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Risk Factors for Symptomatic Hyperlactatemia and Lactic Acidosis Among Combination Antiretroviral Therapy-Treated Adults in Botswana: Results from a Clinical Trial

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Abstract

Nucleoside analogue reverse transcriptase inhibitors are an integral component of combination antiretroviral treatment regimens. However, their ability to inhibit polymerase- γ has been associated with several mitochondrial toxicities, including potentially life-threatening lactic acidosis. A total of 650 antiretroviral-naive adults (69% female) initiated combination antiretroviral therapy (cART) and were intensively screened for toxicities including lactic acidosis as part of a 3-year clinical trial in Botswana. Patients were categorized as no lactic acidosis symptoms, minor symptoms but lactate <4.4 mmol/liter, and symptoms with lactate ≥ 4.4 mmol/liter [moderate to severe symptomatic hyperlactatemia (SH) or lactic acidosis (LA)]. Of 650 participants 111 (17.1%) developed symptoms and/or laboratory results suggestive of lactic acidosis and had a serum lactate drawn; 97 (87.4%) of these were female. There were 20 events, 13 having SH and 7 with LA; all 20 (100%) were female ($p < 0.001$). Cox proportional hazard analysis limited to the 451 females revealed that having a higher baseline BMI was predictive for the development of SH/LA [aHR = 1.17 per one-unit increase (1.08–1.25), $p < 0.0001$]. Ordered logistic regression performed among all 650 patients revealed that having a lower baseline hemoglobin [aOR = 1.28 per one-unit decrease (1.1–1.49), $p = 0.002$] and being randomized to d4T/3TC-based cART [aOR = 1.76 relative to ZDV/3TC (1.03–3.01), $p = 0.04$] were predictive of the symptoms and/or the development of SH/LA. cART-treated women in sub-Saharan Africa, especially those having higher body mass indices, should receive additional monitoring for SH/LA. Women presently receiving d4T/3TC-based cART in such settings also warrant more intensive monitoring.

Introduction

NUCLEOSIDE ANALOGUE REVERSE transcriptase inhibitors (NRTIs) are effective antiretroviral therapies for the treatment of HIV-1-infected adults and are central to effective combination antiretroviral therapy (cART). NRTIs continue to be a critical component of ART regimens as cART is rolled out in resource-limited settings with medications such as stavudine (d4T) being extensively used due to its favorable cost and formulation benefits.¹⁻³ Active intracellular anabolites of approved NRTIs block viral repli-

cation by competing with cellular deoxynucleotide triphosphates (dNTP) for incorporation into proviral DNA, and are specific for HIV-1 reverse transcriptase.⁴⁻⁶ However, their ability to inhibit human mitochondrial polymerase- γ , resulting in impaired synthesis of mitochondrial enzymes that generate ATP by oxidative phosphorylation, has been associated with several long-term mitochondrial toxicities.^{4,6,7} These include lactic acidosis, myopathy, pancreatitis, peripheral neuropathy, and lipodystrophy.⁴ Lactic acidosis is one of the most serious mitochondrial toxicities with a recently published systematic review documenting an

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overall case fatality rate of 48%.⁸ Symptomatic cART-treated patients identified as having significantly elevated serum lactate concentrations (greater than 10.0 mmol/liter) have a documented mortality rate of 80%.⁹

Higher rates of symptomatic hyperlactatemia (20.2 cases per 1000 person-years) and lactic acidosis (10.6 cases per 1000 person-years) have been reported among southern African cART-treated adults,¹⁰ particularly among overweight females.^{10–15} In addition to female sex and obesity, the use of the NRTIs d4T and/or didanosine (ddI), advanced age (greater than 40 years of age), pregnancy, prolonged cART duration, reduced creatinine clearance, and advanced immunosuppression (baseline CD4⁺ cell count values less than 100 cells/mm³) have also been shown to be risk factors for the development of lactic acidosis.^{8–18}

The Adult Antiretroviral Treatment and Drug Resistance (*Tshepo*) study was an open-label, randomized, factorial design study conducted at Princess Marina Hospital in Gaborone, Botswana. Preliminary findings have shown that cART-treated adults enrolled in the *Tshepo* study had higher than expected rates of lactic acidosis, specifically among overweight (body mass indices greater than 25) females.¹⁹ Herein, we report predictive model data of risk factors for the development of moderate to severe symptomatic hyperlactatemia/lactic acidosis for the completed study.

