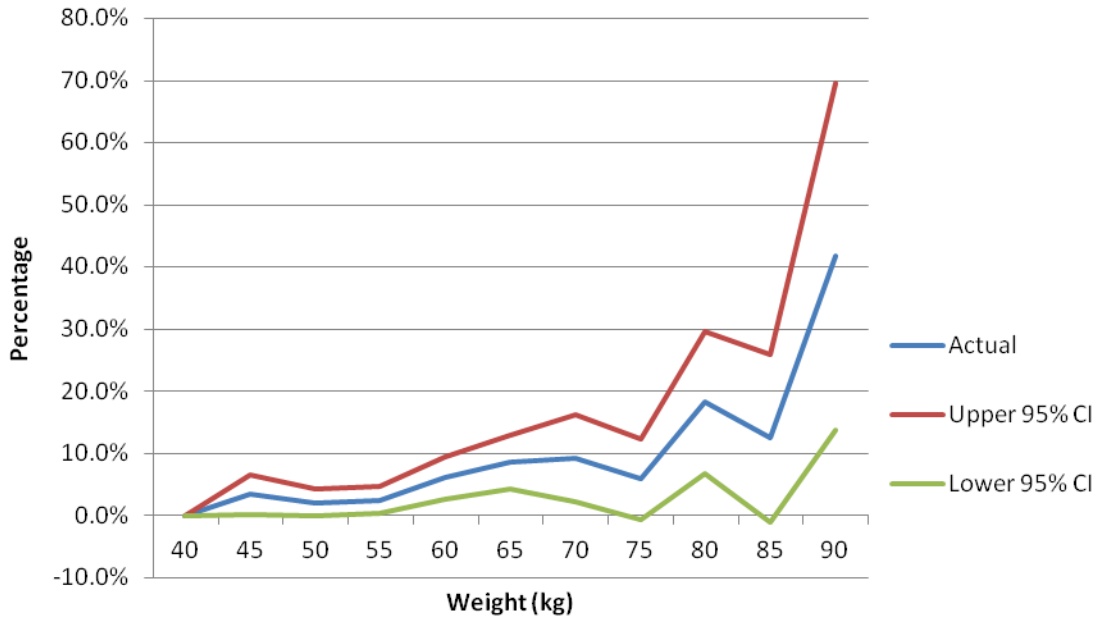
## *3.2 INCIDENCE OF STAVUDINE TOXICITY*

Stavudine induced toxicity requiring a drug substitution occurred in 275 patients giving an incidence rate of 60/1000 pyrs.

LA and SHL were the most common toxicities (Table 2). The incidence of LA in females increased sharply at 60kg, and then rose exponentially in subjects with weights above 80kg (table 2). This is graphically expressed in graph 1.

**Table 2. Cumulative Incidence and Incidence rates of stavudine related toxicities in adults commenced on ART between 2004 and 2007 at Port Shepstone Hospital (N=1 762)**

| Toxicity type | (n) | Total at risk | Incidence | Incidence rate per 1000 pyrs (95%CI) |
|---|---|---|---|---|
| **Lactic acidosis** | 79 | 1762 | 4.5% | 17 (14 to 21) |
| **Females*** | 72 | 1226 | 5.9% | 23 (18 to 28) |
| <40kg | 1 | 24 | 4.2% | 16 (-15to 47) |
| 40-59kg | 4 | 175 | 2.3% | 9 (0 to 17) |
| 50-60 kg | 9 | 383 | 2.3% | 9 (3 to 15) |
| 60-69 kg | 26 | 359 | 7.2% | 28 (18 to 38) |
| 70-79 kg | 9 | 117 | 7.7% | 30 (11 to 48) |
| 80-89 kg | 12 | 76 | 15.8% | 69 (34 to105) |
| >90kg | 9 | 32 | 28.1% | 109 (48 to 167) |
| Kg not recorded | 2 | | | |
| **Males** | 7 | 536 | 1.3% | 5 (1 to 9) |
| <60 kg | 2 | 244 | 0.8% | 3 (-1 to 8) |
| 60-69 kg | 1 | 164 | 0.6% | 2 (-2 to 7) |
| 70-79 kg | 2 | 56 | 3.6% | 14 (-5 to 32) |
| >80 kg | 2 | 40 | 5.0% | 19 (-7 to 46) |
| **Symptomatic hyperlactataemia** | 84 | 1762 | 4.8% | 19 (15 to 22) |
| **Peripheral neuropathy** | 67 | 1762 | 3.8% | 15 (11 to 18) |
| **Lipodystrophy** | 43 | 1762 | 2.4% | 9 (7 to 12) |
| **Pancreatitis** | 2 | 1762 | 0.1% | 0 (0 to1) |
| **Total** | 275 | 1762 | 15.6% | 60 (54 to 67) |




