Short-Course Antenatal Zidovudine Reduces Both Cervicovaginal Human Immunodeficiency Virus Type 1 RNA Levels and Risk of Perinatal Transmission

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Human immunodeficiency virus (HIV) levels in cervicovaginal lavage (CVL) and plasma samples were evaluated in relation to perinatal transmission in a randomized placebo-controlled trial of brief antenatal zidovudine treatment. Samples were collected at 38 weeks' gestation from 310 women and more frequently from a subset of 74 women. At 38 weeks, after a 2-week treatment period, CVL HIV-1 was quantifiable in 23% and 52% of samples in the zidovudine and placebo groups, respectively ($P < .001$). The perinatal transmission rate was 28.7% among women with quantifiable CVL HIV-1 and high plasma virus levels (>10,000 copies/mL) and 1% among women without quantifiable CVL HIV-1 and with low plasma virus levels ($P < .001$). A 1-log increase in plasma HIV-1 increased the transmission odds 1.8 and 6.1 times (95% confidence interval, 0.9–3.5 vs. 2.4–15.4) for women with and without quantifiable CVL HIV-1, respectively ($P = .03$). CVL HIV-1 is an independent risk factor for perinatal HIV-1 transmission.

Recent data indicate that most perinatal human immunodeficiency virus type 1 (HIV-1) transmission occurs during labor and delivery [1, 2]. However, the mechanisms of intrapartum transmission are not well understood. Several observations, including the differential infection rate for vaginally delivered twins [3], the protective effect of cesarean delivery on perinatal transmission [4–6], and the association between prolonged ruptured membranes and perinatal transmission [7, 8], suggest that the newborn's exposure to HIV in cervicovaginal secretions and blood while passing through the birth canal may be an important determinant of transmission. Although HIV-1 has been detected in cervicovaginal secretions of pregnant and nonpregnant HIV-infected women [9–16], the relationship between HIV-1 in cervicovaginal secretions and perinatal HIV transmission has not been well defined.

Zidovudine can reduce the risk of perinatal HIV-1 transmission [17, 18]. In the AIDS Clinical Trials Group 076 Study, in which the mother's treatment began at 14–34 weeks' gestation and the infant was treated for 6 weeks, the mechanism by which zidovudine reduced transmission by two thirds was not clear. Only a small part of the efficacy was explained by reduction in maternal plasma HIV levels [19]. In contrast, in our recent study, short-course maternal zidovudine treatment beginning at 36 weeks reduced transmission risk by half, and we identified maternal plasma HIV level at delivery as the most important determinant of transmission [18]. We have now analyzed virus levels in cervicovaginal lavage (CVL) specimens and have evaluated the interrelationships among HIV-1 levels in CVL and plasma, zidovudine treatment, and risk of perinatal transmission.

Patients and Methods

Patients. This study was part of a clinical trial seeking to evaluate the safety and efficacy of a short antenatal treatment with

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Informed consent was obtained from study patients, and human experimentation guidelines of the US Department of Health and Human Services were followed. The study was approved by the Ethical Review Committee, Ministry of Public Health of Thailand, and the Institutional Review Board, Centers for Disease Control and Prevention.

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oral zidovudine to reduce perinatal HIV transmission. In the trial, 397 HIV-1–infected pregnant women at 2 Bangkok hospitals were randomly assigned to receive zidovudine or placebo [18]. The zidovudine regimen was 300 mg orally twice daily from 36 weeks' gestation until onset of labor and 300 mg every 3 h orally from onset of labor until delivery. Plasma was obtained from all participants at 36 weeks (before starting the study drug), at 38 weeks, and at delivery, and CVL samples were collected at 38 weeks. Of the total study participants, 393 women gave birth to 395 live babies, and 55 (14%) of these women delivered by cesarean section because of obstetrical indications. From February through July 1997, 74 consecutively enrolled women were offered enrollment in a substudy to evaluate more closely the change in plasma HIV RNA level resulting from the study regimen. From the women enrolled in the substudy, additional CVL samples were collected at 36 weeks, 37 weeks, and 1 month postpartum. All women were provided with infant formula and were counseled not to breastfeed. For definitive transmission status, infants were considered HIV infected if any polymerase chain reaction (PCR) test result was positive, and they were considered not infected if their last available PCR test result was negative at 2 months of age or thereafter [18].

