Full title: **An early-infant HIV-risk score for targeted HIV testing at birth**

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Abbreviations:

HIV:	Human immunodeficiency virus
MTCT:	Mother to child transmission of HIV
CD4:	Cluster of differentiation 4
PMTCT:	Prevention of mother to child transmission of HIV
EID:	Early infant diagnosis of HIV
CHER:	Children with HIV Early Antiretroviral Therapy
cART:	Combined 3-drug antiretroviral therapy
PCR:	Polymerase chain reaction
VEID:	Very Early Infant Diagnosis of HIV study
KPTH:	Kalafong Provincial Tertiary Hospital
CRF:	Case report form
MUAC:	Mid-upper arm circumference
SAMRC:	South Africa Medical Research Council
SD:	Standard deviations
IQR:	Interquartile ranges
WHO:	World Health Organisation
VL:	HIV viral load
LDL:	Lower than detectable level
SBC/BIC:	Schwarz Bayesian Information Criterion
ROC:	Receiver-operating curve
FDC:	Fixed dose combination
PROM:	Prelabour rupture of membranes
UTI:	Urinary tract infections
ANC:	Antenatal care
PET:	Pre-eclampsia
TB:	Tuberculosis
IUGR/SGA:	Growth restriction or small for gestational age
TAT:	Turnaround times

EMTCT: Elimination of mother to child transmission of HIV

Table of Contents Summary:

Risk-score models for birth HIV acquisition were developed in this study, and the feasibility of targeted compared to universal birth PCR testing was evaluated.

What's Known on This Subject:

Early HIV testing at or shortly after birth is needed to guarantee timely HIV treatment success for very young infants, but universal HIV PCR testing is expensive.

What This Study Adds:

Models to identify infants at risk for intra-uterine HIV infection were developed. These findings could guide a targeted approach to birth HIV-testing. Targeted PCR testing to diagnose HIV infection in very young infants requires access to maternal viral load testing.

*Author contributions***:**

Prof Du Plessis conceptualized and designed the methodology, developed the software for data collection, collected data, carried out the formal analysis, drafted the original manuscript, and reviewed and edited the final manuscript.

Dr Muller carried out the formal analysis, assisted with data curation, drafted the original manuscript, and reviewed and revised the final manuscript.

Profs Avenant and Pepper conceptualized the study, designed the methodology, critically reviewed the manuscript for important intellectual content, and edited the final manuscript

Prof Goga conceptualized and designed the methodology, assisted with developing the software for data collection, collected data, drafted the original manuscript, and reviewed and edited the final manuscript.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

ABSTRACT

Background

Early HIV testing is needed for treatment success in young infants, but universal testing is expensive. In this study, we examined the feasibility of early-infant HIV-risk scores for targeted PCR testing and early HIV diagnosis.

Methods

A cross-sectional HIV-exposed newborn-cohort was enrolled and PCR tested within 72 hours. We quantified associations between HIV-infection and clinical and laboratory maternal-infant parameters by logistic regression models and determined sensitivity and specificity for derived risk scores.

Results

From August 2014 to December 2016 1759 were enrolled. Mothers without ANC (5.7% (97/1688)) were more likely to deliver PCR-positive newborns (p=0.0005). One-in-five mothers (217/990, 21.9%) had VL > 1000 copies/µL. 432/1655 (26.1%) babies were preterm. LBW was documented in 398/1598 (24.55%) and 13/31 (40.63%) of PCR-negative and -positive newborns, respectively (p=0.0329). 204/1689 (12.08%) were IUGR/SGA, 6/37 (16.22%) PCR-positive. Symptomatic newborns frequently tested positive (p=0.0042). The HIV PCR positivity rate was 2.2% (37/1703).

Two-risk (cART duration, VL), three-risk (cART duration, -VL, symptomatic newborn), and four-risk (cART duration, -VL, symptomatic, SGA newborn) models for HIV-acquisition had predictive probability of 0.28, 0.498, and 0.57 respectively; this could guide targeted birth testing. However, using the three- and four-risk scores (probability 0.02 and 0.04), 20% and 24% will be missed compared with universal testing.

Conclusion

Targeted newborn testing requires access to maternal VL. Even if risk models include parameters such as maternal cART history, birthweight, gestation and symptoms, one-in-five infected newborns will not be targeted. At present; we support universal PCR testing at birth within the South African PMTCT context.

INTRODUCTION

Human immunodeficiency virus (HIV) can be vertically transmitted from mother to child (MTCT) antepartum, during labour and delivery, or postpartum via breastfeeding. [1]. Despite the success of prevention of MTCT (PMTCT) programs in reducing intrapartum HIV infections [2, 3] in South Africa, proportionately more babies are being born with HIV, due to in-utero transmission [4].