Materials and Methods

Study population

The Botswana cohort consisted of patients enrolled in the completed Adult Antiretroviral Treatment and Drug Resistance (“*Tshepo*”) study between December 1, 2002 and December 31, 2007.²⁰ *Tshepo* was an open-label, randomized, 3×2×2 factorial design study conducted at Princess Marina Hospital in Gaborone, Botswana evaluating the efficacy, tolerability, and incidence of drug resistance mutations among six different first-line cART regimens: zidovudine, lamuvidine, plus nevirapine (ZDV/3TC/NVP); zidovudine, lamuvidine, plus efavirenz (ZDV/3TC/EFV); zidovudine, didanosine, plus nevirapine (ZDV/ddI/NVP); zidovudine, didanosine, plus efavirenz (ZDV/ddI/EFV); stavudine, lamuvidine, plus nevirapine (d4T/3TC/NVP); and stavudine, lamuvidine, plus efavirenz (d4T/3TC/EFV). The study also compared two different adherence strategies: intensified adherence arm; standard-of-care plus community-based supervision (ComDOT) versus standard-of-care (SOC) to determine the optimal means of promoting adherence among adults receiving first-line cART.

All study participants were followed for 3 years with monthly scheduled study visits. There were two CD4⁺ cell count strata: (1) study participants either qualified for cART based on existing Botswana national antiretroviral (ARV) treatment guidelines,^{21,22} namely, an AIDS-defining illness and/or CD4⁺ cell count ≤200 cells/mm³, or they met (2) the study eligibility criteria of having a CD4⁺ cell count between 201 and 350 cells/mm³ and a corresponding plasma HIV-1 RNA level >55,000 copies/ml, which was consistent with consensus United States adult treatment guidelines at the time the study was designed.

The primary endpoints of the original trial were development of virologic failure with genotypic drug resistance and development of treatment-related toxicity, as defined by the

first incidence of a grade 3 or higher adverse event. Secondary endpoints were death for any reason and time to non-adherence (namely, an estimated adherence of less than 90%). Primary analyses of efficacy endpoints were performed on an “*intent-to-treat*” basis.

Inclusion criteria

Inclusion criteria were age 18 or greater; Karnofsky Score ≥50; permanent address within 20 km of the study site; positive HIV-1 parallel ELISAs; CD4⁺ cell count of ≤200 CD4⁺ cells/mm³ or a CD4⁺ cell count between 201 and 350 cells/mm³ and a plasma HIV-1 viral load >55,000 copies/ml; hemoglobin ≥8.0 g/dl, absolute neutrophil count ≥1.0×10³/mm³, creatinine level less than two times the upper limit of normal (≤200 μmol/liter), and serum SGPT (ALT) and SGOT (AST) values less than five times the upper limit of normal, 205 U/liter and 170 U/liter, respectively. Inclusion criteria for women of child-bearing potential included the following: nonpregnant, at least 6 months postpartum, and a willingness to maintain active contraception throughout the duration of the study.

Exclusion criteria

Exclusion criteria were presumed or confirmed visceral, but not mucocutaneous, Kaposi’s sarcoma and a diagnosis of ≥Grade 2 peripheral neuropathy within 1 month of enrollment.

All enrolled study participants underwent routinely scheduled safety (toxicity) monitoring. They had comprehensive chemistry and hematology specimens drawn as follows after cART initiation: scheduled comprehensive chemistry and hematology tests monthly for the first 6 months, then every 2 months for the next 6 months (6–12 months following cART initiation), and then every 4 months for the remainder of the study follow-up (12–36 months following cART initiation). In addition, all patients with any one or more out-of-range chemistry and/or hematology results were immediately called in for clinical assessment and confirmatory blood draw.

A serum lactate was immediately drawn on all cART-treated *Tshepo* study participants who developed one or more of the following symptoms and/or laboratory abnormalities suggestive of underlying lactatemia: grade 3 or higher SGPT (ALT) and/or SGOT (AST), grade 3 or higher LDH; serum bicarbonate level less than 20.0 mmol/liter, nausea/emesis, increased fatigue, dyspnea, muscle weakness, and/or paralysis of the lower extremities. Venous plasma lactates were obtained according to the AIDS Clinical Trials Group protocol²³ and were measured by a colorimetric assay using the Roche Integra 400 Plus (Roche Diagnostics, Mannheim, Germany).

