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ORIGINAL RESEARCH

HIV viraemia and mother-to-child transmission risk after antiretroviral therapy initiation in pregnancy in Cape Town, South Africa

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Objectives

Maternal HIV viral load (VL) drives mother-to-child HIV transmission (MTCT) risk but there are few data from sub-Saharan Africa, where most MTCT occurs. We investigated VL changes during pregnancy and MTCT following antiretroviral therapy (ART) initiation in Cape Town, South Africa.

Methods

We conducted a prospective study of HIV-infected women initiating ART within routine antenatal services in a primary care setting. VL measurements were taken before ART initiation and up to three more times within 7 days postpartum. Analyses examined VL changes over time, viral suppression (VS) at delivery, and early MTCT based on polymerase chain reaction (PCR) testing up to 8 weeks of age.

Results

A total of 620 ART-eligible HIV-infected pregnant women initiated ART, with 2425 VL measurements by delivery (median gestation at initiation, 20 weeks; median pre-ART VL, 4.0 $\log_{10}$ HIV-1 RNA copies/mL; median time on ART before delivery, 118 days). At delivery, 91% and 73% of women had VL $\leq$ 1000 and $\leq$ 50 copies/mL, respectively. VS was strongly predicted by time on therapy and pre-ART VL. The risk of early MTCT was strongly associated with delivery VL, with risks of 0.25, 2.0 and 8.5% among women with VL < 50, 50–1000 and > 1000 copies/mL at delivery, respectively ($P < 0.001$).

Conclusions

High rates of VS at delivery and low rates of MTCT can be achieved in a routine care setting in sub-Saharan Africa, indicating the effectiveness of currently recommended ART regimens. Women initiating ART late in pregnancy and with high VL appear substantially less likely to achieve VS and require targeted research and programmatic attention.

Keywords: antiretroviral therapy, HIV, mother-to-child transmission, pregnancy, viral load.

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Introduction

Despite significant advances in prevention of mother-to-child HIV transmission (PMTCT), vertically transmitted HIV infection continues to occur, with the greatest burden of new infections in sub-Saharan Africa [1]. Maternal HIV viral load (VL) is the main determinant of MTCT risk, with transmission being directly proportional to maternal viraemia [2,3]. In turn, reduction in maternal VL is the goal of triple-drug antiretroviral therapy (ART) delivered as part of PMTCT services.

Although recent changes to international guidelines call for use of ART in all HIV-infected pregnant women [4],

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there are few data on maternal VL following ART initiation in pregnancy, particularly from sub-Saharan Africa. Research from Europe and North America has focused on the time to viral suppression (VS) and the proportion of women who reach VS at delivery, a critical window of transmission risk [5,6]. Such studies have been important in demonstrating that women initiating ART earlier in pregnancy have a greater opportunity to achieve VS by delivery [7,8], but less is known about the trajectory and determinants of VL decline during pregnancy and how these relate to early MTCT risk. Understanding changes in maternal VL following ART initiation is particularly important given the growing numbers of pregnant women initiating ART globally [9]. We investigated the patterns and determinants of VL, and early transmission risks, in HIV-infected pregnant women initiating ART within a routine antenatal care (ANC) service in South Africa.

Methods

As part of a larger study of optimization of ART in pregnant and postpartum women [the Maternal Child Health-Antiretroviral Therapy (MCH-ART) study; ClinicalTrials.gov NCT01933477], we recruited and followed a cohort of ART-eligible pregnant women seeking antenatal care in a large primary care facility in the peri-urban community of Gugulethu, Cape Town. The local ANC service, which includes PMTCT interventions, is available free of charge and provides care to more than 4000 women annually. ART has been available through local public sector services since 2004 and the antenatal HIV seroprevalence is approximately 30% [10].

