GSK Medicine: Abacavir, amprenavir, abacavir/lamivudine/zidovudine, Zidovudine, Lamivudine, abacavir/lamivudine, zidovudine/lamivudine, fosamprenavir

Study No.: WWE113329/WE490/EPI40230

Title: Lactic acid levels in children prenatally and perinatally treated with antiretrovirals to prevent HIV transmission.

Rationale: Nucleoside-analogue reverse-transcriptase inhibitors (NRTIs) are substrates for DNA polymerase, the enzyme required for replication of mitochondrial DNA (mtDNA). Decreased concentrations of mtDNA have been observed in cultured cells exposed to NRTIs, and in muscle cells from patients with zidovudine-induced myopathy. Mitochondrial damage is difficult to demonstrate clinically but measurement of lactic acid (LA) is an inexpensive and easy initial screening method. LA in plasma is a sensitive but non-specific marker of mitochondrial dysfunction. Most HIV positive pregnant women are now receiving anti-retroviral therapy (ART) during pregnancy for their own treatment and/or to prevent mother-to-child-transmission (MTCT) of HIV. Recent studies have shown that most of this treatment includes a NRTI backbone and that in more than 90% of cases, these combinations contain zidovudine or lamivudine.

Objectives: To describe and compare the level and occurrence of elevated LA between children from HIV positive (treated with different antiretrovirals) and negative mothers. To assess the association between prenatal and perinatal use of ART classes of drugs and the occurrence of elevated LA levels.

Indication: HIV

Study Investigators/Centers: One center, in Italy

Research Methods:

Data Source: Primary data collection of a clinical cohort of children

Study Design: Cross-sectional and Prospective Cohort Study

Study Population:

This study population comprised newborns of HIV positive and negative mothers, age 1 week to 3 months old, seen at the Pediatric HIV/AIDS Unit in the Department of Pediatrics at the University of Padova, Italy, for whom parental consent for study participation was obtained. The children were divided into two cohorts based on the HIV status of the mothers; children of HIV negative mothers served as controls. Children who presented with septicaemia, cardiomiopathy, inborn errors of metabolism or hypoxia (conditions affecting LA levels) were excluded from the study. Children who were first seen at ages older than 3 months were also excluded.

Study Exposures / Outcomes:

Study Exposures

Exposure was defined as ART administered either during pregnancy (prenatally) and/or during delivery (perinatally). Prenatal ART was categorized into the following drug classes: no antiretroviral therapy, protease inhibitors (PI), nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI). Exposure to prenatal medications within ART drug classes were: PIs – indinavir(IDV), nelfinavir(NFV), saquinavir(SQV), and ritonavir(RTV); NRTIs – zidovudine(AZT), lamivudine(3TC) + AZT, stavudine(d4T), d4T + 3TC, d4T + didanosine(ddI), and AZT + zalcitabine (ddC); NNRTIs - nevirapine(NVP). Perinatal ART exposure was defined as a bolus of AZT given at the time of delivery for HIV positive mothers.

Primary Study Outcome(s)

The primary outcome is the prevalence of an elevated LA level in the children, which is defined as ≥2.5 mmol/liter in a venous blood sample. At least one venous blood sample was taken after the first week of life but within 90 days of birth for HIV children and controls. Children from HIV positive mothers were followed with repeated LA and other measurements during each visit in the first year of life. Controls were followed only if abnormal lactate levels were found.

Secondary Study Outcome(s)

In addition to LA, blood was analysed for electrolytes (sodium, potassium), pyruvate, alanine aminotransferanse (ALT), aspartate aminotransferase (AST), and glucose. Hyperglycemia was a glucoses level ≥5.6 mmol/l, AST was considered abnormal above 80 U/L and ALT was abnormal above 55 U/L. Pyruvate measurements were performed only in children with elevated LA levels and were used to calculate a lactate/pyruvate ratio as this is used as a marker of the oxido-reduction state of the cells and of mitochondrial damage.

Data Analysis Methods:

Levels of LA and other laboratory tests were described by mean, median, standard deviation, and range. Comparison of these metabolic parameters between different groups of children was done by a Student's t-test and non-parametric

tests for non-normally distributed data.

Within the group of HIV negative infants born to HIV positive mothers, the association between exposure of any class of antiretroviral and LA levels was assessed by using multivariate logistic regression analysis that considered patients with first confirmed elevated LA level as cases and patients with normal LA levels as controls.