Definitions

Moderate to severe symptomatic hyperlactatemia (SH). Screened patients found to have a serum lactate level greater than twice the upper limit of normal (≥4.40 mmol/liter) with associated symptoms (as described above), and based on additional laboratory testing (serum bicarbonate and/or arterial/venous pH testing) *without* evidence of acidosis, namely having a serum pH >7.35 and/or serum bicarbonate

>20 mmol/liter, were diagnosed as having moderate to severe symptomatic hyperlactatemia.

Lactic acidosis (LA). Screened patients found to have a serum lactate level ≥ 4.40 mmol/liter with **one or more** positive symptoms **with** evidence of acidosis, namely a serum pH < 7.35 and/or serum bicarbonate less than 20 mmol/liter, were diagnosed as having lactic acidosis.

Statistical considerations

We analyzed time to moderate to severe symptomatic hyperlactatemia (SH) or lactic acidosis (LA) using a Cox proportional hazard model, with the time to the SH or LA event being determined as the time of a first lactate test result of 4.40 mmol/liter or more. Twenty subjects had an event based on this definition. Subjects who did not have the event were censored at the end of the study or time lost to follow-up. Since SH or LA occurred only in women, we analyzed the risk for the development of SH/LA only among female *Tshepo* study participants ($n=451$ total). The model included age, body mass index (BMI), and the nucleoside backbone to which the study participants were randomized (ZDV/3TC versus ZDV/ddI versus d4T/3TC) as a priori defined risk factors. Age and BMI were included as continuous variables. Missing values of BMI [4 (0.06%) of 650] were imputed using multiple imputation.

In addition, we analyzed for the presence of clinical symptoms and/or laboratory abnormalities suggestive of possible underlying SH/LA (“symptoms”) and/or having an SH/LA event (“development of SH/LA”) in the entire study population ($n=650$) using ordered logistic regression. The outcome had three levels: (1) a subject was never screened for lactic acidosis ($n=539$ subjects); (2) a subject was screened because of suggestive clinical symptoms and/or laboratory abnormalities, but the laboratory test result never exceeded 4.4 mmol/liter ($n=91$ subjects); and (3) a subject was screened and the serum lactate result was greater than or equal to 4.4 mmol/liter ($n=20$ subjects). Nucleoside backbone (ZDV/3TC versus ZDV/ddI versus d4T/3TC), nonnucleoside type (NVP versus EFV), adherence strata (SOC versus ComDOT), age, sex, BMI, CD4⁺ cell count, viral load (plasma HIV-1 RNA), and hemoglobin were a priori chosen for inclusion in the ordered logistic model. Missing values of BMI (4), viral load (1), and hemoglobin (13) were imputed using multiple imputation. The viral load was log₁₀ transformed. Age, BMI, CD4⁺ cell count, viral load, and hemoglobin were included as continuous variables. Nonlinearity was assessed for these variables using restricted cubic splines; there was no evidence to suggest nonlinear relationships, so these variables were included as linear terms.

p-values less than 0.05 were considered to be statistically significant. Analyses were conducted using SAS statistical software (version 9.2, SAS Institute, Cary, NC) and R statistical software (version 2.9.0, www.r-project.org). The analysis code is available at <http://biostat.mc.vanderbilt.edu/ArchivedAnalyses/LacticAcidosis2011>.

This study was approved by the Health Research and Development Committee of the Botswana Ministry of Health, the Harvard School of Public Health Human Subjects Committee, and the Vanderbilt University Institutional Review Board.

Results

Baseline characteristics

Overall, 650 adults were enrolled, 451 (69.4%) of whom were female. The median age was 33 years (IQR 29–39). Forty-three percent had advanced WHO clinical disease (Stage 3 or 4) at the time of enrollment. The median CD4⁺ of the entire study population was 199 cells/mm³ (IQR 136–252). Study participants had a median baseline hemoglobin level of 11.6 g/dl (IQR 10.4–12.9) and median BMI of 21.3 (IQR 19.2–24.3), with 22% ($n=139$) having a baseline BMI of >25 and 19% ($n=121$) having a baseline BMI of <18.5 (Table 1). Of note, when broken down by sex, 26.4% (119 of 451) of the females compared to 10.1% (20 of 199) of the males had a baseline BMI of greater than 25. A higher proportion of males (69% versus 56%) had normal baseline BMIs (i.e., between 18.5 and 25). A total of 330 (50.8%) patients were enrolled in the lower CD4⁺ stratum (CD4⁺ cell count value less than or equal to 200 cells/mm³) and 320 (49.2%) patients were enrolled in the upper CD4⁺ cell count stratum (CD4⁺ cell count value between 201 and 350 cells/mm³); 325 participants were randomized to the intensified adherence (Com-DOT) arm.