Collection of CVL samples for HIV RNA PCR. CVL samples were obtained according to procedures of the *DAIDS Virology Manual for HIV Laboratories* [20], with the modification that 3 mL rather than 10 mL of saline was used to flush the cervix and vaginal wall. A transfer pipette was used to recollect as much of this fluid as possible. Samples were transported on ice to the local laboratory and were centrifuged at 750–1000 *g* at 4°C for 15 min. Separate aliquots of the supernatants and pellets were stored at −70°C.

HIV RNA PCR assay. HIV RNA levels in plasma and in CVL samples were determined by using the Amplicor HIV-1 Monitor Test, version 1.5 (Roche Diagnostic Systems, Branchburg, NJ), a quantitative reverse-transcriptase PCR assay. The same procedures were used for testing plasma and CVL supernatants. The lower quantitation limit for this assay was 400 copies/mL.

Statistical analysis. Data were analyzed with version 6.12 of SAS software (SAS, Cary, NC) and version 3.3 of S-Plus software (MathSoft, Cambridge, MA). For analyses using RNA measurements as continuous variables, we set values below the quantitation limit to be 200 copies/mL. However, because a large number of CVL measurements were below the quantitation limit and because CVL RNA levels are measured less precisely than plasma levels are, our statistical analyses emphasized whether CVL HIV RNA was quantifiable, rather than what the quantifiable value was. RNA values are rounded to the nearest 10 copies/mL for CVL and 100 copies/mL for plasma. Two-sided P values $<.05$ were considered statistically significant.

We used data from the subset of women with serial CVL measurements to assess temporal changes in the detection of CVL HIV RNA at the end of pregnancy and the effect of short-course zidovudine treatment. For CVL HIV RNA levels at each time point (the 36-week baseline, 37 weeks, 38 weeks, and 1 month postpartum), we determined the median RNA levels and the percentage of specimens with quantifiable HIV RNA. We compared treatment groups by using the Wilcoxon rank sum test for continuous variables, modified for measurements with a quantitation limit [21], and the χ^2 test for categorical variables. We used logistic regression to test for differences between treatment groups in the proportion of specimens with quantifiable HIV RNA over time. Because these proportions represent repeated measurements from the same subjects, we used the generalized estimating equation method [22]. We used the sign test to evaluate whether CVL HIV RNA levels decreased or increased after 36 weeks for individual women and used the McNemar test for matched pairs to assess changes in the percentage of samples with quantifiable virus levels. For both plasma and CVL HIV RNA, we estimated the coefficient of variation (CV) from the 2 or 3 weekly antenatal samples from women in the substudy placebo group. Each CV estimate was computed as the square root of the pooled estimate of variance divided by the unweighted mean of the women's individual means, restricted to women with $\geqslant 2$ quantifiable samples.

We used data from all women in the trial with CVL samples at 38 weeks to evaluate the relationships between CVL at 38 weeks, plasma HIV RNA at 38 weeks and delivery, and risk of transmission. We used Spearman rank correlation coefficients to assess the relationship of CVL and plasma HIV RNA levels to CD4 count at delivery. We used stratified analysis and logistic regression, adjusted for plasma virus level at delivery, to evaluate the association between CVL virus level at 38 weeks and perinatal transmission.

Results

Baseline characteristics of the 74 women in the substudy (36 zidovudine, 38 placebo) were similar to those of the 310 women in the cohort who had a CVL collection at 38 weeks and to those of the complete cohort of 397 women (not shown) [18]. Of the 83 women in the cohort who did not have a 38-week CVL collection, 72 (87%) delivered before the scheduled collection (4 others were lost to follow-up). The low prevalence of genital ulcer disease was consistent with previous findings [23, 24]. Of the 310 women, 5 (1.6%) were VDRL-positive at their first antenatal visit and were treated before study enrollment, and 1 (0.3%) had a genital ulcer at delivery. Therefore, we did not pursue analyses relating genital ulcer disease to CVL HIV RNA levels. The estimated CVs for log HIV RNA level for successive predelivery samples from the same women in the placebo group were 11.5% and 3.7% in CVL and plasma, respectively.