Study Results:

Sample Size and Baseline Characteristics

Data were collected on 132 children from HIV positive mothers and 23 children from HIV negative mothers (controls). Twenty-eight children of HIV positive mothers did not have a lactate level measurement and in the 104 with lactate levels, 4 had the first LA measurement more than 3 months after birth and were not included in the study. After all exclusions, the final study population comprised 100 children from HIV positive mothers, who had lactate levels measured within the first 3 months of birth, and 23 children from HIV negative mothers. Characteristics of children born to HIV positive mothers are shown in Table 1. Only 2 children were HIV positive and thus excluded from further analysis.

Table 1

Demographics/Baseline Characteristics*	HIV positive children / born to HIV+ mothers	HIV negative children / born to HIV+ mothers	Total N	%
Gender				
Male	2	52	54	54%
Female	0	46	46	46%
Ethnicity				
African	0	46	46	46%
African/ Europe	0	2	2	2.0%
European	2	46	48	48%
Asian	0	1	1	1.0%
South-American	0	3	3	3.0%
Feeding				
Breast	0	1	1	1.0%
Artificial	2	97	99	99.0%
Delivery Mode				
Caesarean section	1	82	83	83%
Vaginal	1	16	17	17%
TOTAL	2	98	100	100%

*Baseline characteristic data not available for control children

Table 2 shows the antiretroviral drug use during pregnancy and/or delivery for HIV positive mothers. Among HIV negative children more than 90% of the mothers had been treated during pregnancy, mostly with NRTIs.

Table 2

		HIV Negative Children		HIV Positive Children	Total (N)	Total (%)
ART therapy during pregnancy		**No.**	**%**	**No.**		
None		9	9.2%	2	11	11.0%
At least one drug		89	90.8%		89	89.0%
Protease inhibitor	no PI	82	83.7%	2	84	84.0%
	IDV	1	1.0%		1	1.0%
	NFV	10	10.2%		10	10.0%
	SQV	4	4.1%		4	4.0%
	RTV	1	1.0%		1	1.0%
NRTI	No NRTI	10	10.2%	2	12	12.0%
	3TC+AZT	61	62.2%		61	61.0%
	AZT	3	3.1%		3	3.0%
	AZT+ddC	1	1.0%		1	1.0%
	d4T	3	3.1%		3	3.0%
	d4T+3TC	17	17.3%		17	17.0%
	d4T+DDI	3	3.1%		3	3.0%
NNRTI	No NNRTI	56	57.1%	2	58	58.0%
	NVP	42	42.9%		42	42.0%
AZT bolus during delivery						
No		1	1.0%		1	1.0%
Yes		97	99.0%	2	99	99.0%
Total		**98**	**100.0%**	**2**	**100**	**100.0%**

PI= protease inhibitor; NRTI= anitiretroviral nucleoside reverse transcriptase inhibitor; NNRTI= antiretroviral non-nucleoside reverse transcriptase inhibitor; IDV=indinavir; NFV=nelfinavir; SQV=saquinavir; RTV=ritonavir; 3TC=lamivudine; AZT=zidovudine; ddC=zalcitabine; d4T=stavudine; ddi=didanosine; NVP=nevirapine.

Lactic Acid Levels

LA levels were significantly higher in HIV negative children born to HIV positive mothers who were treated with ART during pregnancy (mean 3.08; STD 1.57), when compared to controls (mean 1.65; STD 0.40) (T-test: $p<0.001$). LA levels were slightly higher in HIV negative children born to HIV positive mothers who were treated with ART during pregnancy (mean 3.08;STD 1.57) compared to children whose mothers were not treated with ART during pregnancy (mean 2.67; STD 1.57)(but just received AZT bolus at delivery); this comparison was not statistically significant (T-test: $p=0.45$) (Table 3).

Table 3

Cohort	N	Mean LA level mmol/l	STD	Maximum level	Outcome Comparison
LA in Children born to HIV- mothers (Control)	23	1.65	0.40	2.4	
LA in Children born to HIV+ mothers (Study Group)					
Mothers treated with ART in pregnancy	89	**3.08 ***	1.57	8.5	***p<0.001** compared to children born to HIV (–) mothers
Mother not-treated with HAART in pregnancy (excluding bolus AZT during delivery)	9	2.67 ┼	1.57	6.1	┼ p=0.45 compared to children born to HIV (+) mothers treated with ART

In most HIV negative children of HIV positive mothers, the LA levels were first measured in the second month after birth. LA levels decreased from a mean of 3.3 mmol/l in the first month to 2.4 mmol/l in the third month. Also, the prevalence of increased LA levels decreased from 67% in children whose LA levels were measured in the first month to 46% in children whose levels were measured in the third month. Overall, 54% of children had increased LA levels at the first measurement. The mean LA level of HIV negative children from HIV positive mothers who had elevated levels (≥2.5 mmol/l) was 4 mmol/l; the mean LA level of HIV negative children from HIV positive mothers with normal levels (<2.5 mmol/l) was 1.8 mmol/l and comparable with levels from control children.