Follow-up

Study follow-up was approximately 1774 person-years, with a median follow-up time of 156 weeks (IQR 156–157). Ninety-eight percent of all scheduled follow-up visits were attended. During the study, 54 (8.3%) of the 650 enrolled patients were lost to follow-up with regard to primary endpoint information. Of the 54, 26 (48%) had moved out of the study catchment area, 9 (16.7%) declined further participation, and for 19 (35.2%), no further information was available despite repeated attempts by the study team to contact them. The sociodemographic and clinical characteristics of participants who were lost to follow-up did not statistically differ from those who completed the trial.

Moderate to severe symptomatic hyperlactatemia and lactic acidosis outcomes

During the 3 years of follow-up, 111 study participants had a total of 208 serum lactate levels drawn during routine

TABLE 1. BASELINE CHARACTERISTICS OF THE STUDY POPULATION

<i>Characteristic</i>	<i>Median [IQR]</i>
Age	33.3 years [28.9–38.7]
Gender	451 female (69.4%) 199 male (30.6%)
CD4 ⁺ cell count (cells/mm ³)	199 [136–252]
≤ 200 cells/mm ³ (lower strata)	330 (50.8%)
201–350 cells/mm ³ (upper strata)	320 (49.2%)
Plasma HIV-1 RNA (copies/ml)	195,000 [70,350–473,750]
Hemoglobin (gr/dl)	11.6 [10.4–12.9]
Body mass index (BMI)	21.3 [19.2–24.3]
Less than or equal to 25	507 (78%)
Greater than 25	139 (22%)
Less than 18.5	121 (19%)
18.5–25	386 (60%)
Performance status (Karnofsky score)	97.9 \pm 5.2 ^a

^aValue reported as mean \pm standard deviation (SD).

TABLE 2. CHARACTERISTICS OF THE 20 FEMALE STUDY PARTICIPANTS AT THE TIME OF EVENT—MODERATE TO SEVERE SYMPTOMATIC HYPERLACTATEMIA (SH) OR LACTIC ACIDOSIS (LA)

Pt	Age	cART regimen	Duration of cART (months)	CD4 ⁺ cell count (cells/mm ³)	Weight (kg)	BMI	Lactate level (mmol/liter)	Diagnosis	Outcome
1	40	d4T, 3TC, EFV	6.8	234.1	91.5	31.9	6.5	LA/pancreatitis	Died from related causes
2	46	d4T, 3TC, EFV	7.7	706.5	100.0	34.8	5.1	LA	Survived
3	28	ZDV 3TC, EFV	7.7	112.3	62.0	23.7	4.9	SH	Survived
4	33	d4T, 3TC, EFV	7.8	194.5	65.0	25.1	7.9	SH	Survived
5	38	ZDV, 3TC, EFV	1.2	N/A	39.0	18.2	5.0	SH	Died from unrelated causes (pulmonary TB)
6	40	d4T, ddI, NFV	26.4	68.6	70.9	28.4	4.6	SH	Survived
7	49	d4T, 3TC, NVP	6.3	241.8	96.0	34.8	7.0	SH	Survived
8	54	d4T, 3TC, EFV	7.0	178.7	97.0	35.7	10.0	LA/pancreatitis	Died from related causes
9	37	d4T, ddI, NFV	25.5	180.8	68.8	29.4	15.3	LA/pancreatitis	Died from related causes
10	29	d4T, 3TC, NVP	0.6	202.0	57.5	29.7	7.9	SH	Died from unrelated causes (fulminant hepatic failure)
11	32	d4T, 3TC, NVP	9.0	534.0	92.6	33.2	5.1	SH	Survived
12	46	d4T, 3TC, EFV	8.1	527.2	63.0	28.2	10.4	LA	Survived
13	53	d4T, 3TC, EFV	10.0	225.9	79.9	29.7	5.2	SH	Survived
14	35	ZDV, ddI, EFV	8.5	372.8	89.5	35.0	8.2	LA/pancreatitis	Died from related causes
15	46	d4T, ddI, NVP	20.8	203.7	78.0	33.3	11.9	LA	Survived
16	32	ZDV, ddI, EFV	23.0	374.0	95.0	40.1	6.0	SH	Survived
17	45	ZDV, 3TC, EFV	15.4	287.0	84.0	30.9	6.9	SH	Survived
18	36	ZDV, ddI, EFV	24.0	548.0	60.0	21.0	5.4	SH	Survived
19	37	ZDV, 3TC, EFV	18.5	510.0	58.0	20.8	6.2	SH	Survived
20	31	d4T, 3TC, NVP	23.0	479.0	54.0	23.4	4.7	SH	Survived

