Correlates of Virologic Failure

Among Breastfeeding Postpartum Kenyan Women on Option B+

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Abstract

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Background: Option B+ is an HIV/AIDS intervention strategy that streamlines antiretroviral therapy (ART) delivery to pregnant and breastfeeding women in order to improve treatment outcomes, but treatment outcomes have yet to be fully described in field settings. Our study examines the distribution of virologic failure in plasma, breast milk, and endocervical secretions among postpartum breastfeeding mothers using ART while on Option B+ in Kenya, and explores sociodemographic characteristics in this population that were associated with virologic failure.

Methods: We conducted a retrospective analysis on a cohort of HIV-positive, postpartum, breastfeeding women receiving ART while on Option B+, obtained from a parent cohort study in Nairobi, Kenya. The primary outcome of interest was virologic failure in plasma (HIV RNA >1000 copies/mL), in breast milk (>150 copies/mL), and in endocervical secretions (>100 copies/mL) obtained at 6-14 and 18-24 weeks postpartum. Distributions of virologic failure were

described and possible correlates of virologic failure were assessed using univariate tests and multivariate logistic regression.

Results: Of the 42 women in our study, the percentage of women with virologic failure at 6-14 weeks postpartum was 21.4% as detected in plasma; in breast milk, 14.3%, and in endocervical secretions, 23.8%. At 18-24 weeks postpartum, the percentages were 21.1%, 7.1%, and 14.3%, respectively. Younger maternal age, intent to breast feed for longer, and greater weeks of gestation at ART start were significantly associated with virologic failure in plasma ($p < 0.05$ for each). When adjusted for length of time on ART and low CD4+ count at enrollment, the odds of having virologic failure at 6-14 weeks postpartum were 1.25 times higher (95% CI 1.04, 1.51) for each increase in week of gestation at ART initiation. At 18-24 weeks postpartum, these odds were 1.16 times higher (95% CI 1.02, 1.33) for each increase in week of gestation at ART initiation, adjusted for the same variables.

Conclusions: Option B+ was designed to lead to earlier initiation of ART during pregnancy. Despite months of ART, nearly one-quarter of the women in our cohort did not achieve virologic suppression in the postpartum period. However, we demonstrated that earlier initiation of ART in pregnancy was associated with suppressed viral loads in plasma after the antepartum and intrapartum periods. Studies with larger sample sizes and longer postpartum evaluation periods are needed to understand the impact of Option B+ in extended HIV transmission risk, maternal outcomes and future pregnancies.

Introduction

The acquisition of new HIV infections continues to hinder efforts to end the AIDS epidemic. In 2015, of an estimated 1.9 million new infections occurred worldwide, 110,000 occurred in children under the age of 15. Treatment of pregnant and postpartum women with antiretroviral therapy (ART) can greatly reduce perinatal HIV transmission risk and improve maternal health by suppressing viral replication, but limitations in treatment initiation, adherence, and retention in care impede its effectiveness.[1] Strategies for improving ART implementation remain critical for eliminating HIV and AIDS.

Option B+ is an HIV/AIDS intervention strategy designed to streamline ART treatment for pregnant and breastfeeding women. Prior to 2013, the World Health Organization (WHO) recommendations for providing ART to pregnant and breastfeeding women varied according to the woman's CD4+ count, trimester of pregnancy, and intrapartum or postpartum status.[2] Under Option B+, HIV-positive pregnant and breastfeeding women initiate or continue ART regardless of their clinical status or CD4+ cell count, and continue therapy through life. This strategy eliminates treatment delays imposed by CD4+ testing and simplifies the HIV treatment algorithm, facilitating ART initiation and continuation of therapy. By targeting pregnant and breastfeeding women, Option B+ addresses maternal disease progression and mother-to-child transmission (MTCT) of HIV in current and future pregnancies, as well as sexual transmission of HIV.[3,4]

Simplification of ART treatment through Option B+ is believed to confer significant advantages over past ART treatment strategies, but Option B+ has yet to be fully validated in field settings.[2] Assessments of programmatic outcomes of Option B+ have focused predominantly on pregnant women, and successes associated with Option B+ have included earlier ART initiation in pregnancy, earlier clinical stage of women initiating treatment, increased duration of time that women receive ART before delivery, and decrease in the proportion of young infants (up to 12 weeks of age) becoming infected.[5,6] In countries that had adopted the

strategy by December 2013, the number of pregnant women accessing antenatal ART rapidly rose to over 50%, and in Malawi and Lesotho, beyond 90%.[7]

Whether these and other positive outcomes extend beyond pregnancy, and whether they lead to sufficient viral suppression and sustained reduction of sexual transmission and MTCT risk through the breastfeeding period and into future pregnancies, remains to be definitively determined. Women who initiate ART for pregnancy or breastfeeding are consistently shown to have lower ART adherence and retention rates, particularly in the postpartum period, than women who initiate therapy for clinical stage or CD4+ count.[5,8,9] Imperfect adherence to ART has been correlated with a higher probability of incomplete suppression of HIV.[8,10,11] In the postpartum period, insufficient viral suppression places infants at substantial risk for infection through breastfeeding, and could result in as many new pediatric HIV infections as are acquired among fetuses and newborns during pregnancy through delivery.[12]

The distribution of and reasons for incomplete suppression of HIV specific to postpartum mothers on Option B+ have received little attention.[13] Relevant factors may include younger age, fears about a lifetime commitment to ART, and social stigma.[5,8,14] Additionally, virologic suppression in this population may differ by various maternal bodily compartments, with different implications for infant exposure and transmission.

As one of the Global Plan priority countries, Kenya has made significant progress in MTCT of HIV. In 2014, Kenya began scaling up Option B+ rollout, and by October 2015, Option B+ had been adopted by over 90% of all facilities offering programs on maternal or infant health.[15] Between 2009 and 2015, Kenya's MTCT rate at six weeks following birth declined from 8% to 5%, and its final mother-to-child transmission rate, including MTCT in the breastfeeding period, dropped from 21% to 8%.[12] However, much progress remains to be made. In 2014, 29.4% of breastfeeding mothers lacked antiretroviral medication for both themselves and their infants.[16] In a large study conducted between 2014-2015, 6.9% of Kenya's infants tested

positive for HIV, and in 2015, the number of newly HIV-infected children in Kenya was 6600 – the second highest among all Global Plan priority countries.[12,15] A better understanding of the barriers to virologic suppression unique to postpartum breastfeeding women receiving ART in an Option B+ context will inform targeted interventions for the reduction of postpartum MTCT. This study examines the distributions of virologic failure in plasma, breast milk, and endocervical secretions among postpartum breastfeeding mothers using ART while on Option B+ in Kenya, and explores sociodemographic characteristics potentially unique to this population that could be associated with virologic failure.

Methods

Study Design and Population

This is an analysis of postpartum women who were initially enrolled and followed in a prospective parent study on post-partum family planning (PFP study) based in the primary care clinic of Mathare North Health Centre, Nairobi, Kenya. Mathare North is a government clinic that provides antenatal care as well as follow-up care and support services for HIV-positive mothers through a prevention of mother-to-child-transmission (PMTCT) treatment cascade protocol, and has had an ongoing clinical research relationship with the University of Washington (UW) for 20 years. Approximately 300 antenatal patients are diagnosed with HIV at the clinic each year, representing approximately 9% of this clinic's antenatal patient population. PFP's aim is to evaluate the changes in immune modulation associated with use of contraceptive progesterone analogues. Ethical approval for the parent study protocol was granted by the University of Washington Institutional Review Board and the Ethical Review Committee of Kenyatta National Hospital in Kenya. All participants provided written informed consent for participation in the study and for specimen collection and analysis.

Between May 2014 and October 2015, 120 HIV-positive and HIV-negative women who were not pregnant, between 6 weeks and 3 months postpartum, breastfeeding, not already

using family planning, choosing to start contraception with non-hormonal methods or depot medroxyprogesterone acetate (DMPA), asymptomatic for illness, and who had a CD4+ count of over 350 cells/μL, were recruited for the parent study when they brought their infants to Mathare North Health Centre clinic for immunizations (typically at 6, 10, or 14 weeks of age). Initially, only women who were not on highly active ART (HAART) at the time of enrollment were recruited. In August 2014, Option B+ was rolled out to Mathare clinic, and HAART was offered to HIV-positive women who had already been enrolled if they were still breastfeeding. In accordance with WHO Option B+ recommendations, pregnant and breastfeeding women were offered Efavirenz (EFV)-based regimens with Tenofovir (TDF) and Lamivudine, although TDF was substituted for Zidovudine and EFV for Nevirapine as necessary.[17] With the change in clinic regimen, recruitment criteria changed to include all HIV positive women, and women were no longer excluded on the basis of CD4+ count or whether they were already on ART. For this analysis, we included all HIV-positive women on ART who enrolled into PFP on December 1, 2014, and after, at least 3 full months after the date of Option B+ rollout. Three months is the median estimated length of time it takes pregnant women to achieve viral loads of ≤ 1000 copies/mL on ART, and this may also apply to postpartum women[18]. Some women started ART before enrollment and prior to Option B+ roll-out (during their antepartum, intrapartum, or postpartum periods), and a December 1, 2014 cut-off date was chosen to ensure that they had the opportunity to receive Option B+ for sufficient time to achieve viral suppression.