Subset analysis of the effect of zidovudine on CVL virus level. CVL specimens from 72 women at baseline (36 weeks) were assayed for HIV RNA. Of these women, 38 (53%) had quantifiable HIV RNA. Women with and without quantifiable RNA did not differ significantly by age, clinical stage, risk group, hematocrit, CD4 count, or partner's HIV infection status (not shown).

At baseline, CVL RNA levels were similar in the zidovudine and placebo groups (table 1). Median HIV RNA levels decreased during late pregnancy among women receiving zidovudine but were relatively constant in the placebo group. Similarly, in the zidovudine group, 16 (94%) of 17 women and 16 (89%) of 18 women had decreased RNA levels in CVL speci-

Table 1. Human immunodeficiency virus type 1 (HIV-1) RNA in cervicovaginal lavage (CVL) samples during antenatal visits and at 1 month postpartum in subset of study participants, classified by treatment group.

	Zidovudine				Placebo				
			CVL change[a]				CVL change[a]		
Visit	Subjects (*n*)	Median HIV-1 RNA (copies/mL)	Decrease/*n* (%)	*P*[b]	Subjects (*n*)	Median HIV-1 RNA (copies/mL)	Decrease/*n* (%)	*P*[b]	*P*[c]
36 weeks	35	660	—	—	37	470	—	—	.80
37 weeks	34	<400	16/17 (94)	<.001	36	1770	9/23 (39)	.40	.006
38 weeks	35	<400	16/18 (89)	.001	33	580	9/20 (45)	.82	.004
1 month postpartum	36	2700	9/31 (29)	.03	35	3590	7/28 (25)	.01	.54

[a] Values are expressed as decrease/*n* (%), where "decrease" represents the number of subjects whose CVL samples showed decreased HIV-1 RNA levels compared with results recorded at 36 weeks, and *n* represents the total number of CVL samples for which at least 1 of the 2 CVL determinations (at 36 weeks or at the comparison time) was quantifiable.
[b] The sign test was used to assess the number of samples with decreased HIV-1 RNA levels compared with the 36-week baseline.
[c] Wilcoxon two-sample test, modified for quantitation limits, was used in comparing results of zidovudine versus placebo [21].

mens obtained at 37 and 38 weeks, respectively, compared with baseline data. In contrast, CVL RNA levels in the placebo group decreased in only 9 (39%) of 23 women at 37 weeks and in 9 (45%) of 20 women at 38 weeks. Compared with the placebo group, the CVL RNA levels of the zidovudine group were significantly lower at both 37 and 38 weeks. At 1 month postpartum, CVL RNA levels in the zidovudine and placebo groups were similar to each other but had increased from baseline.

The findings were similar when analyzed according to the percentage of CVL samples with quantifiable HIV RNA concentrations (figure 1). At baseline, quantifiable CVL samples were similar in the zidovudine and placebo groups (54% and 51%, respectively). The overall change in the percentage of quantifiable samples from 36 through 38 weeks in the zidovudine group was different from that in the placebo group ($P < .001$). In the zidovudine group, the percentage of quantifiable samples dropped to 35% at 37 weeks ($P = .04$) and to 20% at 38 weeks ($P = .002$; McNemar test). In the placebo group, the percentage of quantifiable samples at 37 and 38 weeks remained similar to the baseline percentage. At 38 weeks, the percentages of quantifiable samples in the zidovudine and placebo groups were similar for the subset and the cohort. Compared with the placebo group, the zidovudine group had a lower percentage of quantifiable CVL samples at 37 weeks and 38 weeks in the subset and at 38 weeks in the cohort. At 1 month postpartum, the percentages of quantifiable CVL samples in both the zidovudine and placebo groups were higher than at 36 weeks ($P = .003$ and $P = .04$, respectively) but were similar to each other.

Correlation between CVL and plasma HIV RNA levels. Of the 310 women in the cohort with CVL samples at 38 weeks, 116 (37%) had quantifiable HIV RNA in CVL samples. The