Routine ART care

Throughout the study period (April 2013 to May 2014), maternal HIV infection status was determined in the PMTCT programme using two rapid antibody tests. ART eligibility changed over time based on local guidelines [9]. Before July 2013, eligibility was based on CD4 cell count ≤ 350 cells/μL [with CD4 cell enumeration using either laboratory-based flow cytometry (Becton Dickinson, East Rutherford, NJ, USA) or a point-of-care device (Pima Analyzer™; Alere Healthcare, Waltham, MA, USA) [11] or World Health Organization (WHO) stage III/IV disease, per WHO 2010 recommendation 'Option A' [12]. From July 2013, eligibility was expanded to include all HIV-infected pregnant women regardless of CD4 cell count or disease status (WHO 2013 recommendation 'Option B+') [9]. Throughout, the first-line ART regimen was tenofovir (TFV) 300 mg + emtricitabine (FTC)/lamivudine (3TC) 300 mg + efavirenz (EFV) 600 mg, once daily, provided as a single fixed-dose combination pill from July 2013. ART initiation and follow-up took place as part of routine ANC. ART was typically initiated either at the first ANC visit or 1–2 weeks thereafter. Follow-up visits were conducted as part of ANC at 1–2-monthly intervals until delivery. In the ANC, nurse-midwives led ART provision with support from doctors, and specialized PMTCT counsellors provided counselling before ART initiation and at follow-up visits. At the time of the study, postnatal infant prophylaxis employed nevirapine daily until 4–6 weeks of age.

Study procedures

The cohort of women initiating ART as part of antenatal care was followed up with research-specific appointments timed and located separately from routine care. For this, consecutive HIV-infected women aged 18 years and older with a confirmed pregnancy making their first ANC visit were approached to participate regardless of gestational age. Consenting ART-eligible women completed an interviewer-administered questionnaire, underwent obstetric ultrasound, and provided 5 mL of venous blood for batched viral load testing conducted by the National Health Laboratory Services using the Abbott RealTime HIV-1 assay (Abbott Laboratories, Chicago, Illinois, USA); results were available retrospectively. We verified that women were not using ART at the time of entry into ANC through review of clinical and laboratory records.

At each follow-up visit, women provided 5 mL of venous blood for batched VL testing and specimen storage. The timing of study visits during pregnancy was based on participants' gestation at the first ANC visit. For women < 31 weeks' gestation at the first ANC visit, further study visits were scheduled for 2 weeks after the first ANC visit, and again at 34–36 weeks' gestation. For women ≥ 31 but < 36 weeks' gestation at the first ANC visit, additional follow-up was scheduled for 34–36 weeks' gestation only; women enrolled into the study after 36 weeks' gestation typically did not have further antenatal follow-up. All women were asked to return to the study as soon as possible after delivery for an additional postpartum study visit. After delivery, we abstracted clinical information from participants' clinic records, including dates of ART initiation.

Early infant diagnosis

Early infant HIV testing was conducted routinely at approximately 6 weeks of age; birth testing was conducted in hospital settings based on criteria to identify infants at high risk of transmission. All infant testing is

conducted by the National Health Laboratory Services using the Roche Cobas AmpliPrep/Cobas TaqMan (CAP/CTM) HIV-1 assay (Roche Diagnostics, Branchburg, NJ, USA).

Analysis

Analyses used STATA Version 13.0 (Stata Corporation, College Station, TX, USA) and R (R Foundation for Statistical Computing, Vienna, Austria). Variables were described using means, medians [with interquartile ranges (IQRs)] and proportions [with 95% confidence intervals (CIs)]. We used the *t*-test, rank–sum test and χ^2 test (replaced in the case of sparse data by Fisher's exact test) for bivariate analyses; all statistical tests were two-sided at $\alpha = 0.05$. VL values were $\log_{10}$-transformed in continuous analyses and categorized for other analyses at ≤ 3.0, > 3.0–4.0, > 4.0–5.0 and > 5.0 $\log_{10}$ copies/mL. Changes in VL values over time were described using fractional polynomial models; results are presented as mean changes in $\log_{10}$ copies/mL with 95% CIs. Mixed-effects linear models were used to identify factors associated with VL decline after ART initiation; regression diagnostics followed standard procedures [13]. VS was defined as ≤ 50 copies/mL, with time to VL ≤ 50 and ≤ 1000 copies/mL examined using product-limit methods. We defined VL at delivery based on the first postpartum VL measurement, replaced by the last antenatal VL measurement if this was taken within 14 days of delivery and the postpartum measurement was available more than 14 days after delivery. Logit models were used to examine factors associated with VS at the time of delivery, including the interaction of pre-ART VL and gestation at ART initiation. Throughout, analyses were stratified by period of ART eligibility ('Option A' or 'Option B+'); as findings did not differ between these periods, we present only results for both periods combined.

Approval

Ethical approval for the study was provided by the Human Research Ethics Committee of the University of Cape Town and the Columbia University Medical Centre Institutional Review Board.