Data Collection

Data for this analysis was obtained from questionnaires that were verbally administered to postpartum women at enrollment (6-14 weeks postpartum), and during study follow-up visits (18-24 weeks postpartum). Questionnaires collected sociodemographic, clinical, and treatment information, and consisted of an initial enrollment questionnaire, up to two interim questionnaires, and up to two follow-up questionnaires. Beginning in January 2015, an Option

B+ questionnaire was additionally administered to all subjects who were still returning for interim and follow-up visits. Physical exams were administered at enrollment, an interim visit, and a follow-up visit. Specimen collection was performed during clinic visits and noted on exam forms. Research staff entered data from all forms into RedCap.

For this analysis, we examined laboratory results from specimens that were collected at the enrollment visit and a follow-up visit. Women were screened for pregnancy via urine pregnancy test at enrollment, received standard counseling for family planning conducted by a family planning nurse, and offered a choice of contraceptive method. Of 20 cc of peripheral blood collected at each visit, 4 cc was used for a CD4+ count conducted by Paediatrics Laboratory at the University of Nairobi, and 5 cc was collected in EDTA and cryopreserved at negative 80ºC; 250 µl of the latter volume was used for HIV-1 RNA measurement. Endocervical secretion specimens were collected using sterile Dacron swabs that were inserted into the cervix, rotated 3 times, and left in the cervix for a total of 30 seconds. Swabs were then placed in viral culture media on ice, then cryopreserved at -80ºC. Breast milk was collected in sterile cups via participant self-expression, centrifuged to separate cells and supernatant, then cryopreserved to -80ºC; 100 µL of supernatant was used to determine HIV viral load. Measurements of HIV-1 RNA for all specimens were conducted at the Overbaugh virology laboratory at the Fred Hutchinson Cancer Research Center, using the Gen-Probe assay.[19]

For subjects with >1000 copies/mL of HIV RNA in plasma at either 6-14 weeks or 18-24 weeks postpartum, frozen blood specimens were sent to the Seattle Children's HIV Specialty Lab for evaluation via the oligonucleotide ligation assay, a HIV genotypic resistance assay that was developed by the Department of Laboratory Medicine at the University of Washington. Viral RNA was reverse-transcribed from plasma and resultant DNA was analyzed using sequence analysis in order to detect reverse transcriptase or protease inhibitor mutations that would confer viral resistance.[20]

Sample Size and Power

Based on our inclusion criteria, 42 women from the parent study qualified for this study. Participants were grouped into those with virologic failure and those with virologic suppression based on HIV RNA thresholds for virologic failure defined in the literature: for plasma, >1000 copies/mL; for breast milk >150 copies/mL, and for endocervical secretions, >100 copies/mL. Preliminary studies estimated that approximately 25% of the participants experienced virologic failure in plasma. Because the number of cases of virologic failure were fixed, the power to detect associations derived from the prevalences of the selected exposures of interest. Based on existing literature, we estimated the prevalences of these exposures to range between 15-60% among participants without plasma virologic failure (ie, those who were virologically suppressed).[4,21] Given our sample size, the minimum odds ratio that could be detected at 80% power was 11.02 if our exposure prevalence among those virologically suppressed was 0.15, and 61.90 if the prevalence was 0.50, indicating that subjects with HIV viral load > 1000 copies/mL would be at least 11.02 or 61.90 times more likely to have the exposure, depending on the prevalence.

Statistical Methods

All analyses were performed using STATA/SE Version 14.2.[22] Our primary outcome was plasma virologic failure. We evaluated participant characteristics at 6-14 weeks postpartum using descriptive statistics. Participants considered to have virologic failure in plasma were compared against those with virologic suppression at the same time point in order to elicit potential sociodemographic and clinical correlates of virologic failure. Comparisons were made for both enrollment (6-14 week) and follow-up (18-24 week) time points, as women's adherence to ART may differ in the early and late postpartum periods. Univariate analyses were performed using Fisher's exact test for categorical variables and the Mann Whitney U test for continuous variables; two-sided p-values were calculated. Cohen's Kappa was calculated in order to

examine concurrency in virologic failure among multiple compartments. Missing values for variables with over 10% of data missing were treated as additional categories in analyses. Viral load measurements of zero were recoded to midway between zero and the assay's lower limit of detection for each specimen, which was 100 copies/mL for plasma and endocervical secretions, and 150 copies/mL for breast milk[19,23] For women for whom self-reported weeks of gestation at ART initiation were missing, this value was calculated using the study infant's birth date. Weeks of gestation under 0 were recoded as 0 and over 45 were recoded as 45.

Variables determined through univariate analysis to be associated with plasma virologic failure at p-values of 0.20 and under, and biologically plausible for association, were considered for multivariate models constructed using both stepwise and backwards elimination methods. Separate analyses were performed for the 6-14 week and 18-24 week postpartum periods using logistic regression and a double-sided significance threshold of 0.05.

While we expected that excluding subjects who enrolled in PFP before Dec. 1, 2014 would minimize the bias in our study, there was no specific date by which we could be certain that all subjects were offered antiretrovirals under Option B+. Additionally, an overly stringent cut-off date would limit the size of our sample and our ability to detect associations. In order to assess whether excluding fewer subjects as dictated by different enrollment cut-off dates would influence observed associations, a sensitivity analysis was conducted using September 1, 2014 as a cut-off date for Option B+ exposure.

Results

Characteristics of the study population

PFP recruited 66 HIV positive women, of whom 59 reported being on ART at either enrollment or follow-up (Figure 1). Of these women, 42 enrolled on or after Dec. 1, 2014, meeting the eligibility criteria for this study. Table 1 provides summary statistics for the entire sample at enrollment (6-14 weeks postpartum). The median number of weeks postpartum was

6.9 weeks (IQR 6.6, 10.9). The mean age was 26.9 (± 4.6) years, most were married (n=35, 83.3%), and 18 participants (42.9%) had received at least some secondary school education. Thirty-five (83.3%) had disclosed their HIV status to their partners. At enrollment, all were breastfeeding and on average, women intended to breastfeed for 11 (±3.1) months. Nearly all (n=40, 95.2%) reported being counseled on the importance of ART adherence and all reported being on ART at enrollment. Most had initiated ART during pregnancy (n=26, 66.7%) at a median of 21.7 (IQR 4.0, 28.0) weeks of gestation while 10, (23.8%), had initiated ART prior to pregnancy. . Thirty-four (81.0%) reported good adherence during pregnancy, never missing more than two doses of ART per month, while nearly all women reported good adherence during breastfeeding (n=41, 97.6%), again missing at most two doses a month. A median of 13.0 weeks (IQR 12.0, 13.1) elapsed between enrollment and follow-up (2 subjects did not return for follow-up). At 18-24 weeks postpartum, one women reported no longer taking ART, and 38 (90.5%) were still breastfeeding.

Distribution of virologic failure

Plasma viral loads above the detectable limit (100 copies/mL) were found in 12 subjects (28.6%) at enrollment; their median HIV RNA level was 14096 copies/mL (IQR 1328, 56046). Nine of these subjects (21.4% overall) had plasma virologic failure (> 1000 copies/mL). Of these, 1 subject was able to suppress plasma HIV to ≤ 1000 copies/mL by follow-up, and a different subject, who was initially suppressed, demonstrated plasma virologic failure at the later time point (Table 2). Of the 9, 8 subjects (21.1%) were not virologically suppressed in plasma at follow-up (Figure 2). Five of the 10 women with plasma virologic failure at either time point harbored virus with resistance mutations; viral RNA in the participant who was able to achieve plasma suppression was not found to have resistance mutations.

For both breast milk and endocervical secretions, the lower limits of detection, 150 and 100 HIV RNA copies/mL, respectively, were also used as the thresholds for virologic failure.