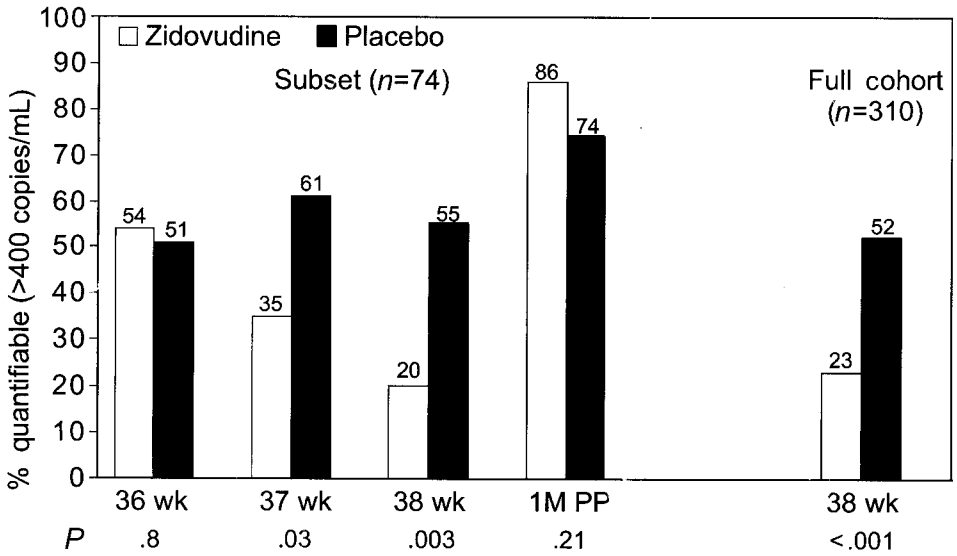

Figure 1. Percentage of cervicovaginal lavage specimens with quantifiable human immunodeficiency virus type 1 RNA, categorized by treatment group, for the subset with multiple collections and for the full cohort at 38 weeks' gestation. *P* values represent statistical significance of differences between the zidovudine *(open bar)* and placebo *(solid bar)* groups. 1M PP, 1 month postpartum; wk, week.