Early infant diagnosis (EID) and treatment of HIV remains a global public health priority. During the Children with HIV Early Antiretroviral Therapy (CHER) trial, infants were randomly assigned to receive deferred therapy versus early combined 3-drug antiretroviral (cART) therapy at a median age of 7.4 weeks [5]. Early HIV diagnosis and antiretroviral therapy, irrespective of clinical stage or CD4 cell count, reduced infant mortality by 76%, and HIV progression by 75% [5]. Whilst EID has focussed on testing exposed infants between the ages of 4 and 6 weeks, more recently birth testing has been considered feasible [6]. Birth testing, using polymerase chain reaction (PCR), can detect in-utero infection and allows for timeous initiation of treatment [7], which is vitally important for these infants who have rapid disease progression and high mortality rates compared to infants who are infected intrapartum [8].

In 2013 and 2015, the South African Department of Health adopted updated guidelines for infant HIV testing to identify all HIV-infected infants as early as possible [3]. The guidelines included HIV-PCR testing at 6 weeks of age and testing of symptomatic or high-risk HIV-exposed infants any time after birth [9]. Additional testing guidelines were added in January 2015 to include PCR testing at birth for high-risk infants, then in June 2015, birth HIV-PCR testing of all HIV-exposed infants together with 10-week PCR testing was recommended [9, 10].

In this study, we tested the feasibility of using models to identify high-risk newborns for intra-uterine HIV-infection for targeted PCR testing. Parameters were extracted from individual or combined clinical and laboratory data. We compare the number of newborn HIV infections identified using a universal and a targeted HIV testing approach at Kalafong Provincial Tertiary Hospital in Pretoria, South Africa.

PATIENTS AND METHODS

A cross-sectional sample of HIV-exposed newborns were recruited to the study. Data abstraction and HIV testing occurred within 72 hours of birth.

Data collection procedures

Trained research staff recruited patients. Researchers identified mothers with known HIV positive or negative status, and mothers of unknown HIV status, daily, from Monday through Friday during working hours, in the obstetric unit pre- and post-delivery. Mothers who were HIV-negative were sent for HIV counselling and testing if their last test was done before 32 weeks gestation, in accordance with national protocols.

Researchers interviewed mothers, and recorded data onto a case report form (CRF). Researchers reviewed medical records for maternal HIV results, infant birth weight, duration of labour, and PMTCT interventions. The following infant parameters were documented: birth history; anthropometric data: weight, length, head circumference, mid-upper arm circumference (MUAC); and the presence or absence of symptoms (*see S1 Appendix for definitions*) that included failing to thrive (includes LBW), birthweight $\leq$ 2.5 kg, congenital pneumonia, hepatosplenomegaly, oral candidiasis, significant lymphadenopathy, and any opportunistic infections. Maternal characteristics documented included antenatal care, labour history, PMTCT interventions and the timing thereof, the last documented maternal viral load,

in accordance with the current South African PMTCT guideline, antiretroviral drug history, and compliance. Poor compliance was defined as <95% dose compliance using self-reported data or visual analogue scale.

Research nurses or qualified medical doctors working at KPTH drew blood from HIV exposed infants, which was tested for HIV infection using total nucleic acid PCR (TNA PCR).

The laboratory reported all HIV PCR positive results to study investigators on a daily basis. Newborns with positive or indeterminate HIV PCR results were referred to HIV clinicians for specialised care and managed by the Immunology (HIV) clinic in accordance with national guidelines. Mothers with high HIV viral loads or other medical problems were referred to the relevant adult services.

Ethical and legal considerations

Management at KPTH granted permission to conduct the study, and the University of Pretoria, Faculty of Health Sciences Research Ethics Committee gave ethical clearance (protocol 285_2014). Each mother gave written informed consent upon enrolment in the study.

CRFs were entered directly into the REDCap (Research Electronic Data Capture, https://www.project-redcap.org/) system hosted at the South Africa Medical Research Council (SAMRC).

Data analysis

Data were analysed using statistical software SAS (version 9.4 TS1M5). Continuous data were expressed as means and standard deviations (SD) or as medians and interquartile ranges (IQR) for skewed distributions. Discrete or categorical data were summarised using frequencies and percentages. Normally distributed data were compared using independent t-tests, otherwise non-parametric alternatives were used.

The weight-for-age z-scores were calculated using CDC 2000 Growth Charts adjusted for gestational age for preterm infants and World Health Organisation (WHO) 2006 growth charts from the Multicentre Growth Reference Study for term infants.. We used univariate and multivariate logistic regression models to identify associations between HIV infection, and maternal and infant parameters. The following predictor variables were considered: preterm gestational age (yes = 1, no = 0), low birth weight < 2,5kg (yes = 1, no = 0), maternal HIV viral load (VL) value (lower than detectable level (LDL) = 1, <1000 =2, ≥1000 =3), maternal HIV viral load (VL) value (LDL = 1, <1000 =2, 1000 – 10 000 =3, >10 000 = 4), maternal HIV seroconversion after 32 weeks gestation (yes = 1, no = 0), maternal cART duration at birth < 4weeks (yes = 1, no = 0), small-for-gestational age (yes = 1, no = 0), maternal tuberculosis (yes = 1, no = 0), symptomatic (yes = 1, no = 0), maternal CD4 cell count value (<200 cells/mm3 = 1, 200-500 cells/mm3 = 2, >500 cells/mm3 = 3). Multivariate regression was initially done on a saturated model (full model) and thereafter reduced to 2-, 3-, and 4-risk models. Model significance was measured with p-values of 0.05 and 0.25.