Among the 6 subjects (14.3%) considered to have breast milk virologic failure at 6-14 week postpartum, the median HIV RNA level was 1568 copies/mL (IQR 340, 2860). Three subjects were able to suppress breast milk HIV levels at follow-up and 1 other initially suppressed at enrollment was no longer suppressed at the later time point. Among the 10 women (23.8% overall) with endocervical virologic failure at 6-14 weeks postpartum, 3 became virologically suppressed; no additional subjects had endocervical virologic failure at follow-up. The median HIV RNA level among detectable endocervical secretion specimens was 444 copies/mL (IQR 143, 7305) at enrollment.

Virologic status in plasma showed fair agreement with virologic status in breast milk and good agreement with virologic status in endocervical secretions (Figure 3, Table 3). At 6-14 weeks postpartum, all of the 9 subjects with plasma virologic failure also had virologic failure in breast milk, endocervical compartments, or both. Percent agreement beyond chance in virologic status was moderate (Cohen's $\kappa = 0.43$) for plasma and breast milk, and almost perfect (Cohen's $\kappa = 0.93$) for plasma and endocervical secretions. Percent agreement beyond chance in virologic status was also notable at 18-24 weeks postpartum; this time poor (Cohen's $\kappa = 0.28$) for plasma and breast milk, and excellent (Cohen's $\kappa = 0.83$) for plasma and endocervical secretions.[24]

Sociodemographic characteristics associated with virologic failure

We were underpowered to detect differences between women who suppressed HIV and women with virologic failure (Table 4). Women with plasma virologic failure and suppression did not differ in marital status, number of past pregnancies, secondary school education, income, and whether they received DMPA. The groups also did not differ in having partners who were aware of their HIV status, ART adherence during breastfeeding and during pregnancy, ART in a previous pregnancy, and the distribution of women who started ART before, during, and after pregnancy. None of the subjects reported missing more than two doses of ART a month during

breastfeeding (the typical threshold for non-adherence is taking less than 90 to 95% of medication per month), so this variable was excluded from further analysis.[11,21,25]

Women with virologic failure in plasma at 6-14 weeks postpartum were significantly younger than women who were suppressed, the former having a mean of 24.2 (±4.4) years of age compared to 27.6 (±4.4) years among the latter (p = 0.05). Additionally, women with plasma virologic failure initiated ART at a later time in pregnancy, at 27.0 weeks (IQR 26.0, 32.0), compared to 20.0 weeks' (IQR 0, 25.0) gestation among suppressed women (p = 0.02). Women with plasma virologic failure trended toward a shorter period of time on ART – 17.3 weeks (IQR 12.1, 30.0) compared to 27.7 weeks (IQR 19.9, 43.9) among those who were suppressed (p = 0.08). No significant differences were detected in CD4+ count between women with failure and suppression at enrollment.

Interestingly, women with plasma virologic failure unexpectedly reported intending to breastfeed for longer, for 13.3 (±4.0) months compared to 10.7 (±2.6) months among virologically suppressed women (p = 0.05). This variable was included in an attempt to determine whether the length of time a woman intended to breastfeed would influence her motivation for taking ART in order to prevent infection to her infant.[8,26] However, this trend is reversed (although not significant) at 18-24 months postpartum, and our significant p-value at the earlier time point may represent a spurious finding. Indeed, a single woman reported wanting to breastfeed for more than 12 months at each time point, raising concern that comparisons of mean estimates beyond 12 months may not be reliable. This variable was therefore excluded from further analysis.

Maternal age and gestation at ART initiation were not significantly associated with plasma virologic failure at 18-24 weeks postpartum. However, 4 women did not have viral load assessments at this time point. The loss of data for 4 subjects may have reduced our power substantially enough to limit detection of the associations present in early postpartum. Nevertheless, we detected a new association: women with plasma virologic failure in late

postpartum were significantly more likely to have a CD4+ count of ≤ 350 cells/mm^3 compared to suppressed women (p = 0.05).

Variables considered for multivariate models included maternal age, gestation at ART initiation, length of time on ART at enrollment (6-14 weeks postpartum), and low CD4+ count at enrollment (Table 5). For each additional week that a pregnancy progressed before ART initiation, the odds of having plasma virologic failure at 6-14 weeks postpartum were 1.25 times higher (95% CI 1.04, 1.51) when adjusted for length of time on ART and low CD4+ count at enrollment. At 18-24 weeks postpartum, the odds of having plasma virologic failure were 1.16 times higher (95% CI 1.02, 1.33) for each later week in pregnancy before ART initiation, adjusted for the same variables.

Sensitivity analysis

We performed a sensitivity analysis using an earlier enrollment cut-off date of Sept. 1, 2014, in order to determine whether including women who enrolled closer to the month of Option B+ rollout (but who were also on ART during the study period) would change the associations that we observed between plasma virologic failure and sociodemographic variables of interest. Twelve women who enrolled between Sept. 1, 2014 and Nov. 30, 2014 were included with our original sample of 42 for sensitivity analysis. At enrollment, all 12 were on ART, their mean maternal age was 26.1 (± 4.1 years), and the median length of time that they had been on ART was 24.2 weeks (IQR 17.2, 41.1). In our sensitivity analysis, weeks of gestation at ART initiation remained significantly associated with plasma virologic failure at enrollment, and not significantly associated with plasma virologic failure in late postpartum. Additionally, low CD4+ count remained significantly associated with plasma virologic failure at follow-up, but not enrollment. However, the association observed earlier between maternal age and plasma virologic failure at enrollment was no longer significant.

Discussion

Among women who were offered Option B+ during pregnancy or breastfeeding, we found that, both in early and late postpartum, 21% had virologic failure in plasma. Of the women with virologic failure in plasma at either time point, most had HIV detected in concurrent breast milk or endocervical secretions. Interestingly, higher odds of plasma virologic failure in early or late postpartum were associated with later gestational age at ART initiation when adjusted for length of time on ART and low CD4+ count (both as measured at enrollment). Although the goal of Option B+ was to promote earlier initiation of ART during pregnancy,[6] our outcome shows that the benefits of starting ART earlier in pregnancy extend into the postpartum period. We demonstrated better suppression among women who started ART earlier in pregnancy even after controlling for length of time on ART.

Our conclusion adds to ongoing debate concerning how pregnancy interacts with ART. Recent literature has suggested that pregnant and non-pregnant women may respond differently to ART. Women on ART have been shown to have a higher risk of plasma virologic failure if they become pregnant during therapy.[27] Myer et al. has also demonstrated that, among women with pre-ART viral loads of > 4.0 log copies/mL, the probability of achieving a plasma viral load of ≤ 50 copies/mL by delivery is greater for women initiating ART earlier in gestation.[18] Our finding of better viral suppression associated with earlier initiation of ART in pregnancy could demonstrate continued better viral suppression extending from improved viral suppression that begins with earlier initiation in pregnancy. Alternatively, separate mechanisms that may involve ART administration and adherence could be responsible for viral suppression in the antepartum and postpartum periods.[27]

Most assessments involving HIV viral load focus on the prenatal period and end at parturition.[14,18] Literature describing the distributions of virologic failure and suppression among postpartum women on Option B+ is sparse, and the circumstances under which woman are evaluated vary widely. In a cohort of 150 Ugandan women who were offered Option B+, 80.7%

were suppressed in plasma, but the threshold for virologic failure was an HIV RNA level of 400 copies/mL, follow-up was performed 3-5 years after initiation, and the cohort was ART-naïve upon ART initiation at 12-28 weeks of gestation.[4] Among 434 women in Kenya who initiated ART regardless of CD4+ cell counts, 80% and 79% had undetectable plasma HIV viral loads at 14 and 24 weeks postpartum, respectively, but the threshold for detectable plasma HIV viral load was 400 copies/mL, the cohort had initiated ART at 34 weeks gestation, and nelfinavir or nevirapine-based triple regimens were administered.[21,17] In contrast, women in our study initiated and continued ART through Option B+ under a variety of conditions: prior to pregnancy (due to low immunological or clinical status or previous pregnancy), during pregnancy, or while breastfeeding; immediately or after some delay after being offered treatment; and at a mix of adherence levels. The distribution of virologic status in our cohort reflects the typical heterogeneity of a population of postpartum women offered Option B+ during pregnancy or breastfeeding.