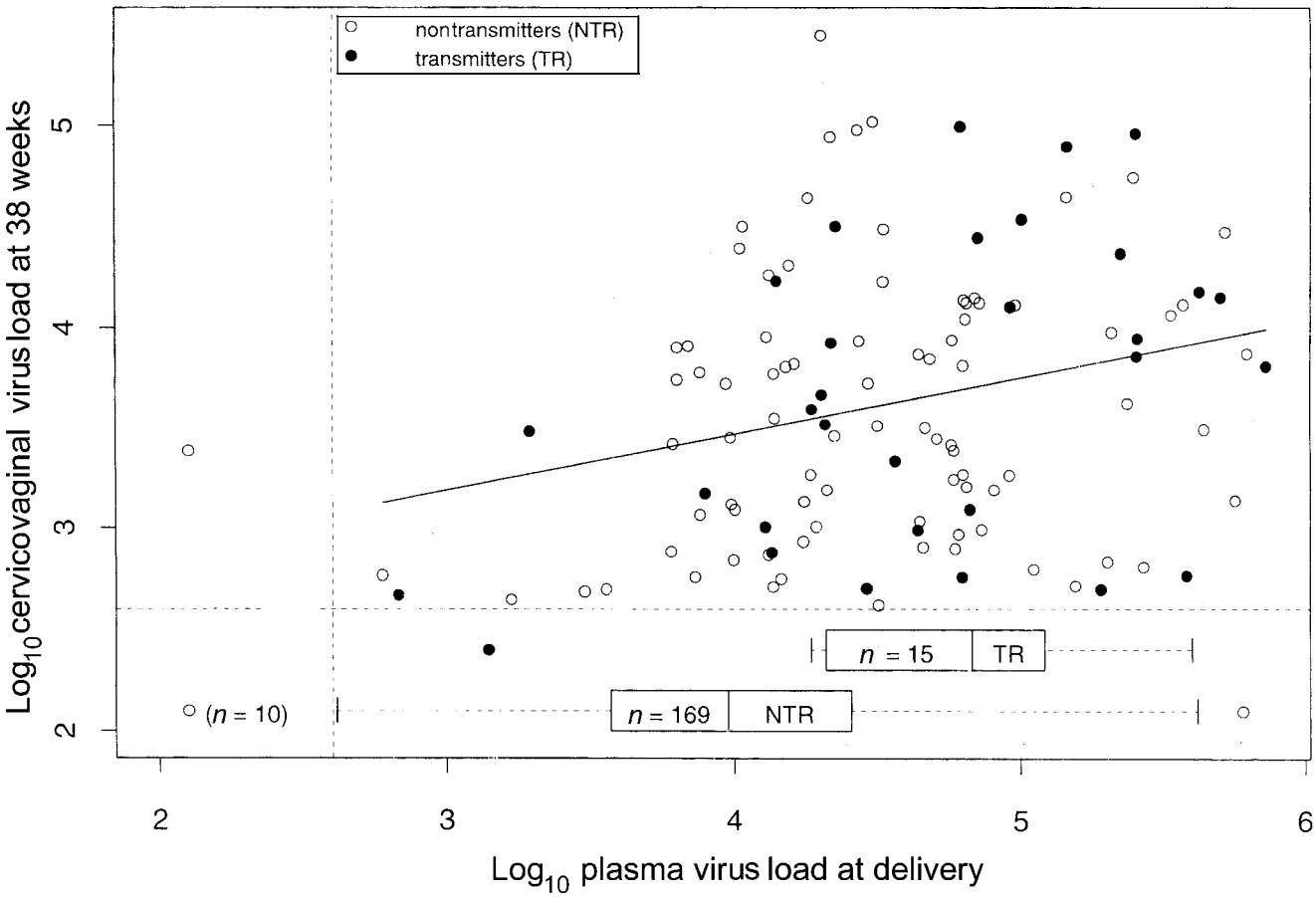

Figure 2. Correlation of human immunodeficiency virus (HIV) type 1 RNA in cervicovaginal lavage (CVL) and plasma with transmission status and ability to quantify CVL HIV. Solid line indicates regression line for women (n=115), with both CVL and plasma HIV RNA levels above the limit of quantitation for assay (r = .27, P = .004). Dashed lines show lower quantitation limits (2.6 log or 400 copies/mL). For CVL samples below quantitation limits, box plots show distribution of plasma virus levels of women who did versus those who did not transmit HIV to their infants. Open circle in lower left quadrant represents 10 nontransmitting women with both plasma and CVL RNA levels below limits of quantitation. To represent all data, 4 women with unknown transmission status are included among the nontransmitting women in the box plot, and 2 women with unknown transmission status are included as nontransmitters in the scatter plot.

median level of the quantifiable samples was 3710 copies/mL. Of 309 women with plasma samples, 299 (97%) had quantifiable plasma HIV RNA at 38 weeks (median, 11,600 copies/mL) and at delivery (median, 16,400 copies/mL). For all available 38-week CVL samples and delivery plasma samples (and with non-quantifiable samples assigned a value of 200 copies/mL), plasma correlated moderately with CVL HIV RNA levels (r = .39, P < .001). As shown in figure 2, the correlation was similar, but less (r = .27, P = .004), when the analysis was limited to women with quantifiable levels; among these women, the correlation was similar for transmitting (r = .36) and non-transmitting (r = .23) mothers. Although statistically significant, the correlation between CD4 count at delivery and CVL HIV RNA level at 38 weeks was weak (r = −.16, P = .006).

Among 18 women in the zidovudine group with baseline CVL HIV RNA above quantitation and a CVL determination at 38 weeks, there was a moderate correlation between the change in CVL and plasma HIV RNA levels at 38 weeks, compared with baseline (r = .56, P = .02; figure 3). All of these women had a decrease in plasma RNA level (median, 0.7-log decrease; range, 0.1–1.3), and all but 2 of these women had a decrease in CVL RNA level (median, ⩾0.8-log decrease; range, 1-log increase to ⩾2.4-log decrease).

Analysis of CVL and plasma HIV RNA levels at 38 weeks' gestation and risk of perinatal HIV transmission. For the overall cohort, controlling for treatment, we found that women who had quantifiable HIV RNA in their 38-week CVL samples were at higher risk of transmitting HIV to their infants than those who did not (26.3% vs. 7.9%; Mantel-Haenszel odds ratio [OR] stratified on treatment, 3.4; P = .001). This association was present in both the zidovudine (OR, 6.7; P = .001) and placebo (OR, 2.5; P = .03) groups (table 2). In each treatment group,