ZDV, zidovudine; 3TC, lamivudine; ddI, didanosine; d4T, stavudine; NVP, nevirapine; EFV, efavirenz; NFV, nelfinavir.

screening for the presence of SH/LA. Fourteen (12.6%) of the 111 screened participants were male, with the remainder ($n=97$; 87.4%) being female. Twenty (19.8%) of these 111 patients had at least one serum lactate level that was greater than twice the upper limits of normal (≥ 4.40 mmol/liter), and therefore met our event definition. These 20 study participants having a defined study event had a total of 74 serum lactates drawn.

Of these 20 total screening events in which moderate to severe serum lactate results (≥ 4.40 mmol/liter) were obtained, 13 (65%) were classified as SH and 7 (35%) were diagnosed as LA (Table 2). All 20 (100%) were female. At baseline, the median age was 37 years (IQR 32–45 years), the median BMI was 28 (IQR 23–33), and the median time on cART was 8.5 months (IQR 7.2–20.4 months). At the time of their SH or LA event, these 20 patients had a median serum lactate level of 5 (IQR 3–6 mmol/liter). Six (30%) of the 20 study participants experiencing an event died, with five of the six deaths (83%) being deemed related to ARV treatment (four lactic acidosis and one fulminant hepatic failure). One death was unrelated to ARV treatment, as this patient died from complications of active pulmonary tuberculosis (Table 2).

Four of the seven (57%) patients diagnosed with LA died as a result of this acute event. Of note, all four of these patients also had a comorbid diagnosis of severe clinical pancreatitis (hemorrhagic) with each having clinical symptoms (nausea, vomiting, and/or abdominal pains) and grade 3/4 serum lipase elevations at the time of their demise. Three (75%) of the four patients experiencing mortality as a result of their lactic acidosis event had been receiving “D” drugs [namely, stavudine (d4T), didanosine (ddI), or both] prior to their death, with two having received d4T/3TC-based cART, one having received ddI/ZDV-based cART, and the fourth having received both ddI and d4T together as part of their second-line (protease inhibitor-containing) regimen (Table 2). Four of the seven (57%) patients diagnosed with LA also had a new diagnosis of grade 2 or higher peripheral neuropathy at the time their diagnosis of lactic acidosis was made.

Sex was a significant predictor of SH/LA as 4.4% (20/451) of females and 0% (0/199) of males had the event ($p < 0.001$, Fisher’s exact test). Among women, having a higher baseline BMI was predictive for the development of SH/LA (aHR = 1.17 per 1-unit increase; 95% CI: 1.08–1.25, $p < 0.0001$) (Table 3).

TABLE 3. RISK FACTORS FOR THE DEVELOPMENT OF MODERATE TO SEVERE SYMPTOMATIC HYPERLACTATEMIA (SH) OR LACTIC ACIDOSIS (LA) AMONG FEMALE STUDY PARTICIPANTS ($n=451$)

Risk factor	Adjusted hazard ratio (HR)	95% confidence interval	p-value
Age (per 10 year increase)	aHR=0.65	[0.28–1.52]	0.33
BMI ^a (per 1-unit increase)	aHR=1.17	[1.08–1.25]	<0.0001

^aRisk factors significantly associated ($p < 0.05$) with the development of SH/LA.