Our study also describes the distributions of women with detectable viral load in breast milk and endocervical secretions. HIV in breast milk poses a MTCT threat to breastfeeding infants, while HIV in endocervical secretions may transmit to maternal sexual partners or a woman's future babies. Although virologic status in breast milk correlated strongly with plasma, breast milk HIV RNA levels have been demonstrated to be episodic[23], and a recommended target threshold for virologic suppression in breast milk has not yet been established. Davis et al. found transmission risk to be 3.8 times higher among women with detectable (>40 copies/mL) compared to non-detectable levels of HIV RNA in breast milk, but all transmitting mothers also had at least one postpartum plasma HIV RNA >3500 copies/mL, thus plasma viral load remained a reliable way of gauging transmission risk.[23,25] Virologic status in endocervical secretions also matched well against that in plasma. However, variability in endocervical secretion viral load is generally found to be higher than in plasma, and the magnitude of viral shedding in endocervical secretions can remain at transmissible levels even when plasma HIV

levels are undetectable. This raises the concern that the appearance of a smaller proportion of women with endocervical virologic failure at late postpartum (compared to early postpartum) does not mean that a smaller proportion of women can sexually transmit HIV.[28] However, Baeten et al. reported a dramatically lower risk of transmission (<1 event per 100 person-years) among women with HIV RNA levels below 240 copies/mL.[29]

The numbers of women in our study with virologic suppression in plasma were essentially unchanged between study time points. This could be because the length of time between viral load measurements (13.0 weeks, IQR 12.0, 13.1) may have been too short to allow for changes in suppression status before the next measurement, or due to factors interfering with viral suppression. Correlates of virologic failure following treatment with ART vary widely across studies, and comparability among studies is limited by differences in methodology, particularly regarding the prevalence and timing of previous ART exposures, length of time on ART, threshold of virologic failure, pregnancy and immunological status of women at ART start, and timing of evaluation.[18,30,31] Many have interpreted the difficulties in achieving excellent postpartum viral suppression with the drug regimen itself and lack of adherence or retention in care.[13,32] Factors cited to influence adherence generally included side effects of HIV, difficulty in obtaining transport to clinic, lack of partner disclosure, and negative treatment by healthcare workers.[33] Few focus on the postpartum period, despite the ongoing opportunity for sexual transmission of HIV, MTCT through breastfeeding and future pregnancies, and development of treatment drug resistance.[12,34] We show that the ability to suppress HIV levels to below 1000 copies/mL in the postpartum period appears to be linked to earlier start of ART in pregnancy, which supports the goals of Option B+. Since we adjusted for duration of time on ART, our findings support the idea that remaining factors possibly involved in a causal pathway between earlier initiation and lower risk of plasma virologic failure in the postpartum include adherence to ART and biological factors that influence the way women physically respond to ART.[21] Concerns that biological changes in pregnant women could attenuate ideal

physical responses to HIV drug regimens are difficult to rule out due to the difficulties in obtaining suitable comparison groups.[18,32]

While adherence was not a factor that achieved significance in our analysis, positive associations between ART adherence and plasma virologic suppression have been consistently documented in the literature.[11,13,35] The proportions of women with plasma virologic suppression have been shown to increase linearly in a dose-dependent fashion from adherence levels of 50% through 100%.[36] However, we did find that younger maternal age, a possible barrier to adherence, was significantly associated with early postpartum plasma virologic failure. While this association was no longer significant in late postpartum or multivariate analysis, it remains plausible. Younger women have been consistently demonstrated to be more likely than older women to be lost to follow-up.[37–39] Older age, receiving an HIV diagnosis prior to pregnancy, and already being on ART at conception have also been associated with lower MTCT.[40] Barriers to adherence and retention in care that are associated with virologic failure may also be more likely to affect younger women. Such barriers have included challenges in same-day initiation of ART and lower levels of education about Option B+, both of which have been associated with poorer ART retention.[8,39,41] However, interventions that have improved adherence when combined with enhanced standard of care have text messaging and phone-based interventions – interventions which may elicit the best response from younger women.[35,42]

Our lack of ability to detect an association between adherence and virologic failure may be due in part to sampling bias as well as data collection limitations.[11,21,25] Treatment non-adherence is typically defined as taking less than 90% to 95% of all pills.[11,35] Because our questionnaires estimated adherence by month or week, our threshold of non-adherence was missing more than two doses a month (ie, taking medication less than 93.3% of the time). According to this measure, 80% of our subjects reported adherence during pregnancy and 97.5% were adherent while breastfeeding. Adherence averages of approximately 73-76% during pregnancy and 53-75% postpartum have been reported in the literature, with many

reporting lower adherence in the postpartum compared to antepartum.[11,13] ART adherence reported by our subjects, particularly while breastfeeding, was substantially higher than normally seen, although a recent meta-analysis used ≤80% as a threshold for analysis, since recent, efavirenz-based, ART regimens are more forgiving when doses are missed.[11,13] In addition, information about ART adherence was only collected at one time shortly post-parturition (and later non-adherence was not captured in our questionnaires), or to actual high adherence in our breastfeeding population. Additionally, in restricting our sample to women who were breastfeeding, our inclusion criteria would have excluded women who had lost babies during pregnancy or in early infancy and women who had stopped breastfeeding early. These women could have been less adherent to therapy, and their exclusion could have reduced our effect size and ability to detect an association. Finally, ART adherence in our study was collected using self-report, which is always considered less accurate than objective pill-counting methods such as pharmacy refills, electronic pill counts, or blood drug levels.[21,25] No subjects in our cohort reported any stock-outs or problems obtaining ART, indicating that drug availability was within their locus of control. Women have been shown to self-report higher than actual adherence, leading to non-differential misclassification of this exposure.[13,43] As a result, some of our sample was likely non-differentially misclassified as adherent, biasing our estimates toward a null association. Non-differential misclassification was likely exacerbated by the fact that women in this cohort could receive different numbers of pills at each of their medication refill visits, depending on the medication set that was available at the time. This variability in pill numbers was not captured in the questionnaire, and possibly had a negative effect on adherence.

Our small sample size imposed several limitations upon our study. We had more missing HIV RNA data at the late postpartum time point, which made it difficult to understand women's viral load trajectories over time. Virologic failure in at least a couple of the specimens for which we were missing 18-24 week postpartum measurements would have resulted in a higher

proportion of women with virologic failure in plasma at this time point compared with the 6-14 week time point. Small sample size limited the generalizability of our results and restricted us from being able to determine whether failures to reject null hypotheses were due to inadequate sample size or true lack of associations. For example, we were unable to detect associations between virologic failure and other factors influencing adherence that have been described in the literature, such as partner awareness of a woman's HIV status, time between HIV diagnosis and ART start, and whether women took ART in a previous pregnancy.[7,35]

The effect sizes of the associations detected between gestational age at ART initiation and plasma virologic failure at 6-14 weeks and 18-24 postpartum were small (adjusted odds ratios of 1.25, 95%CI 1.04, 1.51; and 1.16, 95%CI 1.02, 1.33; respectively). All but one of the gestational ages were given by self-report, which can be inaccurate because it is often calculated using a patient-reported time point – the first day of the mother's last menstrual period, which can be inaccurate due to menstrual cycle irregularity and because menses dates can be difficult to remember. While we may attempt to verify gestational age by calculating it from an infant's date of birth, the calculated gestational age may still be inaccurate, since estimated dates of delivery are not included on the questionnaire, so we cannot account for infants born early or late. In situations like these, non-differential misclassification dampens effect sizes toward the null.

Our study population was drawn from an ART clinic in the Nairobi area that was exceptionally high-performing, and subjects received enhanced support and follow-up for ART adherence. This limited our ability to generalize results to rural, low-performing, or average clinics; or to women not receiving additional ART support as part of a research study.

We were also limited in the window of time within which our viral load measures could be assessed. We did not have data on exposures and HIV viral levels prior to approximately 6 weeks and beyond approximately 24 weeks post-partum. This also restricted our ability to determine when detected resistance mutations occurred – before or after treatment – and draw

further conclusions on causal relationships involving resistance mutations in association with drug regimens and treatment adherence. Longer-term studies are critical for evaluating whether or not the PMTCT cascade under Option B+ promotes virologic suppression throughout the entire breastfeeding period and in future pregnancies, as well as transmission between mothers and their current and future sexual partners. However, the 6-24 week postpartum period that was the focus of this study remains a period of high infant risk for MTCT.[12]

Option B+ remains a strategy that shows promise for protecting pregnant woman and their infants in the pregnancy and early breastfeeding period. Longer-term studies with larger groups of women are needed to understand the impact of Option B+ in extended HIV transmission risk and future pregnancies. However, our results were concerning: among a group of women who reported good ART adherence, faced no drug stock-outs, and who attended the health center regularly, we nevertheless detected more than 1000 copies/ml of HIV in the blood of one in five women. To date, Option B+ programs in Kenya do not routinely use HIV viral load testing to assess HIV viral suppression. Our data indicate that even in high-performing clinics, HIV viral load testing could identify women who would benefit from adherence counseling, ART resistance testing. Furthermore, the partners of these women could benefit from pre-exposure prophylaxis to prevent sexual transmission. Maternal viral load testing could also identify infants who would benefit from ongoing prophylaxis with nevirapine during the breastfeeding period. Option B+ is a promising strategy, but our research indicates that optimal benefit for mothers and babies will be limited without viral load testing to verify that ART is working during the breastfeeding period when infants remain at risk of acquiring HIV.