After fitting logistic regression models, the variables with the highest predictive value were selected using Schwarz Bayesian Information Criterion (SBC or BIC). The performance of the selected models was evaluated using the C-index, or the area under the receiver-operating curve (ROC). We determined the derived risk scores, sensitivity and specificity, as well as false positive and false negative rates for various cut-off values. We developed probability models with the end-point of a positive birth HIV PCR using logistic regression of univariate and multivariate characteristics and risk factors. For modelling purposes, only positive and negative PCR test results were included.

RESULTS

Study population

Between August 2014 and December 2016, 15175 live babies were born at KPTH, 3356 (22.12%) of these to HIV-infected mothers (Figure 1). Informed consent was obtained from 1759/1911 (92.05%) eligible patients. Patients with birth HIV PCR test results were included.

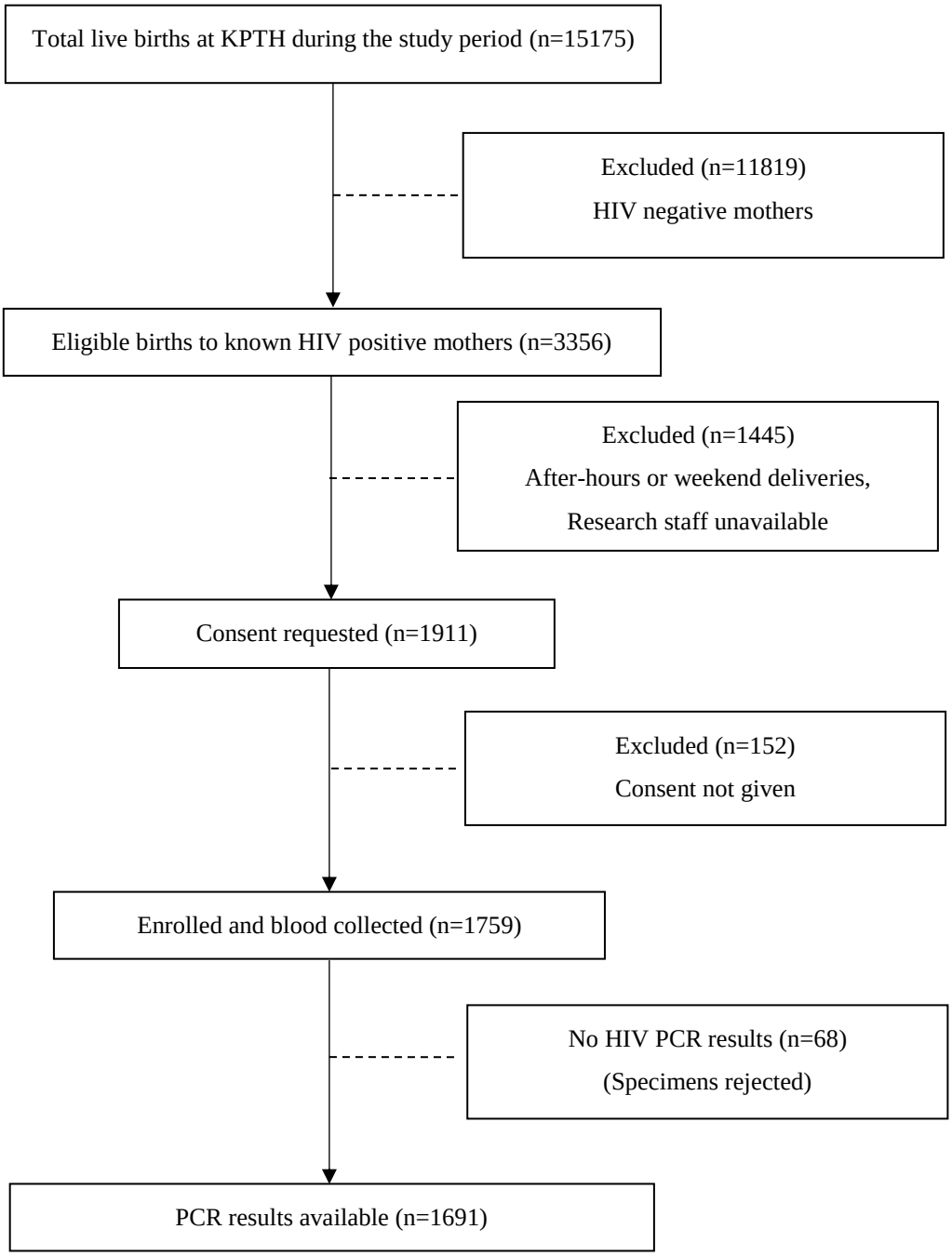

Figure 1: Consort diagram showing the selection of enrolled patients with HIV PCR test results.