Results

A total of 620 ART-eligible HIV-infected pregnant women were enrolled in the study and initiated therapy; 99 (16%) and 521 (84%) women were identified as ART-eligible under 'Option A' and 'Option B+', respectively. The median pre-ART VL overall was 4.0 $\log_{10}$ copies/mL (IQR 3.4–4.6 $\log_{10}$ copies/mL) and 16% ($n = 100$), 35% ($n = 218$), 38% ($n = 240$) and 11% ($n = 70$) had VL ≤ 3.0, > 3.0–4.0, > 4.0–5.0 and > 5.0 $\log_{10}$ copies/mL, respectively. Among women with pre-ART VL ≤ 3.0 $\log_{10}$ copies/mL, the median VL was 2.4 (IQR 1.9–2.9) $\log_{10}$ copies/mL.

Table 1 describes the cohort at the time of ART initiation. The median age was 28 years (IQR 24–32 years) and 18% of women ($n = 111$) were primagravid. The median gestation at ART initiation was 20 weeks (IQR 15–25 weeks) and 51% of women had CD4 cell counts < 350 cells/μL. Overall, 29% of women ($n = 178$) had previous antiretroviral exposure, of whom 4% had defaulted triple-drug ART previously ($n = 23$) and 26% ($n = 161$) and 2% ($n = 9$) had previous exposure to a zidovudine (ZDV)-based prophylaxis regimen and single-dose nevirapine only for PMTCT, respectively. Pretreatment VL appeared significantly higher among women with previous ART use, women with lower CD4 cell counts at ART initiation and women with a history of tuberculosis, but was not associated with sociodemographic characteristics or gestation at ART initiation.

By November 2014, all pregnancies had ended, with 222 woman-years of observation accrued (median duration of follow-up per woman, 19 weeks) and 45 perinatal losses (7%). One participant was censored because of maternal death. The median duration of ART use before delivery was 118 days (IQR 77–151 days). During follow-up, 2425 VL measurements were taken with at least one pre-ART and one post-ART initiation measurement for all women. Most pre-ART specimens were collected on the day of ART initiation (25th percentile, 4 days before ART initiation); the next two specimens were taken a median of 15 and 87 days after ART initiation, respectively, and the post-delivery specimen was taken a median of 130 days after ART initiation. Overall, 591 participants had a VL measurement available postpartum (median time post-delivery, 6 days; IQR 4–9 days) and 587 had a VL measurement included as a delivery VL (95%). Women without a delivery VL measurement available initiated ART at a significantly earlier gestational age compared with women with delivery VL measurements available (16 *vs.* 20 weeks, respectively; $P = 0.002$) but there were no differences in other characteristics, including pretreatment VL (Table S1).

Change in viral loads after ART initiation

Figure 1a describes the changes in VL observed during the first 24 weeks after ART initiation. The mean predicted VL started at 3.9 $\log_{10}$ copies/mL before ART initiation and decreased to 2.7, 2.4, 2.1, 1.8 and 1.7 $\log_{10}$

Table 1 Demographic and clinical characteristics of 620 HIV-infected pregnant women initiating antiretroviral therapy (ART) in Cape Town, South Africa