References

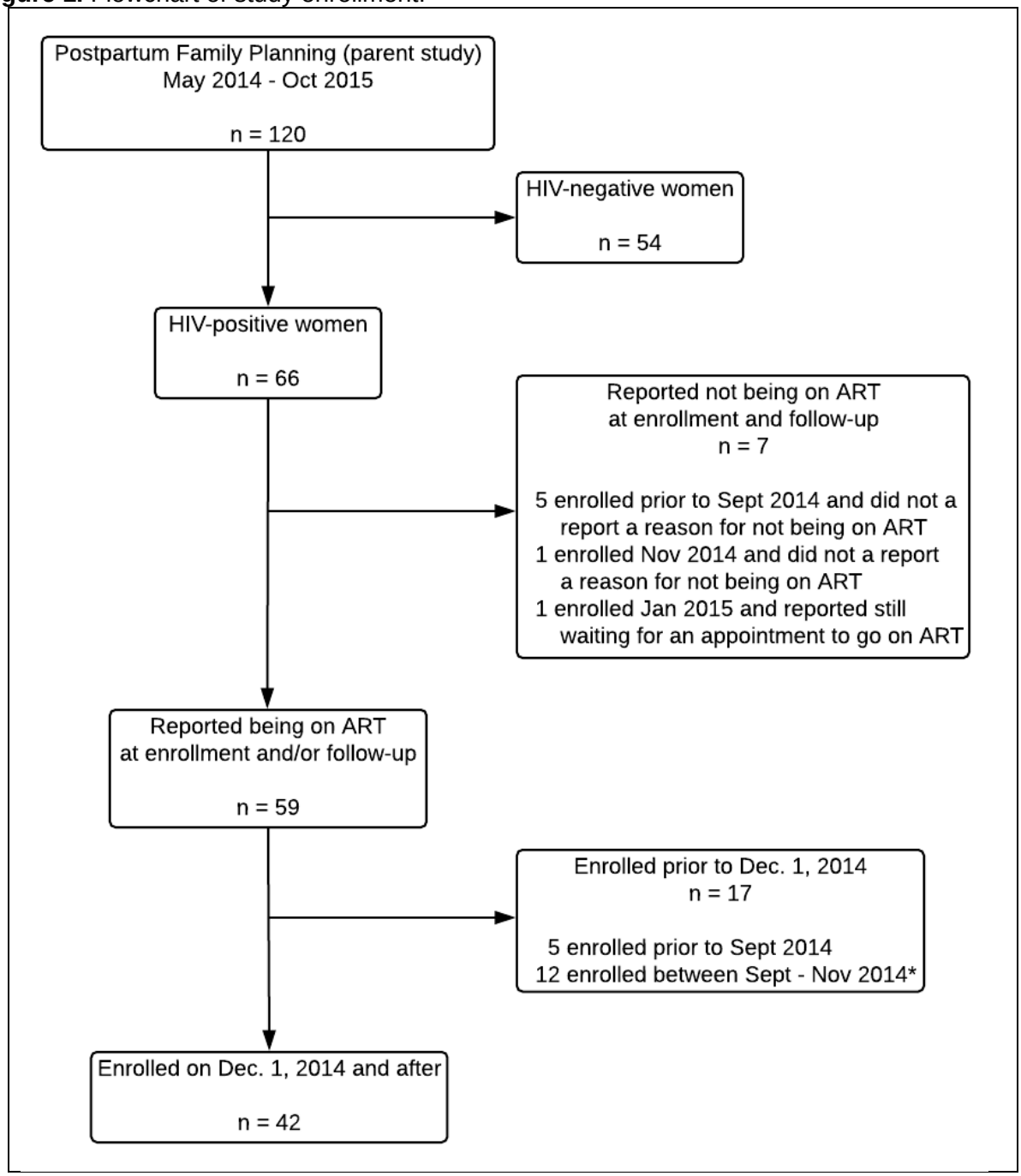
1. UN Joint Programme on HIV/AIDS (UNAIDS). Prevention Gap Report 2016 [Internet]. Available from: http://www.unaids.org/sites/default/files/media_asset/2016-prevention-gap-report_en.pdf
2. World Health Organization (WHO). Programmatic Update: Use of Antiretroviral Drugs for Treating Pregnant Women and Preventing HIV Infection in Infants - Executive Summary [Internet]. 2012. Available from: http://www.who.int/hiv/PMTCT_update.pdf
3. World Health Organization (WHO). Guideline on when to Start Antiretroviral Therapy and

on Pre-Exposure Prophylaxis for HIV. Geneva, Switzerland: 2015.
4. Koss CA, Natureeba P, Kwarisiima D, et al. Viral Suppression and Retention in Care up to 5 Years After Initiation of Lifelong ART During Pregnancy (Option B+) in Rural Uganda. J Acquir Immune Defic Syndr 2017;74(3):279–84.
5. Kamuyango AA, Hirschhorn LR, Wang W, Jansen P, Hoffman RM. One-year outcomes of women started on antiretroviral therapy during pregnancy before and after the implementation of Option B+ in Malawi: A retrospective chart review. World J AIDS 2014;4(3):332–7.
6. Kim MH, Ahmed S, Hosseinipour MC, et al. Brief Report: Impact of Option B+ on the Infant PMTCT Cascade in Lilongwe, Malawi. J Acquir Immune Defic Syndr 2015;70(1):99–103.
7. Kieffer MP, Mattingly M, Giphart A, et al. Lessons learned from early implementation of option B+: the Elizabeth Glaser Pediatric AIDS Foundation experience in 11 African countries. J Acquir Immune Defic Syndr 2014;67 Suppl 4(10):S188-94.
8. Katirayi L, Namadingo H, Phiri M, et al. HIV-positive pregnant and postpartum women's perspectives about Option B+ in Malawi: a qualitative study. J Int AIDS Soc 2016;19(1):20919.
9. Tenthani L, Haas AD, Tweya H, et al. Retention in care under universal antiretroviral therapy for HIV-infected pregnant and breastfeeding women ("Option B+") in Malawi. AIDS 2014;28(4):589–98.
10. Ngarina M, Kilewo C, Karlsson K, et al. Virologic and immunologic failure, drug resistance and mortality during the first 24 months postpartum among HIV-infected women initiated on antiretroviral therapy for life in the Mitra plus Study, Dar es Salaam, Tanzania. BMC Infect Dis 2015;15:175.
11. Haas AD, Msukwa MT, Egger M, et al. Adherence to Antiretroviral Therapy During and After Pregnancy: Cohort Study on Women Receiving Care in Malawi's Option B+ Program. Clin Infect Dis 2016;63(9):1227–35.
12. UN Joint Programme on HIV/AIDS (UNAIDS). On the Fast-Track to an AIDS-free generation [Internet]. 2016. Available from: http://www.unaids.org/sites/default/files/media_asset/GlobalPlan2016_en.pdf
13. Nachega JB, Uthman OA, Anderson J, et al. Adherence to antiretroviral therapy during and after pregnancy in low-income, middle-income, and high-income countries: a systematic review and meta-analysis. AIDS 2012;26(16):2039–52.
14. Gill MM, Hoffman HJ, Bobrow EA, et al. Detectable Viral Load in Late Pregnancy among Women in the Rwanda Option B+ PMTCT Program: Enrollment Results from the Kabeho Study. PLoS One 2016;11(12):e0168671.
15. Mwau M, Bwana P, Kithinji L, et al. Mother-to-child transmission of HIV in Kenya: A cross-sectional analysis of the national database over nine years. PLoS One 2017;12(8):e0183860.
16. National AIDS Control Council. Kenya AIDS Response Progress Report: Progress towards Zero. Nascop, Minist Heal Gov Kenya [Internet] 2014;(March):37. Available from: http://www.unaids.org/sites/default/files/country/documents/KEN_narrative_rep
17. World Health Organization (WHO). Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection. Geneva, Switzerland: World Health Organization (WHO); 2013.
18. Myer L, Phillips TK, McIntyre JA, et al. HIV viraemia and mother-to-child transmission risk after antiretroviral therapy initiation in pregnancy in Cape Town, South Africa. HIV Med 2017;18(2):80–8.
19. DeVange Panteleeff D, Emery S, Richardson BA, et al. Validation of performance of the gen-probe human immunodeficiency virus type 1 viral load assay with genital swabs and breast milk samples. J Clin Microbiol 2002;40(11):3929–37.