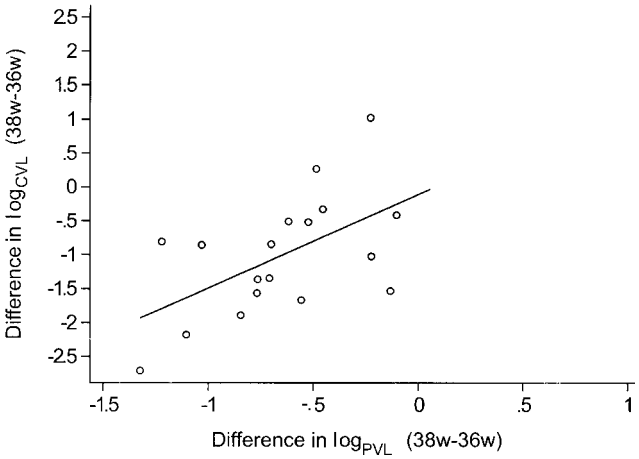

Figure 3. Correlation between changes in human immunodeficiency virus (HIV) type 1 RNA levels in cervicovaginal lavage (CVL) and plasma samples, from 36 weeks' (baseline) to 38 weeks' gestation, in women taking short-course zidovudine. In the zidovudine subgroup, 18 women had quantifiable CVL HIV RNA at baseline and a CVL HIV RNA measurement at 38 weeks (with detection limit set at 200 copies/mL). Solid line represents the regression line ($r = .56$, $P = .015$). PVL, plasma HIV RNA viral load.

women with HIV-infected infants had higher CVL RNA levels than women whose infants were not infected (zidovudine group, $P = .001$; placebo group, $P = .03$).

We further evaluated the association between CVL virus level and perinatal transmission by controlling for plasma RNA level. Because a plasma RNA level >10,000 copies/mL at delivery was a highly significant threshold for transmission risk in this population [6, 18], we stratified findings by plasma RNA levels of ⩾10,000 and <10,000 copies/mL (table 3). CVL with quantifiable RNA was strongly associated with perinatal transmission in both strata. Women with quantifiable HIV RNA in CVL and high plasma RNA levels had the highest transmission rate and 28 times the risk of transmission (95% confidence interval [CI], 3.9–201; $P < .001$) as women without quantifiable CVL and with low plasma HIV RNA. Women with high plasma HIV RNA and nonquantifiable CVL HIV RNA and women with low plasma HIV RNA and quantifiable CVL HIV RNA had similar transmission rates. Having quantifiable CVL HIV RNA increased the risk of transmission 1.9 times (95% CI, 1.1–3.4; $P = .02$) for women with high plasma virus levels and 14.6 times (95% CI, 1.6–133; $P = .016$, Fisher's exact test) for women with low plasma virus levels.

In a multiple logistic regression model, quantifiable CVL HIV RNA and log plasma HIV RNA were associated with risk for transmission, but CD4 count was not. We therefore limited further analysis to plasma and CVL RNA levels. The interaction between quantifiable CVL and plasma HIV RNA was significant ($P = .03$). The combination of quantifiable CVL and this interaction added predictive value to the model containing only plasma HIV RNA (likelihood ratio $\chi^2 = 11.7$ with 2 *df;* $P = .003$). In this model, the risk of transmission increases more rapidly with increasing plasma HIV RNA among women with nonquantifiable CVL than among those with quantifiable CVL. The model predicts that for an increase of 1 log in plasma HIV RNA, the odds of transmission increase by 6.1 (95% CI, 2.4–15.4; $P < .001$) for women without quantifiable CVL HIV and by 1.8 (95% CI, 0.9–3.5; $P = .10$) for women with quantifiable CVL HIV.

Discussion

This study shows that HIV RNA in the cervicovaginal canal is a risk factor for perinatal HIV transmission, even after adjustment for plasma HIV RNA. Furthermore, this study shows that cervicovaginal HIV RNA levels can be substantially reduced by as little as 1–2 weeks of zidovudine administered near the end of pregnancy.

In our main analysis of the Bangkok short-course zidovudine trial, which was conducted in a non–breast-feeding population, we found a strong relationship between plasma virus level and risk of perinatal transmission [18]. Women with low plasma HIV RNA levels (<10,000 copies/mL) had a low risk for transmission (4%) compared with women with higher RNA levels (21%), and 80% of the efficacy of zidovudine was explained by reduction in plasma RNA level at delivery. In this new analysis, we demonstrate the additional effect of CVL RNA level on risk of transmission. Women with low plasma viral RNA levels at delivery and low (below the quantifiable limit) HIV RNA levels in CVL samples at 38 weeks' gestation had an extremely low transmission risk (1%), women with low plasma viral RNA levels but quantifiable HIV RNA in CVL samples had a higher transmission risk (15%), and women with both high plasma viral RNA levels and quantifiable HIV RNA in CVL samples had the highest transmission risk (29%). Expressed another way, women with high plasma HIV RNA levels and quantifiable HIV RNA in CVL samples had 28 times the risk of transmitting HIV to their child as those with low plasma RNA levels and nonquantifiable HIV RNA in their CVL samples.