	All women (n = 620)	Pre-ART viral load (log_{10} copies/mL)				
		≤ 3.0 (n = 95)	> 3.0–4.0 (n = 217)	> 4.0–5.0 (n = 238)	> 5.0 (n = 70)	P-value
Age (years) [median (IQR)]	28 (24–32)	28 (25–32)	28 (24–32)	27 (24–31)	28.5 (25–33)	0.243
≤ 24 years	161 (26)	21 (22)	57 (27)	68 (29)	12 (17)	0.693
25–29 years	222 (36)	37 (39)	72 (33)	84 (35)	28 (40)	
30–34 years	168 (27)	23 (24)	62 (29)	62 (26)	21 (30)	
≥ 35 years	73 (12)	14 (15)	26 (12)	24 (10)	9 (13)	
Gravidity [median (IQR)]	2 (2–3)	2 (2–3)	2 (2–3)	2 (2–3)	2.5 (2–3)	0.271
Primagravida	108 (17)	16 (17)	37 (17)	45 (19)	10 (14)	0.829
Gestation [median (IQR)]	20 (15–25)	21 (15–28)	20 (14–26)	19 (15–24)	21 (17–25)	0.374
≤ 14 weeks	138 (23)	21 (22)	55 (25)	51 (22)	11 (16)	0.291
> 14 to ≤ 28 weeks	370 (60)	51 (54)	122 (56)	149 (63)	48 (69)	
> 28 weeks	109 (17)	22 (23)	40 (18)	36 (15)	11 (16)	
Completed high school	164 (26)	24 (25)	50 (23)	70 (29)	20 (29)	0.458
Currently employed	237 (38)	32 (34)	83 (38)	99 (42)	23 (33)	0.419
HIV diagnosis in current pregnancy	342 (55)	51 (54)	124 (57)	129 (54)	38 (54)	0.910
Married/cohabiting	257 (43)	43 (47)	91 (43)	95 (41)	28 (42)	0.789
Previous antiretroviral exposure	175 (28)	30 (32)	60 (28)	61 (26)	24 (34)	0.453
Past ART use	23 (4)	0	3 (2)	12 (5)	8 (11)	< 0.001
Past PMTCT: NVP only	9 (2)	3 (4)	1 (1)	4 (2)	1 (2)	0.318
Past PMTCT: NVP + ZDV	161 (26)	28 (35)	58 (32)	54 (28)	21 (35)	0.559
Previous history of tuberculosis	62 (10)	7 (7)	13 (6)	26 (11)	16 (23)	0.001
CD4 count at start of antenatal care (cells/μL) [median (IQR)]	342 (234–504)	547 (394–722)	390 (301–550)	279 (198–399)	198 (128–278)	< 0.001
Median during 'Option A'	260	477	288	243	191	< 0.001
Median during 'Option B+'	360	548	419	289	205	< 0.001
CD4 count						
≤ 200 cells/μL	110 (18)	3 (3)	13 (6)	60 (25)	34 (52)	< 0.001
201–350 cells/μL	204 (34)	16 (17)	65 (31)	100 (42)	23 (35)	
351–500 cells/μL	136 (23)	20 (22)	64 (31)	47 (20)	5 (8)	
> 500 cells/μL	153 (25)	53 (58)	67 (32)	29 (12)	4 (6)	

Values are n (%), unless otherwise stated.
IQR, interquartile range; NVP, nevirapine; PMTCT, prevention of mother-to-child HIV transmission; ZDV, zidovudine.

copies/mL within 7, 14, 28, 56 and 84 days of ART initiation, respectively. Declines in VL were heavily influenced by pre-ART viraemia (Figure 1b): by 28 days after ART initiation, the mean predicted VL was 1.7, 1.9, 2.3 and 2.9 log_{10} copies/mL in women with pre-ART VL ≤ 3.0, > 3.0–4.0, > 4.0–5.0 and > 5.0 log_{10} copies/mL, respectively. In a nonlinear mixed-effects model, pre-ART VL and duration of ART use were the principal determinants of VL over time (Table 2).

The median time to VL ≤ 1000 copies/mL in the cohort was 24 days overall but varied significantly by pre-ART VL: women with VL > 3.0–4.0, > 4.0–5.0 and > 5.0 log_{10} copies/mL before ART initiation had median times to VL ≤ 1000 of 20, 27 and 76 days, respectively (P < 0.001; Fig. 2a). The median time to VS ≤ 50 copies/mL in the cohort was longer both overall (94 days) and for each of the corresponding categories of pre-ART VL (18, 64, 114 and 131 days for women with pre-ART VL ≤ 3.0, > 3.0–4.0, > 4.0–5.0 and > 5.0 log_{10} copies/mL, respectively; P < 0.001; Fig. 2b). At 8 weeks prior to delivery, 71% and 48% of women had viral suppression to ≤ 1000 and ≤ 50 copies/mL, respectively, in product-limit analyses; by 4 weeks prior to delivery, these proportions had increased to 79% and 60%, respectively.

During follow-up, 477 women achieved VS ≤ 50 copies/mL before delivery. Following initial VS ≤ 50 copies/mL, 943 woman-months were accrued before delivery and 40 viraemic episodes (> 50 copies/mL) were observed in 27 women who had viral suppression (6% of all women who had suppression; incidence density, 4.2 episodes per 100 woman-months; 95% CI 3.0–5.7). The majority (58%) of these episodes involved viraemia ≤ 1000 copies/mL (median 2.1 log_{10} copies/mL; IQR 1.8–3.6 log_{10} copies/mL) and 12 of the 27 women (44%) showed re-suppression to ≤ 50 copies/mL at a subsequent visit. The incidence of viraemic episodes was not associated with pre-ART VL, CD4 cell count or participant demographics.