20. Beck IA, Mahalanabis M, Pepper G, et al. Rapid and sensitive oligonucleotide ligation assay for detection of mutations in human immunodeficiency virus type 1 associated with high-level resistance to protease inhibitors. J Clin Microbiol 2002;40(4):1413–9.
21. Okonji JA, Zeh C, Weidle PJ, et al. CD4, viral load response, and adherence among antiretroviral-naive breast-feeding women receiving triple antiretroviral prophylaxis for prevention of mother-to-child transmission of HIV in Kisumu, Kenya. J Acquir Immune Defic Syndr 2012;61(2):249–57.
22. StataCorp. Stata Statistical Software: Release 14. 2015;
23. Slyker JA, Chung MH, Lehman DA, et al. Incidence and correlates of HIV-1 RNA detection in the breast milk of women receiving HAART for the prevention of HIV-1 transmission. PLoS One 2012;7(1):e29777.
24. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977;33(1):159–74.
25. Davis NL, Miller WC, Hudgens MG, et al. Maternal and Breast Milk Viral Load: Impacts of Adherence on Peri-Partum HIV Infections Averted - the BAN Study. J Acquir Immune Defic Syndr 2016;73(5):572–80.
26. Marinda P, Chibwe N, Tambo E, Lulanga S, Khayeka-Wandabwa C. Challenges and opportunities of optimal breastfeeding in the context of HIV option B+ guidelines. BMC Public Health 2017;17(1):541.
27. Westreich D, Cole SR, Nagar S, Maskew M, van der Horst C, Sanne I. Pregnancy and virologic response to antiretroviral therapy in South Africa. PLoS One 2011;6(8):e22778.
28. King CC, Ellington SR, Davis NL, et al. Prevalence, Magnitude, and Correlates of HIV-1 Genital Shedding in Women on Antiretroviral Therapy. J Infect Dis 2017;216(12):1534–40.
29. Baeten JM, Kahle E, Lingappa JR, et al. Genital HIV-1 RNA predicts risk of heterosexual HIV-1 transmission. Sci Transl Med 2011;3(77):77ra29.
30. Ngoma MS, Misir A, Mutale W, et al. Efficacy of WHO recommendation for continued breastfeeding and maternal cART for prevention of perinatal and postnatal HIV transmission in Zambia. J Int AIDS Soc 2015;18(1):19352.
31. European Collaborative Study, Patel D, Cortina-Borja M, Thorne C, Newell M-L. Time to undetectable viral load after highly active antiretroviral therapy initiation among HIV-infected pregnant women. Clin Infect Dis 2007;44(12):1647–56.
32. Westreich D, Evans D, Firnhaber C, Majuba P, Maskew M. Prevalent pregnancy, biological sex, and virologic response to antiretroviral therapy. J Acquir Immune Defic Syndr 2012;60(5):489–94.
33. Clouse K, Schwartz S, Van Rie A, Bassett J, Yende N, Pettifor A. “What they wanted was to give birth; nothing else”: barriers to retention in option B+ HIV care among postpartum women in South Africa. J Acquir Immune Defic Syndr 2014;67(1):e12-8.
34. Mancinelli S, Galluzzo CM, Andreotti M, et al. Virological Response and Drug Resistance 1 and 2 Years Post-Partum in HIV-Infected Women Initiated on Life-Long Antiretroviral Therapy in Malawi. AIDS Res Hum Retroviruses 2016;32(8):737–42.
35. Mills EJ, Lester R, Thorlund K, et al. Interventions to promote adherence to antiretroviral therapy in Africa: a network meta-analysis. lancet HIV 2014;1(3):e104-11.
36. Nachega JB, Hislop M, Dowdy DW, Chaisson RE, Regensberg L, Maartens G. Adherence to nonnucleoside reverse transcriptase inhibitor-based HIV therapy and virologic outcomes. Ann Intern Med 2007;146(8):564–73.
37. Landes M, Sodhi S, Matengeni A, et al. Characteristics and outcomes of women initiating ART during pregnancy versus breastfeeding in Option B+ in Malawi. BMC Public Health 2016;15(1):713.
38. Tweya H, Gugsa S, Hosseinipour M, et al. Understanding factors, outcomes and reasons for loss to follow-up among women in Option B+ PMTCT programme in Lilongwe, Malawi. Trop Med Int Health 2014;19(11):1360–6.

39. Knettel BA, Cichowitz C, Ngocho JS, et al. Retention in HIV Care During Pregnancy and the Postpartum Period in the Option B+ Era: Systematic Review and Meta-Analysis of Studies in Africa. J Acquir Immune Defic Syndr 2018;77(5):427–38.
40. Dryden-Peterson S, Lockman S, Zash R, et al. Initial programmatic implementation of WHO option B in Botswana associated with increased projected MTCT. J Acquir Immune Defic Syndr 2015;68(3):245–9.
41. Tsegaye D, Deribe L, Wodajo S. Levels of adherence and factors associated with adherence to option B+ prevention of mother-to-child transmission among pregnant and lactating mothers in selected government health facilities of South Wollo Zone, Amhara Region, northeast Ethiopia, 2016. Epidemiol Health 2016;38:e2016043.
42. Geldsetzer P, Yapa HMN, Vaikath M, et al. A systematic review of interventions to improve postpartum retention of women in PMTCT and ART care. J Int AIDS Soc 2016;19(1):20679.
43. Orrell C, Cohen K, Leisegang R, Bangsberg DR, Wood R, Maartens G. Comparison of six methods to estimate adherence in an ART-naïve cohort in a resource-poor setting: which best predicts virological and resistance outcomes? AIDS Res Ther 2017;14(1):20.

Figure 1. Flowchart of study enrollment.



*Included in sensitivity analysis.

Table 1. Sociodemographic and clinical characteristics of the study population at 6-14 weeks and 18-24 weeks postpartum.

Maternal Sociodemographic and Clinical Characteristics	N (%)*, Median (IQR), or Mean (SD) (N=42)
6-14 weeks postpartum	
Maternal age (years)	26.9 (±4.6)
Married[∞]	35 (83.3%)
Number of previous pregnancies	2.5 (±1.4)
Attended at least some secondary school	18 (42.9%)
Estimated monthly rent (USD[◊])[i]	42 (±41)
Weeks postpartum at enrollment	6.9 (6.6, 10.9)
Received DMPA contraception	29 (69.1%)
Infant's father is HIV positive[†]	17 (40.5%)
Partner aware of woman's HIV status	35 (83.3%)
Infant tested for HIV at ~6 weeks of age[i]	39 (95.1%)
Enrolled in Comprehensive Care Clinic (CCC)	42 (100%)
Months intending to breastfeed	11.3 (±3.1)
Breastfeeding	42 (100.0%)
Counseled about importance of ART adherence	40 (95.2%)
Took AZT or ART in a previous pregnancy	21 (50.0%)
On ART	42 (100%)
Months between HIV diagnosis and ART start[‡]	
≤ 1 month (30 days)	6 (14.3%)
> 1 month (31 days+)	22 (52.4%)
Timing of ART initiation	
prior to pregnancy	10 (23.8%)
during pregnancy through delivery	28 (66.7%)
upon or after delivery	4 (9.5%)
Gestation (weeks) at ART start	21.7 (4.0, 28.0)
ART adherence during pregnancy[i]	
missed ≤ two doses per month	34 (81.0%)
missed > two doses per month	3 (7.1%)
not on ART[α]	4 (9.5%)
ART adherence during breastfeeding[i β]	
missed ≤ doses per month	41 (97.6%)
missed > two doses per month	-
not on ART	-
Length of time on ART at 6-14 weeks postpartum specimen collection, in weeks[iv]	25.5 (17.7, 42.9)
Self-reported CD4 count at ART initiation[iii]	
> 350 cells/mm^3	7 (16.7%)
≤ 350 cells/mm^3	7 (16.7%)
unknown	25 (59.5%)

Maternal Sociodemographic and Clinical Characteristics	N (%)*, Median (IQR), or Mean (SD) (N=42)
Lab-reported CD4 count[i]	
> 350 cells/mm^3	32 (76.2%)
≤ 350 cells/mm^3	9 (21.4%)
Detectable plasma viral load	12 (28.6%)
Virologic failure detected	
in plasma (>1000 copies/mL)	9 (21.4%)
in breast milk[i] (>150 copies/mL)	6 (14.3%)
in endocervical swab specimen (>100 copies/mL)	10 (23.8%)
18-24 weeks postpartum	
Time elapsed since enrollment, in weeks[ii]	13.0 (12.0, 13.1)
On ART[ii]	39 (92.9%)
Breastfeeding[ii]	38 (90.5%)
Detectable plasma viral load[iv]	12 (28.6%)
Virologic failure detected	
in plasma[iv] (>1000 copies/mL)	8 (21.1%)
in breast milk[iv] (>150 copies/mL)	3 (7.1%)
in endocervical swab specimen[ii] (>100 copies/mL)	6 (14.3%)

IQR = Interquartile Range, SD = Standard Deviation
* May not sum to 100% due to missing values.
[∞] Versus single/divorced/separated/widowed.
[°] Using an exchange rate of 92 USD to 1 Kenyan Shilling (Feb. 1, 2015 rate from http://www.xe.com)
[i] 1 missing. [ii] 2 missing. [iii] 3 missing. [iv] 4 missing. [†] 13 missing. [‡] 14 missing.
[α] 1 started ART at delivery, and 3 sometime between delivery and enrollment.
[β] From the time of ART initiation (before enrollment for all women).