Even after we adjusted the data for plasma HIV RNA levels, our stratified and multivariate analyses confirmed that HIV RNA in CVL samples was a risk factor for perinatal transmission. Our analyses also showed interaction between CVL and plasma HIV RNA. CVL HIV RNA levels were a much stronger determinant of transmission among women with low plasma levels of HIV RNA. This effect can be seen directly in our stratified analysis. Among women with low plasma RNA levels, women with quantifiable CVL HIV RNA were at nearly 15 times (95% CI, 1.6–133) the risk of transmission as women who did not have quantifiable CVL HIV RNA. In contrast, among women with high plasma virus levels, women with quantifiable CVL HIV RNA had 2 times (95% CI, 1.1–3.4) the risk of transmission as women who did not have quantifiable HIV

Table 2. Maternal human immunodeficiency virus type 1 (HIV-1) RNA at 38 weeks' gestation in the full cohort, classified by treatment group and infants' HIV infection status.

	Zidovudine			Placebo		
Maternal HIV-1 RNA level	All infants (n = 151)	Infected infants (n = 15)	Infants not infected (n = 136)	All infants (n = 153)	Infected infants (n = 30)	Infants not infected (n = 123)
CVL						
Median[a]	<400	510	<400	580	3160	<400
% quantifiable[b]	23	60	18	52	70	48
Plasma						
Median[a]	5100	8200	4200	25,700	70,100	18,300

NOTE. CVL, cervicovaginal lavage.
[a] Median values are copies/mL.
[b] Lower quantitation limit is 400 copies/mL.

RNA in their CVL samples. This interaction suggests that estimates of the risk of perinatal transmission associated with plasma HIV RNA are more meaningful when they also account for HIV RNA in CVL samples.

Two previous studies were too small to allow inferences about the relationship between cervicovaginal virus levels and perinatal HIV transmission [9, 25]. Our data indicate clearly that the HIV RNA level in the genital tract of an infected pregnant woman near term increases the risk of perinatal HIV transmission, especially if the plasma virus level is low. Furthermore, our findings suggest that reduction in CVL HIV RNA level or exposure to cell-free virus in the cervicovaginal canal decreases the risk of transmission. These findings support the observations of a protective effect of cesarean delivery on perinatal transmission, even for women receiving zidovudine [4, 5]. They also suggest that the risk of perinatal transmission may be further reduced by treatment with more potent antiretroviral drugs or by local interventions that reduce exposure to HIV in the birth canal. However, after adjusting for plasma HIV RNA levels in our main trial, we did not find that mode of delivery or duration of membrane rupture was a significant determinant of perinatal HIV transmission [18]. Furthermore, one study of vaginal cleansing with chlorhexidine did not demonstrate an overall effect in preventing perinatal HIV transmission [26].

Zidovudine use has been associated with decreased likelihood of detecting HIV in cervicovaginal secretions [9, 12, 16]. Our data, based on a longitudinal, randomized design, demonstrate lower CVL HIV RNA levels after 1 and 2 weeks of treatment, compared with baseline and with an untreated group. This finding was confirmed in our larger, cross-sectional analysis of 38-week CVL samples, which showed that women in the zidovudine group were half as likely as women in the placebo group to have quantifiable HIV RNA in their CVL samples. The changes in CVL HIV levels during the course of zidovudine treatment paralleled the changes in plasma HIV RNA levels, which decreased by approximately one-half log within the first week of treatment, dropped further by the second week, and returned to baseline after treatment ended [27]. Our data suggest that the decrease in CVL HIV RNA level correlates with the decrease in plasma HIV RNA level for individual women receiving zidovudine.