Viral suppression at delivery

At the time of delivery, 91% of women (n = 517 of 587; 95% CI 89–93%) had VL ≤ 1000 copies/mL and 73% had suppression to ≤ 50 copies/mL (n = 429 of 587; 95% CI

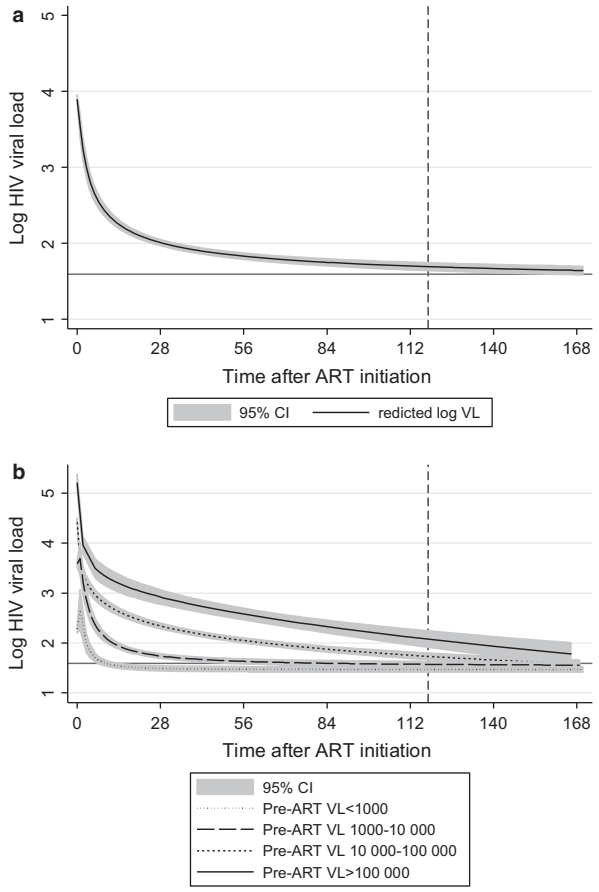

Fig. 1 Fractional polynomial model predicting $\log_{10}$ HIV viral load (VL) after antiretroviral therapy (ART) initiation, (a) in the overall cohort and (b) stratified by pre-ART VL category, based on 2085 VL measurements. In both graphs, the red horizontal lines represent 50 copies/mL and blue dashed vertical lines represent the median duration of ART use before delivery (118 days). CI, confidence interval.

69–77%). Among the 27% of women (n = 158) who had VL > 50 copies/mL at delivery, the median VL was 2.8 $\log_{10}$ copies/mL. Among women with VL > 50 copies/mL at delivery, 19% (n = 30 of 158) had achieved VS ≤ 50 copies/mL earlier in pregnancy, but had detectable VL in their peripartum measurement.

Table S2 compares clinical and demographic characteristics of women who did and did not achieve VL ≤ 50 and ≤ 1000 copies/mL at the time of delivery. For both VL cut-points, ART initiation later in gestation, previous ART exposure, previous history of tuberculosis, being primagravida, and elevated pre-ART VL were associated with raised VL at delivery. In a multivariable model, pre-ART viraemia and gestation at ART initiation remained strongly associated with VS at delivery (Table S3); these associations did not differ appreciably when stratified by ART eligibility period (comparing 'Option A' to 'Option B+').

Table 2 Results for a mixed-effects linear regression model predicting HIV viral load measurements (in $\log_{10}$ copies/mL) based on 2085 viral load measurements taken after antiretroviral therapy (ART) initiation in the cohort

	Coefficient	95% confidence interval	*P*-value
Age (years)	−0.003	−0.009 to 0.003	0.279
Previous ART use	0.143	−0.029 to 0.315	0.104
Pre-ART CD4 cell count			
≤ 200 cells/μL	1.0	(Reference)	–
201–500 cells/μL	−0.038	−0.129 to 0.052	0.405
> 500 cells/μL	−0.097	−0.206 to 0.013	0.083
Pre-ART viral load			
≤ 3.0 $\log_{10}$ copies/mL	1.0	(Reference)	–
> 3.0–4.0 $\log_{10}$ copies/mL	0.999	0.901 to 1.097	< 0.001
> 4.0–5.0 $\log_{10}$ copies/mL	1.728	1.626 to 1.830	< 0.001
> 5.0 $\log_{10}$ copies/mL	2.434	2.298 to 2.570	< 0.001
Time after ART initiation*			
1–7 days	−0.214	−0.227 to −0.201	< 0.001
8–28 days	−0.012	−0.019 to −0.006	< 0.001
29–84 days	−0.006	−0.008 to −0.004	< 0.001
84–170 days	−0.0001	−0.001 to 0.001	0.910

*Time after ART initiation is treated as a spline with knots at 7, 28 and 84 days after ART initiation.