**Graph 1. Incidence of lactic acidosis according to baseline weight in female persons living with HIV commenced on antiretroviral therapy at PSH between 2004 and 2007**

## *3.3 SPECTRUM OF CLINICAL PRESENTATION OF LA*

Of the 63 LA cases with complete records 59 (94%) presented with loss of weight (median loss of 6 kg, inter quartile range 4 to 8 kg), 45 (71%) with abdominal symptoms (nausea, anorexia, pain or distension), 15 (24%) with peripheral oedema and 8 (11%) with dyspnoea. Peripheral neuropathy was recorded in 44 (56%) cases and lipodystrophy in 35 (42%). Loss of weight was the only presenting symptom in 13 (21%) cases. A combination of loss of weight and abdominal symptoms occurred in 43 (68%) cases. LA occurred between eight and 16 months treatment duration in the majority (67/79) of cases (median 10 months, range 5 to 24 months).

Of the 79 LA cases the median serum lactate was 7.5 mmol/l (range 5.0 to 11.7) and mean serum $CO_2$ 17 (range zero to 19). 21 cases (27%) were severe, lactate >10 mmol/l or serum $CO_2$ <15 mmol/l. In a

subgroup of 65 LA cases in which a blood gas analysis had been performed, 49 (75%) had a pH <7.34 and 11 (17%) a pH<7.2.

The overall mortality from LA was 7.6% (6/79). All cases that died had a lactate of >10 mmol/L. Of the patients with lactate >10 forty percent (6/15) died. Seventy five percent (6/8) cases with a serum CO2 <15 mmol died. All cases with serum CO2 <10 mmol/l died. Fifty five percent (6/11) patients with pH <7.2 and 83% (5/ 6) with a pH<7.1 died. Four cases of LA occurred during pregnancy. All of these patients survived. 3 pregnancies were carried to term with babies were born alive and well. There was one intrauterine death.

All patients with stavudine toxicity were changed from stavudine to zidovudine without recurrence of lactic acidosis during the follow up period.

### *3.4 COMPARISON BETWEEN LA CASES AND CONTROLS AT BASELINE*

The clinical characteristics of cases with LA and controls were compared at baseline (Table 3). Female gender and weight were the only two parameters that emerged significantly different between the two groups.

