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Table 1: Risk of MTCT by HIV RNA viral load in mothers on ART

Country	Study population	Documented breastmilk MTCT and comment
MTCT in mothers with undetectable viral load		
Shapiro et.al,[1] Botswana, July 2006-May 2008,	Pregnant HIV-1 infected women with CD4+ ≥200 randomly assigned to Trizivir twice daily or Kaletra with Combivir twice daily starting between 26 and 34 weeks' gestation through weaning or 6 months postpartum (whichever occurred first). If CD4+ <200 cells or AIDS lifelong ART started between 18 and 34 weeks' gestation	Of 8 infants infected with HIV, ***two were infected during breastfeeding, at ages 91 and 94 days***. **Of these 2 infants:** • One of these mothers reported ART adherence challenge: her PVL at delivery, 1 month and 3 months was 257, <50 and <50 copies/mL respectively. Her breastmilk VL (BVL) at months 1 and 3 were <50 and <50. • The other mother had a PVL of <50 copies/mL at delivery, months 1 and 3 and a BVL <50 copies/mL at months 1 and 3 **Comment:** Mothers on ART with suppressed viral loads, who were closely monitored still transmitted HIV to their children postnatally
Giuliano et.al.[2] Malawi, Feb 2008-2009	Observational study of HIV-positive pregnant women attending two antenatal Clinics, Malawi. Women older than 16, naïve to antiretrovirals (with the exception of single-dose nevirapine), willing to breastfeed up to 6 months with no grade 3 or 4 laboratory toxicity and no active tuberculosis were enrolled	8 children became HIV infected: two were detected during month 1, one during month 3, one during month 6 and four during month 12. Plasma VL (PVL) and breastmilk VL (BVL) presented as copies /mL were available for 7 babies, plasma V was consistently less than 67 copies/mL in 4 children. Breastmilk VL was consistently lower than 37 copies/mL in 2 children. **Comment:** MTCT occurred in mothers with documented undetectable/low VL
Davis et.al[3] Malawi, 2004-2010	Analysis included mothers randomised to 28 weeks of postpartum antiretrovirals with ≥1 plasma or breastmilk specimen **AND** all mothers who transmitted HIV to their infants from 2 to 28 weeks (n=31) **AND** 15% of mothers who did not (n=232). Plasma and breastmilk HIV RNA obtained at enrollment, 2, 6, 12, 18, and 24 weeks postpartum. Breastmilk HIV RNA also measured at 4 and 8 weeks postpartum. HIV RNA detected but below quantitation limit were assigned 39 for plasma and 55 for breastmilk. Undetectable RNA concentrations were assigned a value of 20 in plasma and 28 for breastmilk.	Partial and near perfect adherence were associated with a 76% (95% CI 28-92%) and a 62% (95% CI 14-83%) reduction in the odds of having detectable breastmilk VL, respectively compared with poor adherence. • Among 27 HIV infected infants, 1 infant infected at 24 weeks, was born to a mother with undetectable baseline plasma VL, and three high postpartum VL measurements (8,000-108 000 copies/ml). • 5/116 mothers with complete VL information had undetectable plasma and breastmilk VL at all time points. None transmitted HIV to their infants. • Among the 134 mothers with breastmilk VL and infant HIV status, 15 transmission events occurred between 2 and 28 weeks postpartum: 11 (73%) had at least one detectable breastmilk VL before transmission occurred, and 8 (53%) had a detectable breastmilk VL at the last measured time point before transmission. • Detectable breastmilk HIV VL and breastmilk HIV transmission occurred despite apparently perfect (100%) maternal ARV pill count adherence. • No mother who consistently maintained a plasma viral load <100 copies/ml transmitted HIV to her infant during breastfeeding. **Comment:** It is critical to maintain breastmilk VL consistently low and preferably below 100 copies /mL
Myer et.al,[4] South	Cohort of 620 ART-eligible pregnant women recruited and initiated in ART at first ANC	94% children received an HIV test - the median age of testing was 44 days