Table 2. Subjects With Detectable Viral Load at 6-14 Weeks or 18-24 Weeks Postpartum

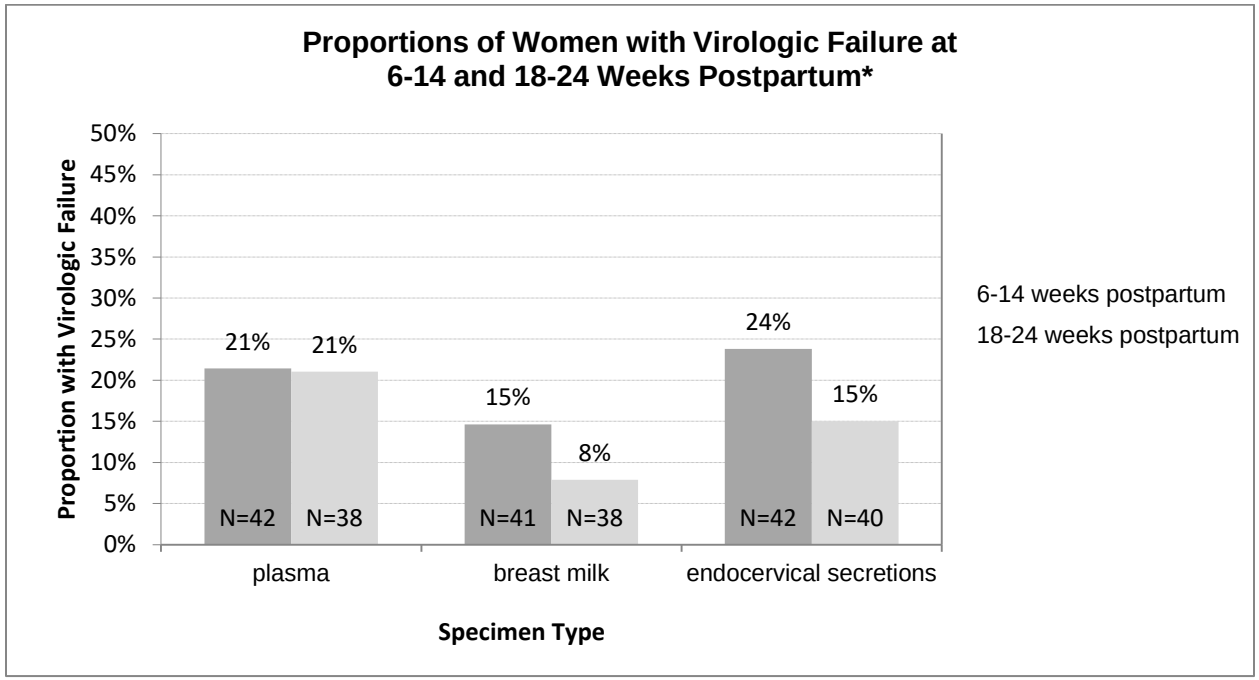
Subject	6-14 weeks postpartum			18-24 week postpartum		
	plasma	breast milk	endocervical secretions	plasma	breast milk	endocervical secretions
1	11706	*75*	9165	134	*75*	*50*
2	*50*	*75*	*50*	400	*75*	*50*
3	34744	2860	190	41910	220	498
4	47748	*75*	105	42168	*75*	*50*
5	150	*75*	*50*	*50*	*75*	*50*
6	16486	*75*	452	24488	*75*	703
7	1836	*75*	122	63802	*75*	6530
8	820	*75*	435	1746	*75*	*50*
9	64344	*75*	5865	*(missing)*	*(missing)*	*(missing)*
10	*50*	*75*	*50*	*50*	750	*50*
11	*50*	*75*	*50*	108	*75*	*50*
12	4178	2615	143	5944	*75*	9765
13	*50*	*75*	*50*	236	*75*	*50*
14	212302	6965	9178	118438	690	6095
15	*50*	180	*50*	*(missing)*	*(missing)*	*50*
16	83116	340	7305	18278	*75*	1375
17	690	520	*50*	*50*	*75*	*50*
No. with virologic failure	9	6	10	8	3	6
No. who eventually achieved virologic suppression[Δ]	1	3	3	-	-	-
No. suppressed at 6-14 weeks with virologic failure at 18-24 weeks postpartum	-	-	-	1	1	0
Median HIV RNA*, IQR	14096 (1328, 56046)	1568 (340, 2860)	444 (143, 7305)	12111 (318, 42039)	690 (220, 750)	3735 (703, 6530)

IQR=Interquartile Range

[Δ] Values given in copies/mL. Thresholds for virologic failure: plasma >1000 copies/mL, breast milk >150 copies/mL, endocervical secretions >100 copies/mL.

* Median HIV RNA calculated using only values above the threshold of detection (100 copies/mL for all specimen types).
Note: Plasma testing for HIV mutations conferring drug resistance was performed for all patients who were found to have plasma virologic failure at either time point. Subjects 3, 6, 8, 9, and 14 were found to have reverse transcriptase or protease inhibitor mutations. Subjects 1, 4, 7, 12, and 16 were found to be negative for these resistance mutations.

Figure 2.



*N represents the total number of each specimen type for which viral load measurements are available. Missings are excluded.

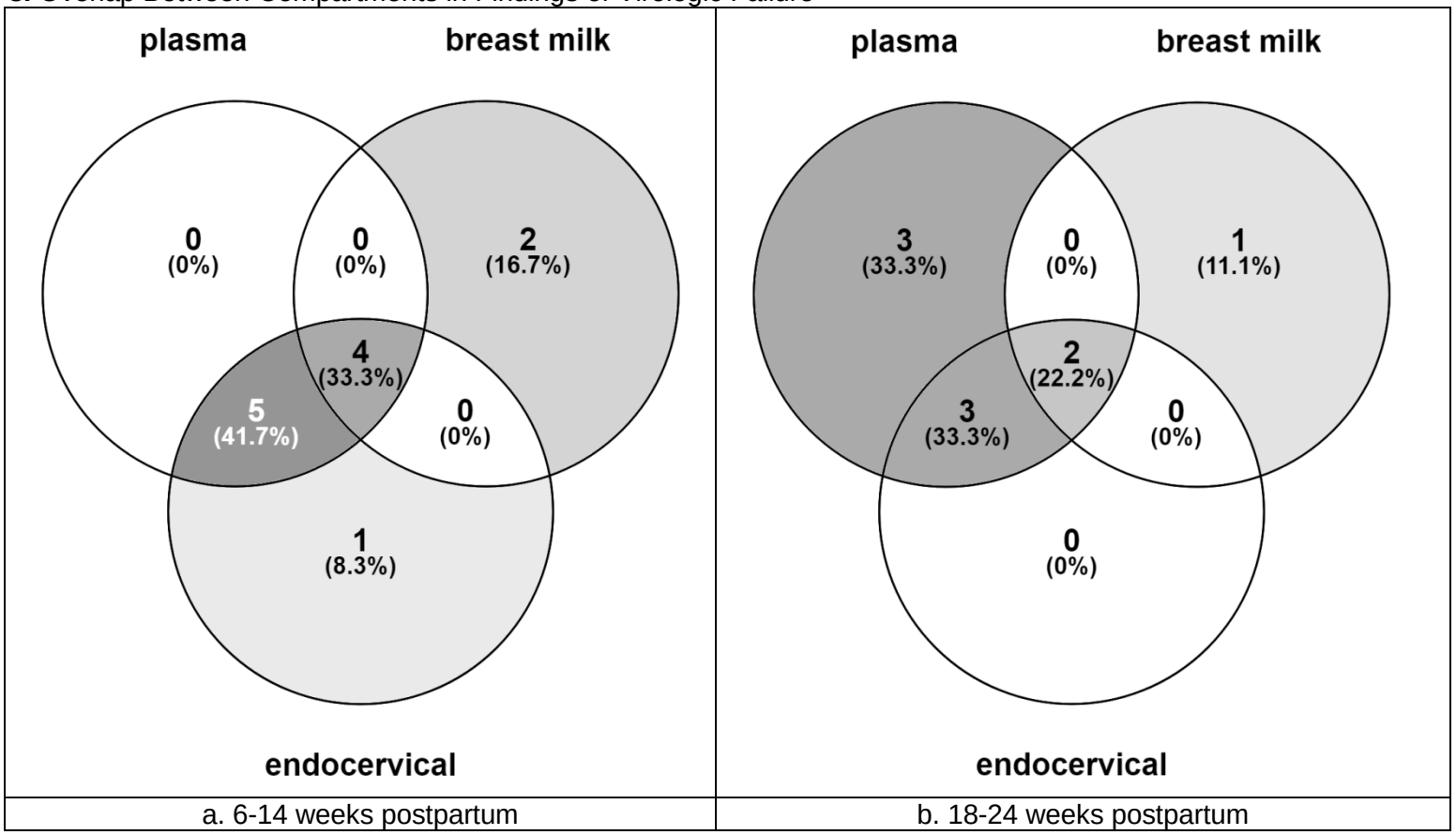
[Δ] Thresholds for virologic failure: plasma >1000 copies/mL, breast milk >150 copies/mL, endocervical secretions >100 copies/mL.