PCR: polymerase chain reaction

Maternal characteristics

Almost half of HIV positive mothers were in their thirties (854/1759, 48.55%) and most had a secondary education (1557/1759, 88.52%). Although most women were not legally married

(1370/1706, 80.30%) almost half received monetary support from a partner (809/1726, 46.87%) and just over a third were employed (599, 34.70%). Almost all mothers reported having access to a cell phone (1625/1720, 94.48%) and household assets such as a television (1415, 82.28%) and fridge (1252, 72.79%) (Table 1).

Most mothers knew their HIV status before delivery (98.77%) and were on cART (1626/1704, 95.29%) namely fixed dose combination (FDC) treatment (1473, 90.09%), with 105 (7.09%) having received FDC for less than one month before delivery. Close to a quarter of HIV positive mothers (376/1687, 22.25%) seroconverted towards the end of pregnancy (> 32 weeks' gestation). Using laboratory data, we could trace HIV viral loads within three months of delivery for 935 (53.16%) mothers. Similarly, we could trace CD4 cell counts for 1196 (67.99%) mothers for the preceding 6-months. We recorded 562 (60.11%) mothers with undetectable HIV viral load (LDL), indicating virological control. About 1 in 5 mothers (208, 22.24%) had an HIV viral load level of more than 1000 copies/mL documented within 3 months before delivery. We observed mild immunological suppression in 588 (49.16%) and 394 (32.94%) mothers who had CD4 counts between 200 and 500 cells/mm^3, and > 500 cells/mm^3, respectively. Maternal HIV viral load and maternal CD4 cell count values were negatively correlated -0.103 (p=0.0053).

Very few mothers had tuberculosis (21/1730, 1.21%), most of whom (13, 65%) were being treated for tuberculosis. Only 5.75% (97/1688) of mothers had no ANC visits, the median visits being 4 (IQR 3 – 5 visits). Babies with positive HIV PCR results were associated with mothers who had no ANC visits (p=0.0005) (Table 1). Babies with positive HIV PCR results were associated with mothers who had mean VLs of 53424 (SD = 116854) copies/mL, while babies with negative HIV PCR results were associated with mothers who had mean VLs of 11361 copies/mL (SD = 66185) (p=0.0511).

Table 1: Descriptive statistics of maternal, general and HIV-related variables of the cohort and of the mother-infant pairs with positive HIV PCR tests, Kalafong Provincial Tertiary Hospital, August 2014 – December 2016.

Variables	**Modalities**	**Birth cohort* n (%)**	**PCR positive n/N (%)**	**p-value** (pos vs neg)**
General maternal information				
Maternal age at delivery (yrs) (N=1729)	<= 20 21-25 26-29 30-39 >= 40	81 (4.68%) 295 (17.06%) 401 (23.19%) 854 (49.39%) 98 (5.67%)	5/81 (6.17%) 5/295 (1.69%) 11/401 (2.74%) 13/854 (1.52%) 0/98 (00.00%)	0.0202
Level of education (N=1638)	No education Primary Secondary Tertiary	20 (1.22%) 121 (7.39%) 1495 (91.27%) 2 (0.12%)	1/20 (5.00%) 3/121 (2.48%) 30/1495 (2.01%) 0/2 (0.00%)	0.4087*
Antenatal visits (N=1566)	No ANC visits 1-2 visits 3-5 >5	90 (5.75%) 300 (19.16%) 867 (55.36%) 341(21.78%)	7/90 (7.78%) 11/300 (3.67%) 11/867 (1.27%) 3/341 (0.88%)	0.0005
Maternal HIV diagnosis				
Maternal HIV status known at birth (N=1630)	Yes No	1610 (98.77%) 20 (1.22%)	30/1610 (1.86%) 3/20 (15.00%)	0.0068*
Seroconversion after 32 weeks of gestation (N=1618)	Yes No	360 (22.25%) 1258 (77.75%)	10/360 (2.78%) 22/1258 (1.75%)	0.2047*
Maternal HIV treatment and compliance				
On cART at birth (N=1635)	Yes No	1558 (95.29%)* 77 (4.71%) **1473 (90.09%) of cART were FDC*	27/1558 (1.73%) 6/77 (7.79%)	0.0036*
cART duration <4 weeks at birth (N=1481)	Yes No	105 (7.09%) 1377 (92.91%)	7/105 (6.67%) 20/1377 (1.45%)	0.0020*

Variables	**Modalities**	**Birth cohort* n (%)**	**PCR positive n/N (%)**	**p-value** (pos vs neg)**
Self-reported compliance to cART (%) (N=1290) Median = 96% IQR 1% – 98%	0-9 10-19 20-89 90-100	68 (5.27%) 7 (0.54%) 172 (13.33%) 1043 (80.85%)	3/68 (4.41%) 2/7 (28.57%) 0/172 (0.00%) 16/1043 (1.53%)	0.0312*
Maternal viral load and CD4 parameters				
Maternal HIV viral load ≤ 3 months of delivery (absolute value, copies/mL) (N=935)	LDL ≤ 1000 ≥ 1000	562 (60.11%) 165 (17.65%) 208 (22.24%)	1/562 (0.18%) 7/165 (4.24%) 27/208 (12.98%)	< 0.0001
Maternal CD4 count within 6 months of delivery (cells/mm3) (N=1196)	< 200 200-500 > 500	214 (17.89%) 588 (49.16%) 394 (32.94%)	12/214 (5.61%) 12/588 (2.04%) 7/394 (1.78%)	0.0089