Africa, April 2013-May 2014	(median 20 weeks gestation) from a peri-urban primary care facility, Cape Town, South Africa. ART regimen was Tenofovir 300 mg+emtricitabine /lamivudine 300 mg+efavirenz 600 mg.	(IQR 42–49 days); 25 infants (5%) were tested within 24 h of birth. MTCT was 1.3% (95% CI 0.5–2.6%)=7 infants. **Early MTCT (6-weeks post-delivery) differed significantly by maternal plasma VL (P<0.001):** • VL <50: 0.25% (n=1 of 406), • VL 50-1000: 2.0% (n=2 of 102) and • VL >1000: 8.5% (n=4 of 47) In infants testing positive, pre-ART maternal VL was higher, gestation at ART initiation was later, and duration of maternal ART use before delivery was shorter, compared with infants testing negative. • One of the 7 transmissions occurred in a mother who previously achieved viral suppression (VS) and then experienced subsequent viraemia around delivery. • 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 **Comment:** Women with VL <1000 copies/mL accounted for 43% of MTCT.
Gill et.al.[5] Kabeho study	600 HIV-positive women with delivery data, enrolled in follow-up (April 2013 - January 2014). The most common regimen was Tenofovir 300 mg+lamivudine 300 mg+efavirenz 600 mg.	• Of the four infants infected with HIV postnatally (detected at 9 or 24 months) one was born to a mother with a VL <1000 at delivery and one to a mother with no VL at delivery. • Among the four, the median time on maternal ART at delivery varied from 2.5 to 91.1 months, with high reported adherence. **Comment:** Reasons for MTCT, despite early initiation of ART included challenges with ART adherence, long interval between VL tests or post or per-partum viral rebound. Consequently, regular VL testing with immediate results, or additional preventive measures or both are needed to eliminate breastmilk MTCT in mothers on ART.
Landes et.al.[6] Malawi, Oct 2014-May 2016	Nested cross-sectional study of women enrolled in longitudinal follow up in the National Evaluation of the Malawi PMTCT Programme (NEMAPP) study with a VL result at enrolment (median time 1.8 months postpartum, range 4-26 weeks). Thirteen outpatient clinics. ART regimen was Tenofovir 300 mg+emtricitabine /lamivudine 300 mg+efavirenz 600 mg.	34 (51.5%) infant HIV infections occurred in mothers on ART. MTCT occurred in 8 (0.9%, 95% confidence interval (CI) CI 0.3-1.5%) and 6 (7%, 95% CI 1.6-124%) infants born to mothers with undetectable (<40 copies / mL) or low detectable (≥40-1000copies/mL) VL at enrolment, on ART, accounting for 23.5% and 17.6% of MTCT, respectively. **Comment:** The authors estimate that in Malawi, women with low detectable VL on ART, contribute an excess of 460 new infant HIV infections annually, translating into 60 additional infections per 100 000 births annually in Malawi.

Table 2: Viral load monitoring in women on ART

Country	Study Population	Time of plasma VL measurement and key finding
Gill et.al.[7] Kabeho study, Rwanda, April 2013 - January 2014	608 HIV positive pregnant (3rd trimester) or early postpartum (within 2 weeks) women enrolled from 14 facilities in Rwanda. This paper reports enrolment data. Women were on ART for a median of 13.5 months (IQR 3.0 ±48.8), - median time on their current regimen being 8.8 months (IQR 2.3 ±34.9); 76.1% (n=462) of women were already on ART at their first ANC visit.	**VL measured at enrolment - 3rd trimester or within 2 weeks postpartum:** 52.2% (n=315/603) had undetectable VL (≤20 copies/mL); 84.6% (n=510) had a VL of less than 1,000 copies/mL. **Undetectable VL at enrolment varied by ART duration: No ART:** 15.4% (n=2/13); **ART ≤4 months:** 29.7% (n=63/212); **ART >4 months:** 66.1% (n=250/378); **ART for >36 months:** Detectable VL in 37.7% (n=72/191) versus 29.9% (n=56/187) in women on ART for 4-36 months (AOR=0.70, 95% CI: 0.45 ±1.10, P=0.12). **Among women on ART >12 months,** 65.9% (n=201/305) had undetectable VL; 89.8% (n=274/305) with VL <1,000 copies/ml.
Myer et.al.[4]South Africa, (April 2013- May 2014	Cohort of 620 ART-eligible pregnant women recruited and initiated in ART at first ANC (median 20 weeks gestation) from a peri-urban primary care facility, Cape Town, South Africa. VL measured at every follow-up: Follow-up schedule: • **<31 weeks at enrolment:** 2-weeks later, 34-36 weeks and as soon as possible (asap) after delivery • **≥31 - <36 weeks at enrolment:** 34–36 weeks' gestation and asap after delivery • **>36 weeks:** asap after delivery • • **VL at delivery** based on first postpartum VL measurement - replaced by last antenatal VL measurement if this was within 14 days of delivery and postpartum measurement was more than 14 days after delivery.	Viral suppression (VS) defined as ≤50 copies/mL. Median time to plasma VL <1000 copies/mL or VS, respectively was: • 24 days, increasing to 76 days (≈2.5 months) if plasma VL >5.0 log10 copies/mL at ART initiation (P<0.001). • 94 days increasing to 131 days (≈4.5 months) if plasma VL >5.0 log10 copies/mL at initiation (P<0.001). **At 8 weeks before delivery:** 71% had VL ≤1000 and 48% ≤50 copies/mL **By 4 weeks before delivery:** 79% had VL ≤1000 and 60% ≤50 copies/mL 477 women achieved VS ≤50 copies/mL before delivery; 40 viraemic episodes (>50 copies/mL) were observed in 27 (6%) women who had viral suppression: 58% of these episodes involved viraemia ≤1000 copies/mL (median 2.1 log10 copies/mL; IQR 1.8–3.6 log10 copies/mL); 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 **At delivery**: 91% of women (517/587; 95% CI 89–93%) had VL ≤1000 copies/mL and 73% had VL ≤50 copies/mL (429/587; 95% CI 69–77%). Among the 27% of women (n=158) with VL >50 copies/mL at delivery 19% had achieved VS ≤50copies/mL earlier in pregnancy. In a multivariable model, pre-ART viraemia and gestation at ART initiation remained strongly associated with VS at delivery **Probability of VS** was predicted as >80% if ART was initiated before 15 weeks, regardless of pre-ART VL, and at pre-ART VL <3.5 log10 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% for women initiating ART after 28 weeks' gestation with pre-ART VL >5.0 log copies/mL. However, this latter sub-group constituted <5% of the overall cohort