**Table 3. Baseline characteristics of LA cases and controls at Port Shepstone hospital between 2004 and 2007 (n=79)**

| Characteristic | | | Analysis of matched pairs adjusted for gender and weight | | |
|---|---|---|---|---|---|
| | Cases | controls | n* | p value | AOR (95% CI) |
| Age (years)† | 35.8 | 35.9 | 70 | 0.929 | 1.0 (0.9 to 1.1) |
| Female gender | 91.1% | 70.5% | 70 | 0.006 | 5.3 (1.6 to 17.8) |
| Efavirenz regimen | 51.8% | 53.2% | 70 | 0.881 | 0.9 (0.3 to 2.6) |
| Previous ARVs | 12.7%% | 6.3% | 70 | 0.371 | 0.7 (0.3 to 1.5) |
| Diabetes | 3.8%% | 1.3% | 69 | 0.625 | ‡ |
| Currently pregnant | 5.1% | 0.0% | 68 | ‡ | ‡ |
| Hepatitis B surface antigen positive | 9.1% | 12.2% | 16 | 0.883 | 1.1 (0.3 to 4.4) |
| Weight (kg)† | 70.2 | 57.0 | 69 | 0.001 | 1.1 (1.1 to 1.2) |
| <60kg | | | | | Reference (1) |
| 60-70 kg | | | | 0.006 | 6.6 (1.7 to 25.0) |
| 71-80 kg | | | | 0.018 | 6.9 (1.4 to 34.3) |
| >80 kg | | | | 0.001 | 95.5 (6.9 to 1318.4) |
| CD4 (cells/µL)† | 126 | 113 | 70 | 0.677 | 1.0 (1.0 to 1.0) |
| Viral load (copies/ml)† | 167850 | 1812968 | 29 | 0.446 | 1.0 (1.0 to 1.0) |
| Haemoglobin (g/dl)† | 11.3 | 11.3 | 66 | 0.167 | 0.9 (0.7 to 1.0) |
| Alanine transaminase (iu/l)† | 30 | 26 | 60 | 0.511 | 1.0 (0.9 to 1.0) |
| Albumin (g/l)† | 34 | 32 | 56 | 0.338 | 0.9 (0.90 to 1.0) |
| Urea (mmol/l)† | 3.52 | 3.69 | 54 | 0.353 | 1.1 (0.89to 1.5) |
| Creatinine †(µmol/l) | 81 | 77 | 54 | 0.954 | 1.0 (0.9 to 1.0) |
| Cholesterol † (mmol/l) | 3.6 | 3.6 | 52 | 0.919 | 0.9 (0.6 to 1.7) |
| Triglycerides† (mmol/l) | 1.3 | 1.2 | 52 | 0.125 | 1.7 (0.9 to 3.3) |

* Only pairs with results were included in the analysis

†Mean values

‡ McNemar's chi square test could not be computed due to low numbers; Conditional logistic regression model could not be achieved due to small sample size

## *3.5 COMPARISON OF CASES AND CONTROLS SIX MONTHS INTO ART*

See table 4 for comparison of clinical variables between cases and controls six months into ART. A higher ALT or TRIG was significantly associated with LA. There was no correlation between the ALT and TRIG (Spearmen Correlation Coefficient = 0.083, p=0.372).

**Table 4. Comparison of selected parameters between LA cases and controls six months into ART, at Port Shepstone hospital between 2004 and 2007 (n=79)**

| | **Description of groups** | | **Analysis of matched pairs adjusted for gender and weight** | | |
|---|---|---|---|---|---|
| **Characteristic** | **Lactic acidosis cases** | **Matched Controls** | **n*** | **P value** | **AOR (95% CI)** |
| CD4 (cells/ul)† | 253 | 267 | 56 | 0.220 | 1.0 (1.0 to 1.0) |
| Log Viral load)† (copies/ml)† | 630 | 18096 | 47 | 0.488§ | 1.0 (1.0 to1.0) |
| Haemaglobin (g/dl)† | 12.9 | 13.3 | 57 | 0.221 | 0.8 (0.6 to 1.1) |
| Alanine tranaminase (IU/L)† | 44 | 33 | 52 | 0.029 | 1.0 (1.0 to 1.1) |
| >50IU/l | 30.2% | 9.2% | | 0.015 | 11.1 (1.6 to 77.7) |
| Urea (mmol/L)† | 3.5 | 3.6 | 55 | 0.828 | 1.1 (0.7 to 1.6) |
| Creatinine (umol/L) † | 74 | 73 | 55 | 0.806 | 1.0 (1.0 to 1.0) |
| Cholesterol †(mmol/L) | 4.5 | 4.2 | 51 | 0.111 | 1.6 (0.9 to 2.8) |
| Triglycerides (mmol/l)† | 1.41 | 0.88 | 52 | 0.039 | 8.7 (1.1 to 67.1) |
| <=0.5 | 10.5% | 23.9% | | | Reference (1) |
| 0.5-1 | 47.4% | 54.9% | | 0.117 | 3.9 (0.7 to 21.3) |
| 1.1-1.5 | 21.1% | 6.8% | | 0.043 | 11.2 (1.1 to118.6) |
| >1.5 | 21.2% | 14.1% | | 0.311 | 4.9 (0.2 to 103.2) |