**Birth cohort includes all enrolled patients. **p values were calculated between the PCR-positive and PCR-negative study groups of the cohort using either *Fisher or Chi-square calculations.*
PCR: polymerase chain reaction, ANC: antenatal care; PROM: preterm rupture of membranes; cART: combination antiretroviral therapy; LDL: lower than detectable levels; TB: tuberculosis

Infant characteristics

Of the infants enrolled in the study, 53.16% (935) were boys. More than a quarter of enrolled infants (432/1655, 26.10%) were born at less than 38 (37 completed) weeks, with a median gestation of 35 weeks (IQR 32-36 weeks) (Table 2).

Table 2: Descriptive statistics of infant characteristics of the cohort and PCR-positive babies born to mother that are HIV positive, Kalafong Tertiary Provincial Hospital, August 2014 – December 2016.

Variables	**Modalities**	**Birth cohort n (%)**	**PCR positive n/N (%)**	**p-value**
Gestational age at birth (weeks) (N=1588)	< 34 weeks	105 (6.61%)	3/105 (2.86%)	0.0015 (Fisher)
	34 – 37 weeks	209 (13.16%)	10/209 (4.78%)	
	>=38 weeks	1526 (96.10%)	19/1526 (1.25%)	
Any birth symptom/sign (N=1689)	IUGR/SGA	204 (12.08%)	6/204 (2.94%)	0.0042
	Pneumonia	28 (1.66%)	1/28 (3.57%)	
	Anaemia	5 (0.30%)	0 (0.00%)	
	Thrombocytopaenia	2 (0.12%)	1/2 (50.00%)	
	Hepatomegaly	2 (0.12%)	0 (0.00%)	
	Splenomegaly	1 (0.06%)	1/1 (100.00%)	

IUGR/SGA: intra-uterine growth restriction / small-for-gestational age

Babies had a mean birth weight of 2.83kg. Low birth weight (<2.5kg) was documented in 398/1598 (24.55%) of the HIV PCR-negative infants and 13/32 (40.63%) in the positive group (p=0.0329 (Chi-square)). The median z-score for weight was -0.33 (IQR -1.06 – 0.38).

Fewer than 15% (14.33%) of enrolled infants displayed clinical symptoms at birth. No newborns had generalised lymphadenopathy or extensive oral candidiasis. Evidence of growth restriction or small for gestational age (IUGR/SGA) were documented in 204/1689 (12.08%) of the enrolled infants, of whom six were PCR positive. Pneumonia and anaemia were documented in 1.7% and 0.3% of infants, respectively (Table 2). Newborns that were symptomatic more frequently tested HIV positive (p=0.0042).

HIV PCR results

Of the 1691 infants with birth PCR results, 31 (1.8%) were HIV positive, 1646 (97.3%) were negative, and 14 (0.83%) were indeterminate; 3.87% samples were rejected (68/1759). According to the National HIV testing protocol, the indeterminate HIV PCR results were

repeated in 12 of the 14 patients, two infants could not be traced. Half of the repeated HIV PCR tests (6/12) tested positive and half negative, increasing the positivity rate to 2.2% (37/1703).

The mean turnaround times (TAT) for the PCR results was 68.27 hours (IQR 46.05 – 93.88 hours).

HIV-risk score models

Univariate (unweighted) model

We used univariate regression models to identify associations between HIV PCR positive outcome, and ten infant and maternal characteristics.

Newborns with positive PCR results were significantly associated with maternal viral load levels <1000 (OR = 26.53; 95% CI 1.353 to 520.310, p=0.002) and ≥1000 (OR = 123.67; 95% CI 7.385 to >999.99); maternal cART duration less than 4 weeks (OR = 0.146, 95% CI 0.057 to 0.373; p<0.0001); and symptomatic newborns (p = 0.237, 95% CI 0.100 to 0.561; p=0.0011).

Multivariate models

The saturated multivariate regression model only identified two significant risk factors (p<0.05), namely maternal viral load (p=0.0002) and symptomatic newborns (p=0.02). We retained variables with a p < 0.25 in further models to increase predictive value; therefore, cART < 4 weeks (p=0.17) and SGA (p=0.25) were added to risk models. These four parameters were combined in 2-, 3- and 4-risk regression models. Table 3 illustrates risk models, starting with the individual unweighted model, followed by two 2-risk models (model 1 & 2), a 3-risk (model 3) and a 4-risk (model 4) model and finally the full model that used nine parameters in one weighted model. Maternal HIV viral load and infant symptoms were the first risks in the 2-risk model, whilst the second 2-model score modelled 2 maternal parameters, maternal viral load and duration of maternal cART. The 3-risk model incorporated maternal viral load, cART

duration, and infant symptomatology; the 4-risk model added infant size for gestational-age to the mentioned 3 risks.