In children with elevated LA levels, pyruvate was also assessed to compute the lactate-to-pyruvate ratio. All ratios were < 8, suggesting no major mitochondrial damage.

Change in LA levels over time was assessed in HIV negative children of HIV positive mothers who had elevated LA levels. Thirty percent of the children with elevated levels did not have follow-up LA measurements, but among those that did LA levels decreased with increasing age of the children. Among those that were reassessed within 6 months after birth, only 4 (10.8%) still had elevated LA levels. One year after birth all children had normalized LA levels.

Secondary Study Outcomes

Table 4 shows results of the secondary outcome variables for HIV negative children from HIV positive mothers by normal (< 2.5 mmol/l) and high (≥ 2.5 mmol/l) LA levels. High LA levels were associated with increased potassium levels, however, sodium, glucose and ALT did not statistically significantly differ by LA levels. AST was statistically significantly different by LA levels with non-parametric test results.

The prevalence of abnormal AST, ALT and glucose levels was low (from 0 to 8%) and not different by normal and high LA levels in HIV negative children born to HIV positive mothers. Among children with high LA levels a high percentage (4/50, 8%) had low glucose levels (<3.3 mmol/l) compared to children with normal LA levels (0/44, 0%); the prevalence difference was almost statistically significant (p=0.06).

Table 4

	N	Mean	STD	Median	Min	Max	Outcome Comparison between LA <2.5 mmol/l & ≥2.5mmol/l (p-value T-test/ Mann-Whitney)
Lactic Acid levels < 2.5 mmol/l							
Na+	44	137.3	2.6	138	128	141	
K+	44	5.1	0.5	5.1	3.9	7.2	
Glucose	44	4.6	0.5	4.5	3.3	6.0	
AST	43	35.9	34.9	30.0	19	256	
ALT	44	37.8	107	18.0	6	721	
Lactic Acid Levels ≥ 2.5 mmol/l							
Na+	52	138.2	2.1	138	134	143	0.053 / 0.13
K+	50	5.5	0.6	5.4	4.1	7.1	0.003 / 0.001
Glucose	50	5.2	5.7	4.4	2.4	44.0	0.49 / 0.37
AST	50	37.7	21.2	34	19	158	0.76 / 0.04
ALT	53	21.2	8.9	19	9	57	0.26 / 0.64

ART use and LA levels

Table 5 shows the association between ART treatment during pregnancy and LA levels among HIV negative children of HIV positive mothers. ART drug use was not statistically significantly associated with the occurrence of elevated LA levels. Use of NRTI's resulted in an increase of risk (adjusted Odds Ratio = 1.77; 95% CI 0.40-7.9), but the confidence intervals were wide to due the small size of the non users group.

Table 5

ART Tx during pregnancy	No elevated LA	Elevated LA	Odds Ratio	95% CI	Adjusted Odds Ratio*	95% CI
No PI	37 (84.1%)	45(83.3%)	ref.			
PI	7(15.9%)	9(16.7%)	1.06	036-3.1	0.93	0.27-3.2
No NNRT	25(56.8%)	31(57.4%)	ref.			
NNRTI	19(43.2%)	23(42.6%)	1.0	0.44-2.2	0.91	0.36-2.31
No NRTI	6(13.6%)	4(7.4%)	ref.			
NRTI	38(86.4%)	50(92.6%)	1.97	0.52-7.5	1.77	0.40-7.9

*Adjusted for other ART drugs and age at first measurement

Clinical Outcome

None of the children studied with elevated LA levels showed any clinical manifestation which could be associated to mitochondrial damage or any other type of disorder.

Limitations:
Baseline demographic data on type of delivery, gender and feeding were not available from the control group of children born to HIV negative mothers, making the comparison of both groups difficult. This study was performed in a single center in Italy thus limiting generalizability of findings to other populations of infants born to HIV positive mothers. The control group had a small sample size.
Conclusions:
Study results show that there is an increase of LA levels in most infants exposed to ART (including those exposed only perinatally and after delivery) as compared to controls, which is particularly evident in the first weeks of life. This increase is not associated with any clinical symptoms or increased levels of hepatic enzymes and other biochemical markers. LA levels decrease upon increasing age and are usually normal around 6 months of age. No significant association was found between increased LA and type of ART drug used during pregnancy, however, there is a slight trend toward increased risk following the use of NRTIs, although not significant. These data confirm that there is a transitory increase in LA levels in the first weeks of life in children exposed to ART for prophylactic mother-to-child-transmission, possibly reflecting slight mitochondrial damage. However, the lactate / pyruvate ratio (key marker of mitochondrial damage) was always normal and there was no association between increased lactate and clinical or biochemical impairment