* Only matched pairs with results were included in the analysis

†Mean values

§ Log value used in analysis

## *3.6 COMPARISON OF THE CHANGE IN SELECETED PARAMETERS BETWEEN CASES AND CONTROLSINTHEFIRST SIX MONTHSOF ARTY*

Changes in clinical parameters over 6 months of ART are compared between cases and controls in Table 5. An increase in TRIG, compared to a decrease in controls was nearly found to be significant (p=0.065).

**Table 5. Comparison of the median/mean change in characteristics in LA cases and controls over first six months of ART, at Port Shepstone hospital between 2004 and 2007 (n=79)**

| | | | **Analysis of Matched pairs adjusted for gender and weight** | | |
|---|---|---|---|---|---|
| | **Cases** | **Controls** | **n** | **P value** | **AOR (95% CI)** |
| Weight (kg) | 4 | 5 | 63 | 0.088 | 1.1 (1.0 to 1.2) |
| CD4 (cells/ul) | 102 | 153 | 56 | 0.319 | 1.0 (1.0 to 1.0) |
| Viral load (copies/ml) | 167220 | 1794872 | 20 | 0.753 | 1.0 (1.0 to 1.0) |
| Hb (g/dL) | 1 | 2 | 52 | 0.614 | 1.0 (0.8 to 1.1) |
| ALT (IU/L) | 14 | 6 | 39 | 0.306 | 1.0 (1.0 to 1.1) |
| By >20iu/l | | | | 0.758 | 1.5 (0.1 to 24.3) |
| Urea (mmol/l) | 0.02 | 0.09 | 45 | 0.561 | 0.9 (0.7 to 1.2) |
| Creatinine (umol/l) | -7 | -4 | 45 | 0.853 | 1.0 (1.0 to 1.0) |
| Cholesterol (mmol/l) | 1.4 | 0.6 | 42 | 0.149 | 2.2 (0.8 to 6.2) |
| Triglycerides (mmol/l) | 0.11 | -0.33 | 44 | 0.065 | 1.4 (1.0 to 2.1) |

# CHAPTER 4: DISCUSSION

This study was inspired by the large number of reports (2, 3, 4,5) of toxicity associated with anti-retroviral treatment, following the rollout of ART on a massive scale by the national department of health in SA. The drug most implicated was stavudine (2, 3, 4, 5). Here we describe the epidemiology, incidence, clinical presentation and risk factors associated with LA and go further to explore biochemical markers that are potential predictors of LA.

## *4.1 INCIDENCE OF STAVUDINE TOXICITY*

The incidence rate of **LA** (17/1000 pyrs) is higher in our cohort compared to the developed world (0.6-10/1000 pyrs) (6, 7). This is consistent with other local SA cohorts (4, 5, 8, 31).

The incidence of **SHL** (19/1000 pyrs) in our cohort is in the upper range reported in the developed world (3,5 to 20/1000 pyrs (6, 11, 22) and is similar to other local cohorts (4, 5, 8, 31). This is difficult to comment on due to the lack of a standardized definition for SHL (6, 12).