Table 3: Associations between maternal and infant characteristics and weighted newborn HIV acquisition in HIV-exposed infants in the Very Early Infant Diagnosis of HIV study (VEID) study

Characteristics	**Unadjusted OR (95% CI)** ***P-value***	**Adjusted OR Model 1** ***P-value***	**Adjusted OR Model 2** ***P-value***	**Adjusted OR Model 3** ***P-value***	**Adjusted OR Model 4** ***P-value***	**Adjusted OR Full model** ***P-value***
Preterm gestational age	0.55 (0.23 to 1.30) *0.17*	-	-	-	-	0.51 (0.13 to 1.98) *0.33*
Low birth weight (LBW)	0.42 (0.18 to 1.01) *0.05*	-	-	-	--	2.11 (0.40 to 11.06) *0.38*
Maternal VL (1) (LDL/<1000/≥1000)						
Comparing values:						
<1000 vs LDL	26.53 (1.35 to 520.31)	24.48 (1.27 to 472.49)	30.92 (1.62 to 590.21)	28.11 (1.50 to 527.33)	27.84 (1.55 to 501.43)	25.05 (1.74 to 359.98)
≥1000 vs LDL	123.67 (7.39 to >999.99) *0.0002*	103.17 (6.22 to >999.99) *0.0005*	141.50 (8.66 to >999.99) *0.0002*	117.69 (7.28 to >999.99) *0.0004*	113.82 (7.32 to >999.99) *0.0004*	100.45 (7.98 to >999.99) *0.0002*
Maternal HIV seroconversion >32 week's gestation	0.71 (0.28 to 1.79) *0.46*	-	-	-	-	0.81 (0.28 to 2.28) *0.68*
Maternal cART duration <4 weeks	0.15 (0.06 to 0.37) *<0.0001*	0.31 (0.12 to 0.83) *0.02*		0.45 (0.16 to 1.29) *0.14*	0.42 (0.14 to 1.24) *0.12*	0.46 (0.15 to 1.39) *0.17*
Small-for-gestational age	0.48 (0.19 to 1.23) *0.12*	-	-	-	0.66 (0.22 to 1.97) *0.46*	0.51 (0.16 to 1.60) *0.25*

Maternal TB	0.94 (0.05 to 18.32) *0.97*					1.20 (0.04 to 39.56) *0.92*
Symptomatic	0.24 (0.10 to 0.56) *0.001*		0.18 (0.07 to 0.46) *0.0004*	0.21 (0.08 to 0.57) *0.002*	0.24 (0.09 to 0.66) *0.006*	0.22 (0.06 to 0.82) *0.02*
Maternal CD4 cell count *(<200/200–500/>500 cells/mm^3)* *Comparing values:* *<200 vs >500* *200-500 vs >500*	0.35 (0.12 to 0.99) 0.31 (0.12 to 0.83) *0.06*					0.83 (0.26 to 2.62) 0.71 (0.25 to 2.01) *0.81*
Maternal VL (2) *(LDL/<1000/1000-10000/>10000)* *Comparing values:* *<1000 vs LDL* *1000-10000 vs LDL* *>10000 vs LDL*	26.528 (1.353 to 520.279) 76.990 (4.177 to >999.999) 166.890 (9.799 to >999.999) *0.0002*					

1600 observations were read, 33 of these from PCR-positive patients, and 634 observations were used

AIC/BIC/R2 for model 1: 135.08/152.89/0.08; model 2: 128.56/146.37/0.09; model 3:126.11/148.37/0.09; model 4: 125.16/151.87/0.10; final model: 123.86/177.29/0.10

AIC: The Akaike information criterion (AIC) is an estimator of the relative quality of statistical models for a given set of data. Given a collection of models for the data, AIC estimates the quality of each model, relative to each of the other models.

BIC (SC): Bayesian information criterion (BIC) / Schwarz criterion (also SBC, SBIC) is a criterion for model selection among a finite set of models; the model with the lowest BIC is preferred. R^2: Variation in y explained by the model. (Cox-Snell measures)

Probability testing of each risk-model

Mothers with an HIV viral load ≥1000 copies/mL who deliver a symptomatic newborn have a 38% chance of a positive PCR HIV test result at birth (model 1 – 2 risks, Figure 3a). Another 2-risk model, containing only maternal characteristics, maternal viral load and cART duration, showed a probability of 28% for a newborn PCR-positive test if maternal viral load is ≥1000 copies/mL and the mother has not received cART treatment for more than 4 weeks (model 2 – 2 risks, Figure 3b). In model 3, newborns have a probability of 0.49 of a positive PCR test if the newborn is symptomatic, the mother had received cART for less than 4 weeks before delivery and if the mother had a HIV viral load of ≥ 1000 copies/mL (Figure 3c). The four-risk model included maternal characteristics - viral load and cART duration - and infant characteristics - symptomatic newborn and SGA; in this model newborns have the highest probability of a positive HIV PCR if maternal viral load ≥1000 copies/mL, cART duration of less than 4 weeks before delivery, and an infant that is symptomatic and small-for-gestational age (model 4, Figure 3d).