Figure 3 shows a contour plot of the interaction of pre-ART VL and gestation at ART initiation in predicting probability of VS ≤ 50 copies/mL at the time of delivery (based on model outputs shown in Table S4); absolute proportions of women in each category of pre-ART VL and gestation at ART initiation (in trimesters) are overlaid. High probabilities of VS ≤ 50 copies/mL (> 80%) were predicted in women initiating ART before 15 weeks' gestation regardless of pre-ART VL, and at pre-ART VL < 3.5 $\log_{10}$ copies/mL regardless of gestation. The predicted probability of VS ≤ 50 copies/mL at delivery decreased to < 50% for women initiating ART after 20 weeks' gestation with pre-ART VL > 4.0 log copies/mL, and reduced to < 10% (denoted by red colour) for women initiating ART after 28 weeks' gestation with pre-ART VL > 5.0 log copies/mL. However, this subgroup of women with < 10% probability of achieving VS ≤ 50 copies/mL at delivery constituted < 5% of the overall cohort.

Early MTCT risk

Data on infant HIV testing up to 56 days of age were available for 555 infants (94%); the reasons for no test data available were unknown for the majority of infants without a test result (67%; n = 24), with smaller proportions of children who died before testing (8%; n = 3) or emigrated out of the province (25%; n = 9). For children with test results, the median age of testing was 44 days (IQR 42–49 days); 25 infants (5%) were tested within

24 h of birth. Overall, seven infants tested positive (including one infant tested at birth), giving an early transmission risk of 1.3% (95% CI 0.5–2.6%). Transmission risk was 0.25% (n = 1 of 406), 2.0% (n = 2 of 102) and 8.5% (n = 4 of 47) among women with VL < 50, 50–1000 and > 1000 copies/mL at delivery, respectively (P < 0.001). In infants testing positive, the pre-ART maternal VL was higher, the gestation at ART initiation was later, and the duration of maternal ART use before delivery was shorter, compared with infants testing negative. However, none of these comparisons achieved statistical significance (Table S5). One of the seven transmissions occurred in a mother who previously achieved VS and then experienced subsequent viraemia at the time of delivery, and another transmission was observed in a woman who achieved VS soon after initiation and sustained this through pregnancy and delivery; the remaining five transmissions occurred in women who did not achieve VS before delivery (Fig. S1).

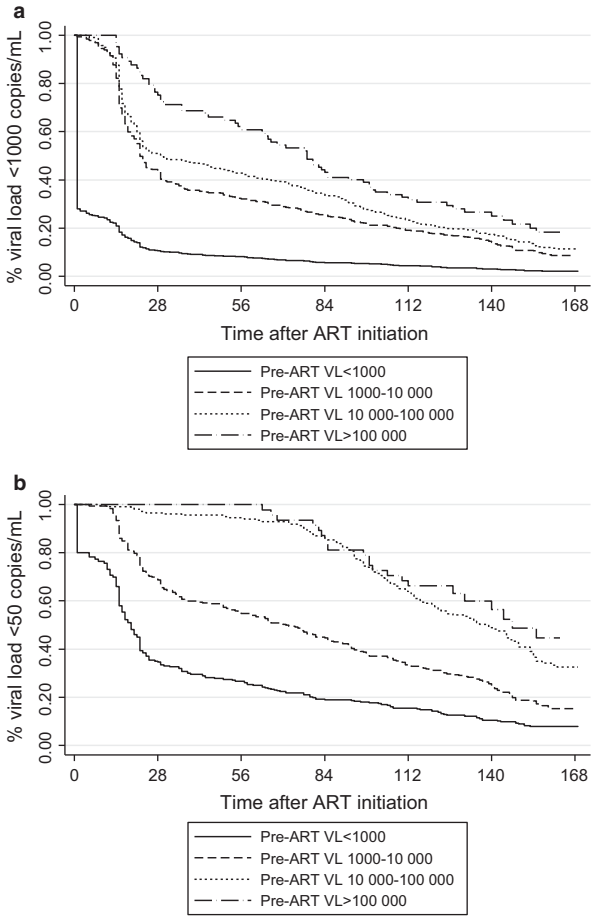

Fig. 2 Kaplan–Meier plot of time to viral suppression defined as (a) ≤ 1000 copies/mL and (b) ≤ 50 copies/mL, following antiretroviral therapy (ART) initiation, according to pre-ART viral load (VL) levels.