The incidence of **lipodystrophy and peripheral neuropathy** that warranted discontinuation of stavudine (9/1000 pyrs and 3.8/1000pyrs respectively) found in our cohort is lower than other local cohorts (3, 8). This may be due to the shorter duration of observation in our cohort, median of 18 months compared to 3 years (3, 8) and that peripheral neuropathy and lipodystrophy are late side effects (24).

## *4.2 POTENTIAL BASELINE RISK FACTORS FOR LA*

Female gender and increased body weight have been clearly associated with LA (2, 16). Our study again confirms **female gender** as a strong risk factor. The risk in females (incidence rate of 23/1000

pyrs) in our cohort, similar to other local studies (2), was about five times that in males (incidence rate of 5/1000 pyrs). The high incidence of LA in SA may be explained by the high dominance of females in SA cohorts (3, 4, 5) and also the high prevalence of female obesity in SA (41).

Our study also confirms **excess weight** as a strong risk factor for LA (2, 16, 32). The relationship between weight and LA, particularly in females, appears to be complex. A dramatic increase in incidence (Table 2, fig 1) and risk (Table 3) is observed when baseline weight exceeded 60kg, followed by a second exponential increase with baseline weights above 80kg. One might postulate that the increase in incidence with weights >60kg is due to the higher **dose of stavudine** recommended for this weight (16) keeping in mind that mitochondrial toxicity are dose related (33). However, the exponential increase in risk above 80kg is likely due to other factors. Interestingly we demonstrate that female patients <60kg who received 30mg stavudine also had a high incidence of LA relative to reports from the developed world. This suggests that further dose reductions should be considered to reduce the risk of LA. Clearly such a strategy will have to be balanced against the anti-viral potency of lower doses.

**Diabetics** and pregnancy are considered risk factors for LA. This risk might be related to weight. Due to small number of patients with these conditions we were unable to evaluate these risks.

The lack of association between **CD4 counts** and LA here is consistent with other similar case control studies (16, 29). This is in contrast to the finding of Mathews *et al* (2) and others (30). Possible explanations include study design issues and under-representation of subjects with higher CD4 counts in our cohort due to criteria for ART initiation in SA (40).

Age, baseline albumin, renal function, liver transaminase levels, lipid levels and HBV infection was not associated with LA in this study. This was consistent with some studies but contrary to others (2, 16).

## *4.3 PREDICTIVE LABORATORY MARKERS AFTER SIX MONTHS*

Since most cases of LA occur 6 to 18 months into ART (5, 16), we considered factors at the six months time point that may serve as predictors of LA.

We showed that an elevated **ALT** >50 IU/L six months into ART predicted increased risk for LA (Table 4). This finding was not unique to our study (16). The raised ALT may represent significant mitochondrial dysfunction manifesting as steatohepatitis.

Higher **triglyceride levels** at the six months time point (Table 4) also predicted higher risk for LA. An increase in TRIG value over six months was also nearly found to be nearly significant (Table 5). This is the first study to assess this as a possible risk factor for LA. The finding is not entirely unexpected if one considers that elevated TRIG levels have been previously associated with hyperlactataemia (18, 19). There was no correlation between the increase in ALT and TRIG levels, suggesting it is an independent predictor of risk. The risk may be related to the association between high TRIG and hepatic steatosis (metabolic syndrome), a common (possibly etiologic) feature of LA (refer to chapter 1, liver abnormalities and LA). The significance is difficult to interpret clinically, as the highest risk was for a TRIG in the upper normal range and became less, although still present, at values above this. This needs further investigation.

The actual, or change in, CD4 count and viral load at 6 months has been implicated as a predictor of LA by some (2,16). This was not supported by our study, which is consistent with others (16).

## *4.4 PRESENTING FEATURES OF LA CASES*

Clinical features of LA including; loss of weight (94% in our cohort), abdominal symptoms (71%) and the presence of other stavudine related toxicities such as peripheral neuropathy (56%) and lipodystrophy (42%) supports claims made by others (12, 16) that these are useful indicators of LA.