Moyo et.al,[8] South Africa, June 2018 - March 2019	At delivery, routine point-of-care (PoC) maternal HIV VL and early infant diagnosis (EID) testing were implemented at 3 tertiary obstetric units in Johannesburg and 1 in Tshwane district, South Africa.	Among 8147 live births to WLHIV, 2769 (34.0%) women and 4333 (53.2%) neonates had valid viral load point of care results. Median VL at delivery (n=2769) was <40 copies/mL (interquartile range: 0–398). The proportion of women with a VL <50, 50 to <1000, and ≥1000 copies/mL was 63.6%, 13.9% and 22.4%, respectively. Only 77.5% and 63.6% of WLHIV had VL <1000 copies/mL, respectively. This study did not measure breastmilk MTCT, only in-utero MTCT. Of the 1449 mother-neonate pairs with both VL and EID results, in utero transmission by VL threshold was 3/946 (0.3%), 6/187 (3.2%), and 25/316 (7.9%) for VL <50, 50- <1000, and ≥1000 copies/mL, respectively (P<0.001)
Landes et.al.[6] Malawi, October 2014-May 2016	Nested cross-sectional study of women - 90.6% on ART - enrolled in longitudinal follow up in the National Evaluation of the Malawi PMTCT Programme (NEMAPP) study with a VL result at enrolment (median time 1.8 months postpartum, range 4-26 weeks). Thirteen outpatient clinics.	**At 1.8-months (IQR 1.5-3.1 months) postpartum:** Among women on ART with available VL (n=1124), 988 (87.9%) achieved VL <1000 copies/mL. Of these: • 902 (91.3%) had an undetectable VL (<40 copies per mL) and • 86 (8.7%) had a low detectable VL (40-1000 copies/mL). Undetectable VL at study enrolment was significantly higher in women who started ART before pregnancy
Myer et.al.[9] South Africa, April 2013-May 2015	523 pregnant/postpartum women on ART ever achieving VL suppression, were followed at 7 days postpartum, six weeks postpartum, then 3 monthly from 3 until 12 months postpartum	**Postnatally from 7-days to 12 months:** • 70% sustained VL ≤50 copies/mL; • 22% had at least one VL >1000 copies/mL, and 58% of the latter had more than one VL measurement VL >1000 copies/mL. Each additional month postpartum was associated with an 11% (incidence risk ratio 1.11 (95% CI 1.07-1.15) increased incidence of viraemia
Gill et.al.[5] Kabeho study: Rwanda, April 2013- - January 2014)	600 HIV-positive women on ART with delivery data, enrolled in follow-up.	Maternal VL was evaluated at enrolment within 2 weeks of delivery and 24 months among women who delivered **At enrollment:** 52.5% had undetectable plasma VL (<20 copies/mL); 84.7% had VL <1000copies/mL **At delivery:** Prevalence of VL <20 copies/mL was 55.3%; of VL <1000 copies/mL was 88.4%, **Among the 70% of women with data at 24 months:** VL <20 copies/mL was 78.2% and VL <1000 copies/mL was 91.2%
Yotebieng et.al.1[0] Democratic Republic of the Congo, Nov 2016-June 2018	Population-level cross-sectional study of 1623 HIV positive women on ART recruited from 105 clinics in 35 Kinshasa provincial health zones (November 2016 to June 2018)	**Among the 1623 HIV positive women recruited during pregnancy (54%), 1-3 days post-delivery (23%) or while breastfeeding during well child visits (23%):** 62% had VL <1000 copies/mL and 53% had VL <40 copies/mL respectively

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