TABLE 4. RISK FACTORS FOR THE SYMPTOMS AND/OR THE DEVELOPMENT OF MODERATE TO SEVERE SYMPTOMATIC HYPERLACTATEMIA (SH) OR LACTIC ACIDOSIS (LA) AMONG ALL SCREENED STUDY PARTICIPANTS ($n=650$)

Risk factor	Adjusted hazard ratio (HR)	95% confidence Interval	p-value
NRTI backbone			
ZDV/3TC ^a	1.0	N/A	N/A
ZDV/ddI	1.49	[0.87–2.55]	0.15
d4T/3TC ^b	1.76	[1.03–3.01]	0.04
NNRTI backbone			
EFV ^a	1.0	N/A	N/A
NVP	0.92	[0.60–1.41]	0.71
Adherence arm			
SOC	1.0	N/A	N/A
ComDOT	1.20	[0.78–1.83]	0.41
Sex ^b			
Female ^b	2.11	[1.09–4.08]	0.028
Male ^a	1.0	N/A	N/A
Age (per 10 years)	0.93	[0.69–1.24]	0.60
BMI ^b (per 1 unit increase)	1.09	[1.04–1.14]	<0.001
CD4 ⁺ cell count (per 100 cells/mm ³ increase)	0.93	[0.72–1.21]	0.58
Log plasma HIV-1 RNA (per 1.0 log unit increase)	1.001	[0.70–1.43]	0.99
Hemoglobin ^b (per 1 unit (g/dl) decrease)	1.28	[1.1–1.49]	0.002

^aReference group; for dual NRTI comparisons = zidovudine (ZDV) plus lamivudine (3TC); for NNRTI comparisons = efavirenz (EFV); and for sex comparisons = male.

^bRisk factors significantly associated ($p < 0.05$) with the development of SH/LA.

ZDV, zidovudine; 3TC, lamivudine; ddI, didanosine; d4T, stavudine; NVP, nevirapine; EFV, efavirenz; SOC, standard-of-care; ComDOT, intensified adherence strategy, namely SOC plus community-based cART supervision; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor.

Additional analyses categorized outcomes as (1) no symptoms, (2) being “screened” for SH/LA due to symptoms but lactate < 4.4 , or (3) SH/LA. Female sex and higher baseline BMI were predictive of symptoms and/or the development of SH/LA in these analyses [adjusted odds ratio (aOR) = 2.11, 95% CI: 1.09–4.08, $p = 0.028$; and 1.09, 95% CI: 1.04–1.14, $p < 0.001$, respectively]. Randomization to d4T/3TC-based cART (aOR = 1.76 relative to ZDV/3TC; 95% CI: 1.03–3.01, $p = 0.04$) and having a lower baseline hemoglobin (aOR = 1.28 per 1-unit decrease; 95% CI: 1.1–1.49, $p = 0.002$) were also predictive of symptoms and/or the development of SH/LA (Table 4).

Subjects who survived their episode of SH or LA resumed ART with a regimen that included one of the following dual NRTI combinations (tenofovir/emtricitabine, tenofovir/lamivudine, abacavir/lamivudine, or tenofovir/abacavir) plus lopinavir/ritonavir (*Kaletra* or *Aluvia* when this heat-stable formulation became available). All patients continued to have plasma HIV-1 viral load levels below 400 copies/ml, along with excellent immunologic recovery.

Discussion

Preliminary data from Botswana and South Africa have shown that cART-treated adults have higher than expected rates of lactic acidosis (1.0–1.1%) when compared to rates previously described elsewhere.^{10–15,19,23} There also appears to be a strong association between the risk for this potentially life-threatening toxicity and sex and body habitus, with overweight (BMI > 25) females being at a significantly higher risk for toxicity.^{10–15,19,23} More in-depth studies are needed to further elucidate risk, as the strong association with female sex appears to be largely driven by baseline body compositional differences; approximately 2.5 times more females (26.4% of females

compared to 10.1% of males) had baseline BMIs greater than 25. Host genetic predisposition may also contribute to these distinct risk susceptibility differences, especially as risk for the development of lactic acidosis has been previously associated with genetic variation among those with inherited mitochondrial DNA disorders [i.e., mitochondrial encephalopathy, lactic acidosis, and stroke (MELAS) syndrome and the A3243G point mutation] as well as persons treated with other potentially causative medications (i.e., the association between the mitochondrial DNA A2706G polymorphism and linezolid-induced lactic acidosis and plasma lactate concentrations and SLC22A2 gene 808 G/T variants among metformin-treated adults).^{24–26}