As observed by others the majority of patients presented between 8 and 16 months on treatment (range 5 to 24 months, median 10 months) are consistent with that of other studies (5, 16).

### *4.5 MORTALITY AND EARLY LA CASE IDENTIFICATION*

The mortality in this study was unusually low compared to similar large cases control studies (5, 16). This may be due to enhanced clinical vigilance, with earlier detection of LA cases following the report of a high incidence of LA in 2005 and 2006 (3, 5). Patients with loss of weight loss were routinely screened for LA from 2006 onwards at PSH. This is supported by the fact that most patients LA cases had lactate levels less than 10.

The associations of mortality, such as the level of lactate and severity of metabolic disturbance (17, 21) at presentation, were also observed here.

### *4.6 RECOMMENCEMENT OF ART*

Our study confirmed zidovudine as a safe alternative (4, 15, 17). Tenofovir is probably an even better alternative, given that it has even lower association with LA (42).

### *4.7 STUDY LIMITATIONS*

Being a retrospective study we had to contend with incomplete record keeping.

A small number of patients (5%) did not have baseline weights captured on the electronic database, and were therefore reported as unknown (Table 1). Due to the small number they had a negligible effect on the calculation of incidence according to weight bands (Table 2). Incomplete chart records were also noted.

Blood results were incomplete in both the case and control group.

Another limitation is that BMI could not be calculated since heights were not measured.

# CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

## *5.1 CONCLUSIONS AND RECOMMENDATIONS FOR CLINICAL CARE*

Clinical presentation is non-specific and requires a high index of suspicion. Timing of presentation and presence of other stavudine related toxicities are useful pointers to the diagnosis of LA.

This study provides supports the WHO guideline revision in 2006, recommending the reduction in dose of stavudine from 40mg to 30mg irrespective of weight (43). It also supports the WHO revision in 2010, to prefer the use of zidovudine or tenofovir in first line regimens (44), and the SA National guideline decision to replace stavudine with tenofovir, in the first line regimen in 2010 (45).

Female gender and excess weight are the main risk factors. Diabetes and pregnancy were not confirmed as risk factors in this study due to the low numbers. Stavudine should be used with extreme caution and with close monitoring, if at all.

Patients with raised ALT or TRIG levels at 6 months into ART should be carefully observed for development of LA. A strategy worth considering in such patients would be to commence regular lactate screening.

## *5.2 RECOMMENDATION FOR FURTHER STUDY*

The incidence of LA and other stavudine toxicities needs to be evaluated at the 30mg dose, particularly in higher risk groups. The correct dose of stavudine needs further evaluation, given that 20mg has been suggested to provide adequate antiviral efficacy (34).

ALT and TRIG levels at the 6 months time point, as a predictor of LA needs, further evaluation.

# APPENDIX

## *VARIABLES CAPTURED FOR LA CASES*

**Baseline variables**: ART initiation date, LA diagnosis date, NNRTI regimen (efavirenz / neviripine), age, gender (m/f), diabetes (y/n), pregnant (y/n), already on ART (y/n), weight, CD4 count, viral load, urea, creatinine, triglyceride, cholesterol, ALT and albumin levels.

**Variables at the six months follow up visit**: weight, CD4 count, viral load, urea, creatinine, triglyceride, cholesterol, ALT and albumin levels.

**Data captured at time of LA diagnosis**: date, duration of therapy, change in weight over last 4 months (kg), specific symptoms recorded in the clinical notes with regards to nausea (y/n), abdominal pain (y/n), abdominal distension (y/n), lipodystrophy (y/n), peripheral neuropathy (y/n), loss of appetite (y/n) and dyspnoea (y/n) or death (y/n). Biochemical features captured included; formal venous lactate, serum $CO_2$ and standard bicarbonate and pH level on the blood gas analysis.

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