Discussion

These results from a routine clinical setting in South Africa demonstrate that the vast majority of women (91%) achieved VL ≤ 1000 copies/mL before delivery, although fewer (73%) reached VL ≤ 50 copies/mL. These high levels of viral suppression in routine care indicate the effectiveness of nonnucleoside reverse transcriptase inhibitor (NNRTI)-based regimens used according to PMTCT guidelines that prescribe universal ART initiation in pregnant women. Pre-ART VL, coupled with time on treatment, were the primary determinants of VL during pregnancy, with women initiating treatment during the third trimester with VL > 4.0 $\log_{10}$ copies/mL at greatest risk of failing to achieve VS ≤ 50 copies/mL by delivery. The early MTCT risk was low overall, but increased significantly in women with VL > 1000 copies/mL at delivery.

Few studies globally have examined VL changes over time during pregnancy, and comparisons are limited by

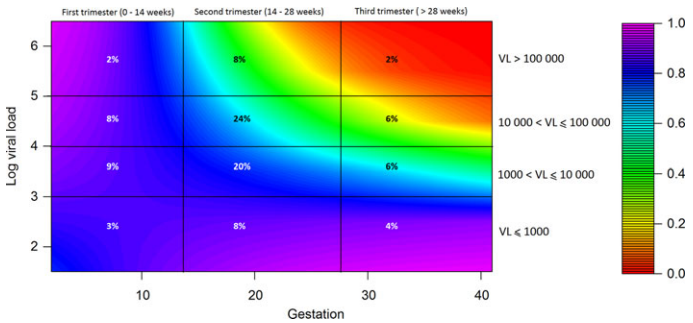

Fig. 3 Contour plot from the logit model predicting probabilities of viral suppression (VS) ≤ 50 copies/mL at delivery according to pre-antiretroviral therapy (ART) viral load (VL) (in $\log_{10}$ copies/mL) and gestation at ART initiation (in weeks). Red indicates the lowest probability of VS at delivery (< 10% probability) and purple indicates the highest probability (> 90% probability). Percentages in the graph represent the proportion of women initiating ART in the sample in each category of pre-ART VL and trimester of initiation.

methodological variations in the dating of pregnancies and definitions of VS. Nonetheless, our observation that 73% of South African women achieved VS ≤ 50 copies/mL by delivery is broadly consistent with findings from Europe [7,8] and the USA [6], although this percentage of women achieving suppression is higher than those reported in Kenya and Malawi [14,15]. With frequent measurements of VL following ART initiation in pregnancy, these data offer several important insights. First, rapid declines in viraemia were observed soon after ART initiation regardless of pre-ART VL, such that the vast majority of women achieved VL ≤ 1000 copies/mL within 28 days on treatment. While substantially longer periods on ART were required to achieve VS ≤ 50 copies/mL, in keeping with the multiphase viral decay observed in nonpregnant adults [16], this finding demonstrates the potency in pregnancy of the first-line regimen currently used in South Africa and in many countries (TFV + FTC/3TC + EFV) in reducing VL. Viral suppression, commonly defined as ≤ 50 copies/mL, is the goal of ART; however, the MTCT risk below 1000 copies/mL is extremely low (0.6%) [17]; in turn, the rapid early declines in VL observed here underscore the importance of triple-drug regimens in efforts to eliminate MTCT.

In addition to pre-ART viraemia and duration of ART, we found that higher pretreatment CD4 cell counts were independently associated with VS at delivery. Related to this, women with a history of tuberculosis, and those who had defaulted ART previously, appeared less likely to achieve VS at delivery, although these associations did not remain statistically significant in adjusted models. Taken together, these observations suggest that pregnant women initiating ART with more advanced HIV disease may have greater difficulty achieving viral suppression for reasons that are independent of their viraemia. While the immunological and/or socio-behavioural reasons for this require further attention, these data suggest that women with advanced disease may represent an important subgroup which requires particular attention in PMTCT programmes.

Frequent VL monitoring in pregnancy is routine in Europe and North America as part of individualized patient care, with the VL measurement conducted in the mid-third trimester being used to help make decisions regarding mode of delivery and infant prophylaxis [18,19]. While these data are based on VL measurements taken for research purposes, the findings suggest that intensive VL monitoring in pregnancy may not be essential for programmes delivering ART for PMTCT to be effective. Here, frequent VL testing identified primarily transient episodes of viraemia after VS in 6% of pregnant women and, in turn, a notable proportion (19%) of women with viraemia at delivery had viral suppression previously during pregnancy; most of the viraemic episodes identified were < 1000 copies/mL. Transient viraemia is well documented in nonpregnant adults on ART and the source of particular concerns around viral resistance [20,21], but the significance of low-level transient viraemia for transmission is unclear. Following from this, more evidence is required on the role of intensive VL monitoring in pregnancy in prognosticating VL at delivery (and *in utero* transmission risk) for programmes providing ART to pregnant women in resource-limited settings. The role of intensified VL monitoring in resource-limited settings, as well as the interventions that may be used to manage women with elevated VL late in gestation, need further attention.