Among urban Botswana cART-treated clinical trial participants, being female and overweight (i.e., having a higher baseline BMI) were strongly predictive for the development of SH or LA. As national care and treatment programs continue to expand in sub-Saharan Africa, it will be of paramount importance to establish and maintain longitudinal toxicity registries in order to monitor rates of these and other potentially life-threatening toxicities (i.e., nevirapine cutaneous hypersensitivity and hepatotoxicity, as well as tenofovir-associated bone mineral density abnormalities and renal toxicity). In the majority of sub-Saharan African countries, proportionately more females are receiving cART than men, even when accounting for the higher HIV-1 prevalence rates among women. In addition, as national programs scale up, higher proportions of cART-treated adults will be receiving care at primary and secondary-level healthcare facilities, where limited laboratory capacity exists for obtaining serum lactate levels. In such settings, until d4T prescription has been fully phased out, the routine use of validated point-of-care lactate devices for screening persons at risk for the development of SH/LA warrants serious consideration.

One potential limitation includes the possibility of selection bias, due to the fact that all patients experiencing an SH or LA event were female. Of note, all 650 patients randomized to receive cART in this clinical trial were intensively screened for the presence of ARV-related toxicities, including the development of lactic acidosis, as the development of a treatment-modifying toxicity (specifically, the first incidence of a grade 3 or higher adverse event) was a primary outcome of the study. The majority of patients (87.4%) undergoing additional screening were female, specifically, the 111 having signs/symptoms and/or laboratory abnormalities suggestive of underlying lactic acidosis and having a serum lactate level drawn. However, 14 males did have serum lactate levels drawn, with similar proportions of them (as compared to females) having clinical sign/symptoms and/or laboratory abnormalities as their triggering event, realizing that the presence of one or more grade 3 or higher laboratory events [SGPT (ALT), SGOT (AST), LDH; and/or serum bicarbonate level less than 20.0 mmol/liter] was entirely nonsubjective, i.e., meeting the threshold for additional screening based on these protocol-mandated laboratory cutoff values was all based on existing Division of AIDS toxicity/laboratory grading scales, thus limiting the chance for selection bias in this uniformly screened study population. Certainly, treating study clinicians could have had "last-case" bias when screening their next patient for the presence of lactic acidosis based on the clinical and/or laboratory characteristics of the patient they most recently cared for that did experience an SH or LA event (i.e., being female and/or overweight). We are also not able to compare SH/LA event rates among overweight cART-treated females in our study to those not receiving cART in our setting, as our team intensively monitored clinical trial participants only once they initiated cART (at the time of study enrollment/randomization).

In summary, being female and having a higher baseline BMI are predictive for the development of SH/LA. Our results suggest that d4T/3TC-based cART and anemia may also be associated with the symptoms and/or the development of SH/LA. In southern Africa, it may be prudent not to prescribe d4T-based cART to "at-risk" patients. Additional studies including an evaluation of host genetic factors are ongoing to further elucidate this risk.

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References

1. Gilks CF, Crowley S, Ekpini R, *et al.*: The WHO public-health approach to antiretroviral therapy against HIV in resource-limited settings. *Lancet* 2006;368:505–510.
2. Idigbe EO, Adewole TA, Eisen G, *et al.*: Management of HIV-1 infection with a combination of nevirapine, stavudine, and lamivudine: A preliminary report on the Nigerian antiretroviral program. *J Acquir Immune Defic Syndr* 2005;40(1): 65–69.
3. Laurent C, Kouanfack C, Koulla-Shiro S, *et al.*: Effectiveness and safety of a generic fixed-dose combination of nevirapine, stavudine, and lamivudine in HIV-1 infected adults in Cameroon: Open-label multicentre trial. *Lancet* 2004;364: 29–34.
4. Carr A and Cooper DA: Adverse effects of antiretroviral therapy. *Lancet* 2000;356:1423–1430.
5. Modica Napolitano JS: AZT causes tissue-specific inhibition of mitochondrial bioenergetic function. *Biochem Biophys Res Commun* 1993;194:170–174.
6. Brinkman K, ter Hofstede HJM, Burger DM, Smeitink JAM, and Koopmans PP: Adverse effects of reverse transcriptase inhibitors: Mitochondrial toxicity as a common pathway. *AIDS* 1998;12:1735–1744.
7. Lewis W and Dalakas MC: Mitochondrial toxicity of antiviral drugs. *Nat Med* 1995;1:417–422.
8. Arenas-Pinto A, Grant AD, Edwards S, and Weller IV: Lactic acidosis in HIV infected patients: A systematic review of published cases. *Sex Transm Infect* 2003;79(4):340–343.
9. Falco V, Rodriguez D, Ribera E, *et al.*: Severe nucleoside-associated lactic acidosis in human immunodeficiency virus-infected patients: Report of 21 cases and review of the literature. *Clin Infect Dis* 2002;34:838–846.
10. Bolhaar MG and Karstaedt AS: A high incidence of lactic acidosis and symptomatic hyperlactatemia in women receiving highly active antiretroviral therapy in Soweto, South Africa. *Clin Infect Dis* 2007;45(2):254–260.
11. Lactic Acidosis International Study Group: Risk factors for lactic acidosis and severe hyperlactatemia in HIV-1 infected adults exposed to antiretroviral therapy. *AIDS* 2007;21(18): 2455–2464.
12. Geddes R, Knight S, Moosa MY, Reddi A, Uebel K, and Sunpath H: A high incidence of nucleoside reverse transcriptase inhibitor (NRTI)-induced lactic acidosis in HIV-infected patients in a South African context. *S Afr Med J* 2006;96(8):722–724.
13. Fabian J, Venter WD, Mkhabela L, Levin JB, Baker L, and Naicker S: Symptomatic hyperlactatemia in adults on antiretroviral therapy: A single-centre experience. *S Afr Med J* 2008;98(10):795–800.
14. Osler M, Stead D, Rebe K, Meintjes G, and Boulle A: Risk factors for and clinical characteristics of severe hyperlactatemia in patients receiving antiretroviral therapy: A case-control study. *HIV Med* 2010;11(2):121–129.