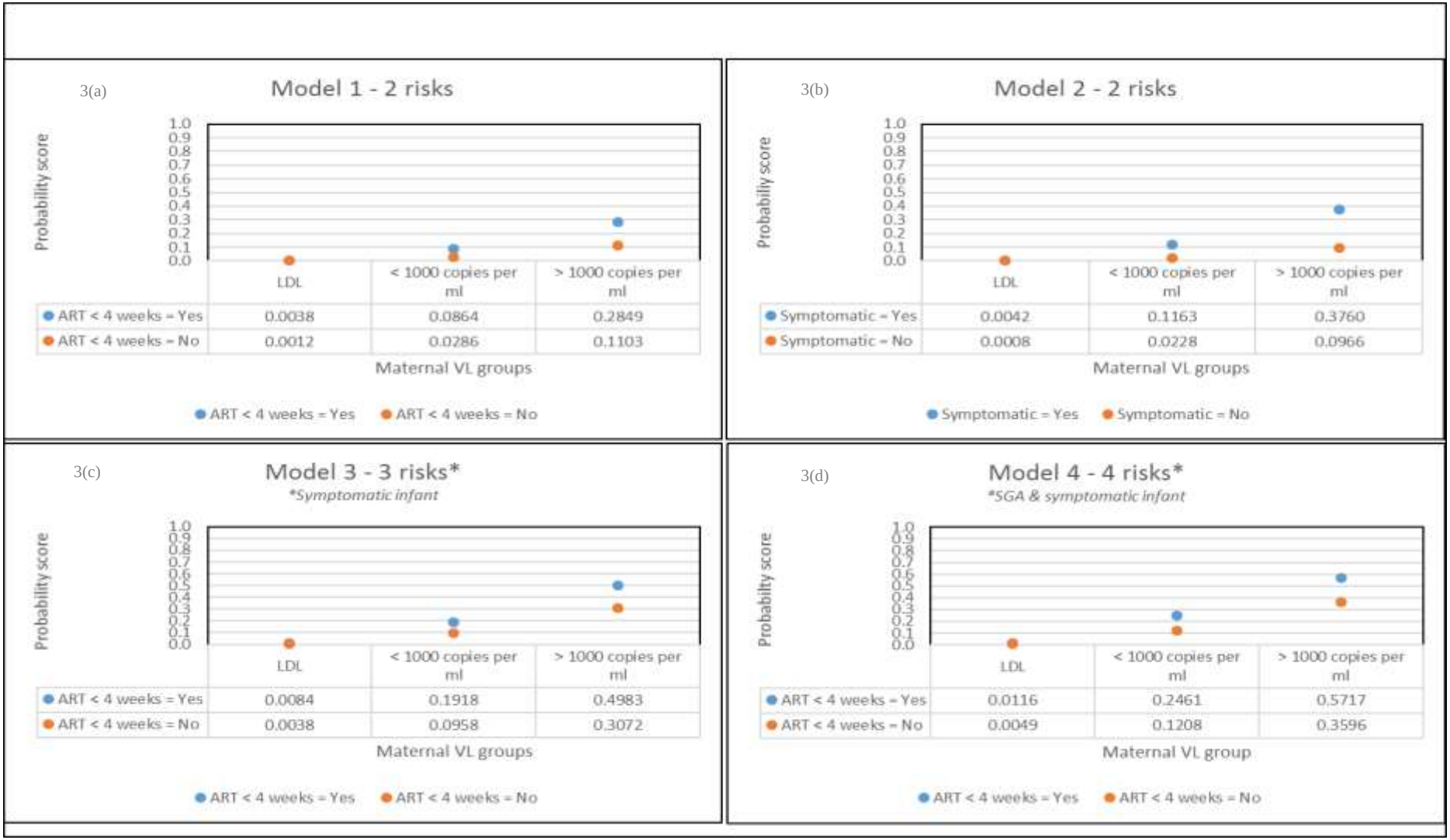

Figure 3: Probability of a HIV-infected newborn by risk factors using multivariable analysis as per models as illustrated in 3(a),3(b),3(c) and 3(d).

Mat_vl_grp = maternal viral load group (1 = lower than detectable, 2 = less than 1000 copies/mL, 3 = equal & more than 1000 copies/mL); sympt = symptomatic; art_less_4w_del = combination antiretroviral therapy less than 4 weeks' duration; SGA = small-for-gestational age

Each model can be used, at a risk-score probability cut off, to determine targeted PCR testing algorithms. When using both the 3- and 4-risk model scores and considering a probability of 0.02 and 0.04 as an indication for targeted birth testing, the sensitivity is 80% and 76% respectively (Table 4).

Table 4: Sensitivity and specificity values of both 3- and 4-risk models as probability levels 0.02 and 0.04.