Table 3. Comparisons of Virologic Suppression Status Between Compartments*

plasma	breast milk[i]		endocervical secretions	
	> 150 copies/mL (suppression)	≤ 150 copies/mL (failure)	> 100 copies/mL (suppression)	≤ 100 copies/mL (failure)
6-14 wks postpartum (N = 42)				
≤ 1000 copies/mL (suppression)	30 (71.4%)	2 (4.8%)	32 (76.2%)	1 (2.4%)
> 1000 copies/mL (failure)	5 (11.9%)	4 (9.5%)	0 (0%)	9 (21.4%)
Cohen's κ	0.43		0.93	
18-24 wks postpartum (N = 38)[iv]				
≤ 1000 copies/mL (suppression)	29 (76.3%)	1 (2.6%)	30 (79.0%)	0 (0%)
> 1000 copies/mL (failure)	6 (15.8%)	2 (5.3%)	2 (5.3%)	6 (15.8%)
Cohen's κ	0.28		0.83	

* Percentages are calculated for each combination of specimens.
[i] One missing measurement at 6-14 weeks postpartum.
[iv] Four missing plasma measurements at 18-24 weeks postpartum.

Figure 3. Overlap Between Compartments in Findings of Virologic Failure[Δ1]



a. 6-14 weeks postpartum | b. 18-24 weeks postpartum

Numbers of patients with virologic failure are given. Percentages are out of the total numbers of patients with virologic failure in any specimen at each time point.

[Δ] Thresholds for virologic failure: plasma >1000 copies/mL, breast milk >150 copies/mL, endocervical secretions >100 copies/mL.

[1] Oliveros, J.C. (2007-2015) Venny. An interactive tool for comparing lists with Venn's diagrams. http://bioinfogp.cnb.csic.es/tools/venny/index.html

Table 4. Correlates of Virologic Failure.

Maternal Sociodemographic and Clinical Characteristics	6-14 wks postpartum			18-24 wks postpartum		
	N (%)*, Median (IQR), or Mean (SD)			N (%)*, Median (IQR), or Mean (SD)		
	>1000 copies/mL (failure) (n=9)	≤1000 copies/mL (suppression) (n=33)	p-value	>1000 copies/mL (failure) (n=8)	≤1000 copies/mL (suppression) (n=30)	p-value
Maternal age (years)	24.2 (±4.4)	27.6(±4.4)	**0.05**	25.9 (±4.7)	27.1 (±4.2)	0.43
Married[∞]	8 (88.9%)	27 (81.8%)	1.00	7 (87.5%)	26 (86.7%)	1.00
Number of past pregnancies	2.3 (±1.2)	2.5 (±1.5)	0.97	2.8 (±1.3)	2.3 (±1.3)	0.23
Attended at least some secondary school	5 (55.6%)	13 (39.4%)	0.46	3 (37.5%)	13 (43.3%)	1.00
Estimated monthly rent (USD[◊])	66 (±82)	36 (±15)	0.67[i]	67 (±88)	37 (±16)	0.55[i]
Weeks postpartum at enrollment	7.0 (6.7, 7.7)	6.7 (6.6, 10.9)	0.77	6.9 (6.6, 7.4)	6.9 (6.6, 11)	0.58
Received DMPA contraception	7 (77.8%)	22 (66.7%)	0.70	6 (75.0%)	21 (70.0%)	1.00
Partner aware of participant's HIV status	8 (88.9%)	27 (81.8%)	1.00	7 (87.5%)	26 (86.7%)	1.00
Months intending to breastfeed	13.3 (±4.0)	10.7 (±2.6)	**0.05**	13.5 (±4.2)	10.8 (±2.6)	0.06
Took ART in a previous pregnancy	4 (44.4%)	17 (51.5%)	1.00	3 (37.5%)	17 (56.7%)	0.44
Timing of ART initiation			0.29			0.11
prior to pregnancy	1 (11.1%)	9 (27.3%)		2 (25.0%)	6 (20.0%)	
during pregnancy through delivery	6 (66.7%)	22 (66.7%)		4 (50.0%)	23 (76.7%)	
upon or after delivery	2 (22.2%)	2 (6.1%)		2 (25.0%)	1 (3.3%)	
Gestation (weeks) at ART start	27.0 (26.0, 32.0)	20.0 (0, 25.0)	**0.02**[i]	26.0 (12.0, 36.0)	20.0 (8.0, 27.0)	0.20
ART adherence during pregnancy			0.21[i]			0.22[i]
missed ≤ two doses per month	6 (66.7%)	28 (84.9%)		6 (75.0%)	25 (83.3%)	
missed > two doses per month	1 (11.1%)	2 (6.1%)		2 (25.0%)	3 (10.0%)	
not on ART	2 (22.2%)	2 (6.1%)		0 (0%)	1 (3.3%)	
Length of time on ART at 6-14 weeks postpartum specimen collection	17.3 (12.1, 30.0)	27.7 (19.9, 43.9)	0.08[iv]	26.0 (9.9, 66.7)	24.3 (18.9, 39.3)	0.77[ii]
Lab-reported CD4 count at 6-14 weeks postpartum			0.38[i]			**0.05**[i]
> 350 cells/mm^3	6 (66.7%)	26 (78.8%)		4 (50.0%)	25 (83.3%)	
≤ 350 cells/mm^3	3 (33.3%)	6 (18.2%)		4 (50.0%)	4 (13.3%)	

Maternal Sociodemographic and Clinical Characteristics	6-14 wks postpartum			18-24 wks postpartum		
	N (%)*, Median (IQR), or Mean (SD)		p-value	N (%)*, Median (IQR), or Mean (SD)		p-value
	>1000 copies/mL (failure) (n=9)	≤1000 copies/mL (suppression) (n=33)		>1000 copies/mL (failure) (n=8)	≤1000 copies/mL (suppression) (n=30)	

IQR = Interquartile Range. SD = Standard Deviation

*May not sum to 100% due to missings. [i]1 missing. [iii]3 missing. [iv]4 missing.

[°°]Versus single/divorced/separated/widowed.

[°] Using an exchange rate of 92 USD to 1 Kenyan Shilling (Feb. 1, 2015 rate from http://www.xe.com).

Table 5. Odds Ratios of Virologic Failure in Plasma[Δ]

Maternal Sociodemographic and Clinical Characteristics	6-14 weeks postpartum			18-24 weeks postpartum		
	N	multivariate aOR (95% CI)	p-value	N	multivariate aOR (95% CI)	p-value
Maternal age (years)	37	*		34	*	
Gestation at ART start (weeks)		1.25 (1.04, 1.51)	**0.02**		1.16 (1.02, 1.33)	**0.03**
Length of time on ART at enrollment		1.03 (1.00, 1.06)	**0.04**		1.03 (1.00, 1.05)	**0.02**
Low CD4 count at 6-14 wks postpartum		3.74 (0.37, 38.0)	0.27		24.54 (1.54, 391.02)	**0.02**

N=number of OR=Odds Ratio. aOR=adjusted Odds Ratio. All listed variables were considered for the multivariate model.
[Δ] Threshold for virologic failure in plasma: >1000 copies/mL.
* Excluded from the final multivariate model.