We found an increase in CVL HIV levels postpartum, but our data suggest that the increase is not due to "rebound" of the virus associated with stopping zidovudine treatment at delivery. The postpartum increase in CVL HIV RNA observed in both groups might be explained by normal blood, lochia, or serous contamination during uterine healing or by physiologic changes in the mucosa after delivery. Although it does not pose a risk for perinatal transmission, this postpartum increase in CVL HIV RNA may represent an increased risk for heterosexual transmission of the virus.

At 36 weeks' gestation (before the study drug was started), HIV RNA was detected in the CVL samples of approximately half of the 72 HIV-infected pregnant women in our subset, which was similar to the percentage detectable in the full placebo group at 38 weeks. This rate falls in the middle of the ranges for detection reported elsewhere (24%–73%) [9–16]. The wide range in reported detection rates of HIV in CVL samples could be due to variability in collection procedures and in HIV quantification techniques. Although collection methods are not standardized, and collection procedures that include the use of a cervical wick may be more sensitive, the CVL collection procedure that we used appears to be quite useful for epidemiologic and, perhaps, clinical purposes.

Earlier reports of studies with smaller samples suggested that the detection of HIV in cervicovaginal secretions was associated with plasma virus levels [9, 14, 16], although one study found no relationship [12]. In our cohort, we found a moderate correlation between plasma and CVL virus level and a similar correlation within treatment groups. In contrast, the correlation of HIV-1 in CVL with CD4 count was low, suggesting that CD4 count would not be useful in predicting CVL virus level, especially in the context of treatment.

Our data may have several limitations. We used the 38-week

Table 3. Perinatal human immunodeficiency virus (HIV) type 1 transmission risk classified by HIV RNA in cervicovaginal lavage (CVL) at 38 weeks' gestation and plasma HIV RNA level at delivery.

Plasma virus level[a]	HIV in CVL quantifiable[b]	Subjects (*n*)	Transmission rate (95% CI)	Relative risk (95% CI)
⩾10,000	Yes	94	28.7 (19.8–40.0)	27.9 (3.9–201)
	No	93	15.0 (8.5–24.0)	14.6 (2.0–109)
<10,000	Yes	20	15.0 (3.2–37.9)	14.6 (1.6–133)
	No	97	1.0 (0.0–5.6)	1.0 (reference)

NOTE. CI, confidence interval.
[a] Values are copies/mL.
[b] Lower quantitation limit is 400 copies/mL.

cervicovaginal virus level as a marker for the infant's exposure to mucosal HIV in the birth canal at delivery. Although CVL RNA levels at delivery may be different from those at 38 weeks, it would be difficult to obtain CVL samples close to or at the time of delivery without causing contamination by blood and other fluids and without posing unnecessary risks. The 38-week collection schedule provided a standardized time for CVL collection, which allowed us to determine the differences in CVL HIV RNA levels between the zidovudine and the placebo groups after 2 weeks of treatment and to assess mucosal virus levels close to the time of delivery. Blood exposure in the birth canal is likely to pose a further risk in addition to that of exposure to HIV from cervicovaginal secretions, but we could only assess this additional risk indirectly, by adjusting for plasma virus level in our analysis. Given these limitations, we may be underestimating the infant's exposure to HIV during delivery. Finally, quantitation of HIV RNA in CVL samples is inherently more variable and less sensitive than quantitation of HIV RNA in plasma, because it relies on a saline flush of an open compartment and on recovery of a variable amount of mucosal secretions and saline. Because of this limitation, quantitative CVL HIV RNA values should not be overinterpreted. With our collection method and range of values, the question of whether CVL HIV RNA was quantifiable or was below detection limits was more helpful for analysis than were the specific values.

Our findings that HIV RNA in CVL samples is a risk factor for perinatal transmission, in addition to HIV RNA in plasma, and that HIV RNA levels in CVL samples are reduced by short-course antenatal zidovudine treatment have direct implications for perinatal intervention strategies. Researchers evaluating perinatal HIV interventions should consider the effect of the interventions on cervicovaginal HIV RNA and the exposure of the infant to cervicovaginal secretions. Besides zidovudine, interventions such as vaginal microbicides, cesarean section, treatment of genital ulcers, and more potent antiretroviral treatments might also be expected to reduce perinatal transmission risk by reducing intrapartum exposure to HIV. In summary, exposure of the newborn to HIV in cervicovaginal secretions should be minimized to reduce the risk of perinatal HIV infection.

Bangkok Collaborative Perinatal HIV Transmission Study Group

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