The early MTCT risk observed here in women initiating ART as part of routine antenatal care (1.3%) is encouraging and comparable to transmission rates seen in other cohorts of women from Europe and North America. While the highest risks of transmission (8.5%) were associated with delivery VL > 1000 copies/mL, we note that this group of women comprised a minority (9%) of women and as a result accounted for only slightly more than half of all infant infections observed. Conversely, women with delivery VL < 1000 copies/mL at delivery had approximately one-tenth the risk of transmission but comprised the overwhelming majority of pregnancies, and in turn accounted for 43% of all infections observed. While the number of transmissions here is small and resulting estimates imprecise, this phenomenon – with almost half of all new infections observed in the very large proportion of women with low-level viraemia at delivery – indicates an emerging challenge facing population-level MTCT efforts under expanding policies of universal ART in high-prevalence settings. In addition, the incidence of perinatal loss observed in this population (7% of all women initiating ART) is relatively high, and substantially more than the 2–3% perinatal mortality rate estimated nationally in South Africa [22]. However, the absence of local comparison groups, involving either HIV-negative pregnancies or pregnancies in HIV-infected women who have already conceived on ART, makes direct attribution uncertain and this is an important avenue for ongoing investigation.

Several strengths and limitations require consideration. The location of this study within a routine public sector health care service allows insights into trajectories of VL and early MTCT on ART in a 'real-world' context beyond highly selected clinical trial populations. The uniform ART regimen used means that variability in antiretrovirals was not a source of heterogeneity, and along with pregnancies dated via ultrasound and frequent VL testing

during the first 200 days on ART, the study is unique in the precision of its measurements. The lower limit of viral detection (50 copies/mL) allowed documentation of low-level viraemia that has not been included in previous analyses [6,8]. However, the analyses were constrained by the absence of objective measures in several areas of interest, including previous exposure to specific antiretroviral agents, which was based on participant self-report. While previous ART use (i.e. having defaulted ART previously) was associated with elevated pre-ART VL, this was not an independent predictor of time to VS, although the prevalence of these antiretroviral exposures was low and in turn this analysis was underpowered to detect associations. The role of past antiretroviral use in influencing viral suppression in pregnant and postpartum women (re-)initiating ART remains an important question for ongoing research [23]. It is also important to note that the delivery VL measurement here was usually taken postpartum (75% within 9 days of delivery), and we cannot rule out elevated VL observed in these specimens as a result of women's waning adherence immediately postpartum despite being counselled on lifelong therapy. Furthermore, our early MTCT rates are based on 6-week testing which may include early postnatal transmission through breastfeeding, while it is also possible that early transmission rates may be underestimated because of the use of infant antiretroviral prophylaxis.

A range of interventions have been mooted to help achieve and sustain VS earlier in pregnancy to reduce MTCT risk, from promotion of earlier antenatal care to strategies to initiate ART more rapidly in pregnancy [24]. There is also interest in how specific classes of antiretrovirals, particularly integrase inhibitors, may influence the timing of VS in pregnancy [25], although evidence on the safety and efficacy of these agents in pregnancy is required [26]. Furthermore, provision of antiretroviral prophylaxis to HIV-exposed infants provides an additional prevention opportunity, but under some approaches there exists a need to identify high-risk infants for intensive prophylactic regimens. Here, women who initiated therapy after 28 weeks' gestation had a high probability of viraemia at delivery, and this may represent an important subgroup of infants who require postnatal prophylaxis for longer duration and/or use of multiple agents.

In summary, this study of routine PMTCT services in South Africa demonstrates rapid declines in VL after ART initiation in pregnancy using a WHO-recommended regimen, with the vast majority of women achieving VL $\leq$ 1000 copies/mL by delivery and in turn low overall MTCT risk. Women initiating ART in pregnancy with high VL and late in gestation appear at risk of viraemia at the time of delivery and MTCT, however, and this high-risk subgroup requires ongoing research and programmatic attention.

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