15. Matthews LT, Giddy J, Ghebremichael M, *et al.*: A risk-factor guided approach to reducing lactic acidosis and hyperlactatemia in patients on antiretroviral therapy. *PLoS One* 2011;6(4):e18736.
16. Marceau G, Sapin V, Jacomet C, *et al.*: Frequency, risk factors, and outcome of hyperlactatemia in HIV-positive persons: Implications for the management of treated patients. *Clin Chem* 2003;49(7):1154–1162.
17. Bonnet F, Balestre E, Bernardin E, *et al.*: Risk factors for hyperlactatemia in HIV- infected patients, Aquitaine Cohort, 1999–2003. *Antivir Chem Chemother* 2005;16(1):63–67.
18. Boubaker K, Flepp M, Sudre P, *et al.*: Hyperlactatemia and antiretroviral therapy: The Swiss HIV Cohort Study. *Clin Infect Dis* 2001;33:1931–1937.
19. Wester CW, Okezie OA, Thomas AM, Bussmann H, Moyo S, *et al.*: Higher than expected rates of lactic acidosis among highly active antiretroviral therapy-treated women in Botswana. *J Acquired Immune Defic Syndr* 2007;46(3):318–322.
20. Wester CW, Thomas AM, Bussmann H, *et al.*: Non-nucleoside reverse transcriptase inhibitor outcomes among combination antiretroviral therapy-treated adults in Botswana. *AIDS* 2010;24(Suppl 1):S27–S36.
21. Botswana National ARV Programme (“MASA”) press release/circular dated September 2006, Vol. 20, Ministry of Health, Botswana; with updated country-wide HAART statistics.
22. Botswana Guidelines on Antiretroviral Treatment: Ministry of Health, Botswana. Anabwani G, Jimbo W (eds.); originally released 2002; revised and latest 2005 available in January 2005.
23. Adult Clinical Trials Group (AACTG) website: AACTG Toxicity Evaluation Group (TOX-EG) working reference document from the AACTG website, with specific instructions for obtaining plasma lactate levels, dated July 23, 2002; available at <http://aactg.s-3.com/>; last accessed November 2010.
24. Fayssoil A: Heart diseases in mitochondrial encephalomyopathy, lactic acidosis, and stroke syndrome. *Congest Heart Fail* 2009;15(6):284–287.
25. Carson J, Cerda J, Chae JH, Hirano M, and Maggiore P: Severe lactic acidosis associated with linezolid use in a patient with the mitochondrial DNA A2706G polymorphism. *Pharmacology* 2007;27(5):771–774.
26. Li Q, Liu F, Zheng TS, Tang I, Lu HJ, and Jia WP: SLCC22A2 gene 808 G/T variant is related to plasma lactate concentration in Chinese type 2 diabetics treated with metformin. *Acta Pharmacol Sin* 2010;31(2):184–190.

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