	Probability level	**True positive (Sensitivity)**	**True negative (Specificity)**
3- & 4-risk scores	0.02	20/25 (80%)	494/773 (64%)
	0.04	19/25 (76%)	603/773 (78%)

DISCUSSION

In this study, we identified associations between maternal and infant characteristics and positive HIV PCR tests for newborns. We combined significant variables to build predictive models for early-infant HIV-risk scores to detect HIV infection at birth. According to our models, newborns had the highest (0.57) probability for a positive HIV PCR positivity if newborns were symptomatic and SGA, and were born to a mother who received cART for <4 weeks and had a VL≥1000. Newborns had a 0.28 probability of a positive HIV PCR if only maternal VL≥1000 and cART<4 weeks were included in the model. Our study population had high HIV prevalence among mothers (22.12%) and overall PCR positivity in newborns was 2.2%, which is higher than the national average of 1.1% [3].

Mothers who gave birth to newborns with in-utero acquired HIV more frequently had either no (p=0.0023) or fewer than three ANC visits (p=0.02). At the time of the study, basic antenatal care guidelines in South Africa recommended an early ANC visit (<12 weeks gestation) followed by four follow-up ANC visits for low risk pregnancies. In mid-2016, eight follow-up visits were recommended, and mothers who test HIV positive during

pregnancy should initiate cART a week later [11]. Mothers who are infected with HIV should receive continuous education about pregnancy-related issues. Antenatal care should complement HIV monitoring and treatment in these mothers.

Almost all mothers knew their HIV status before delivery (99%) and were on cART (95%). Encouragingly, these values are line with two of the current UNAIDS 90-90-90 goals, namely, 90% of all HIV-infected patients should be aware of their HIV status and 90% of HIV-infected patients should receive cART [12]. Mothers who had been on cART for shorter than 4 weeks were significantly associated with newborns who had a positive PCR HIV test at birth. Despite most mothers knowing their status and receiving cART, only 60% had achieved viral suppression, far less than the target of 90% of HIV patients on cART who should be virally suppressed to reach the 90-90-90 goals.

In-utero HIV infection in newborns was significantly associated with a detectable maternal viral load. Both VL levels < 1000 and ≥ 1000 copies/mL proved significant risks for in-utero HIV acquisition ($p = 0.0002$ and $p < 0.0001$ respectively), in both univariate and multivariate analyses. Myer et al. emphasized the importance of diligent maternal viral load monitoring and management during pregnancy and breastfeeding. Viral load testing 4 weeks before or at delivery can provide valuable information to guide targeted interventions from birth, including birth-PCR testing [13, 14]. Healthcare facilities providing ANC services should focus on the importance of providing cART for mothers at least 4 weeks before delivery. Mothers who are HIV positive should be enrolled in PMTCT programmes, which should consider repeat HIV testing, treatment, and viral load monitoring at 34-36 weeks' gestation. In future, the value of maternal VL point-of-care testing should be assessed.

In our study, HIV-infected newborns were more likely to be preterm (< 38 weeks gestation), low birth weight, and small-for-gestational age, although none of these parameters were

significantly associated with a positive HIV PCR test in the univariate regression models. We included small-for-gestational age values in the 4-model multivariate analysis, which added predictive value to targeted testing models. We recommend that all healthcare facilities with maternity services have infant gestational age tables and infant-scales to determine weight-for-age measurements for HIV-exposed newborns.

The newborn HIV PCR positivity rate was 1.83% (31/1691) with less than 5% of samples rejected (68, 3.9%). After repeat testing of the indeterminate results, an additional six patients tested HIV positive, increasing the positivity rate to 2.2% (37/1703). With a maternal HIV positivity percentage of 22% and 1.8% MTCT, 396 per 100 000 live births were HIV-infected in this study. Goga et al. [15] looked at early (4-8 weeks) MTCT risk in South Africa from 2011-2012 and 2012-2013 in relation to the South African PMTCT strategy, which is in line with WHO Option A. In 2011-2012, MTCT was 2.7% and 2.6% in 2012 – 2013, varying between provinces (1.9-5.4%). Mothers who started cART during or before the first trimester of pregnancy had low risk of early MTCT (1.2%) [2]. We recorded a similar rate of MTCT, indicating that most MTCT events are therefore in-utero and intrapartum. The risk has not changed significantly in the past five to six years. The current South African MTCT case rate is not close to the elimination of MTCT (EMTCT) target of ≤50 per 100 000 live births [16].

CONCLUSION

In this study, we present models for targeted birth PCR testing which may be useful in resource constrained settings. Our findings indicate that maternal VL testing is vital for a targeted birth PCR approach. Our 3- and 4-risk models achieved a sensitivity of close to 80%, indicating that 1-in-5 HIV-infected newborns will not be offered targeted birth testing. Globally, EMTCT and early infant diagnosis and treatment are prioritised, thus we support universal birth testing within the South African PMTCT programme.

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SUPPORTING INFORMATION

S1 Appendix: Definitions for infant- and maternal characteristics captured during the very early infant diagnosis (VEID) study

S1 Tables: Probability scores of infant HIV acquisition per infant risk model