

## MATERNAL LEVELS OF PLASMA HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 RNA AND THE RISK OF PERINATAL TRANSMISSION

PATRICIA M. GARCIA, M.D., M.P.H., LESLIE A. KALISH, D.Sc., JANE PITT, M.D., HOWARD MINKOFF, M.D.,  
THOMAS C. QUINN, M.D., SANDRA K. BURCHETT, M.D., JANET KORNEGAY, PH.D., BROOKS JACKSON, M.D.,  
JOHN MOYE, M.D., CELINE HANSON, M.D., CARMEN ZORRILLA, M.D., AND JUDY F. LEW, M.D.,  
FOR THE WOMEN AND INFANTS TRANSMISSION STUDY GROUP\*

### ABSTRACT

**Background** The importance of plasma levels of human immunodeficiency virus type 1 (HIV-1) RNA in pregnant women in relation to the other factors known to influence the risk of transmission of infection to their infants is incompletely defined. We studied the relation of maternal plasma HIV-1 RNA levels to the risk of perinatal transmission and the timing of transmission.

**Methods** We measured plasma HIV-1 RNA serially in 552 women with HIV-1 infection who had singleton pregnancies. The status of infection in their infants was assessed by culture of blood and further classified as early (if a culture of blood obtained within the first two days of life was positive) or late (if a culture of blood obtained in the first seven days of life was negative but subsequent cultures were positive). The rates of transmission at various levels of maternal plasma HIV-1 RNA were analyzed by tests for trend, with adjustment for covariates by stratification and logistic regression.

**Results** Increasing geometric mean levels of plasma HIV-1 RNA were associated with increasing rates of transmission: the rate was 0 percent among women with less than 1000 copies per milliliter (0 of 57), 16.6 percent among women with 1000 to 10,000 copies per milliliter (32 of 193), 21.3 percent among women with 10,001 to 50,000 copies per milliliter (39 of 183), 30.9 percent among women with 50,001 to 100,000 copies per milliliter (17 of 54), and 40.6 percent among women with more than 100,000 copies per milliliter (26 of 64,  $P < 0.001$ ). The treatment status of one woman was unknown. The highest rate of transmission was among women whose plasma HIV-1 RNA levels exceeded 100,000 copies per milliliter and who had not received zidovudine (19 of 30 women, 63.3 percent). Neither higher HIV-1 RNA levels early in pregnancy nor higher levels late in pregnancy were associated with the timing of infection in the infants.

**Conclusions** In pregnant women with HIV-1 infection, the level of plasma HIV-1 RNA predicts the risk but not the timing of transmission of HIV-1 to their infants. (N Engl J Med 1999;341:394-402.)

©1999, Massachusetts Medical Society.

LITTLE was known of the biology of perinatal transmission of human immunodeficiency virus type 1 (HIV-1) when zidovudine treatment was proposed and subsequently proved effective for reducing the risk of neonatal infection.<sup>1</sup> Clarification of the relative contribution of maternal plasma HIV-1 RNA levels and other factors known to influence the risk of transmission might provide insight into the timing of transmission of the virus to the infant.

We undertook this study to determine the relation between maternal plasma HIV-1 RNA levels and the risk and timing of perinatal transmission of HIV-1 infection, using data from one of the largest prospectively assembled and well-characterized cohorts of infected women and their infants in the United States. We sought to test the hypothesis that higher maternal plasma HIV-1 RNA levels independently increase the risk of transmission. We also assessed whether the risk of transmission was correlated more closely with the presence of higher levels earlier in pregnancy rather than later.

### METHODS

#### Study Population

The Women and Infants Transmission Study is an ongoing multicenter, prospective study of the perinatal transmission of HIV-1 and the natural history of HIV-1 infection in pregnant women and their infants.<sup>2</sup> Pregnant women with HIV-1 infection and their infants have been studied at eight centers in four states and Puerto Rico since 1989. The women are enrolled at any time during pregnancy, and their infants are enrolled at birth or shortly thereafter. Our study was approved by the institutional review committees at each center, and informed consent was obtained from each woman.

The study subjects consisted of women who gave birth to sin-

From the Department of Obstetrics and Gynecology, Northwestern University, Chicago (P.M.G.); New England Research Institutes, Watertown, Mass. (L.A.K.); the Department of Pediatrics, Columbia College of Physicians and Surgeons, New York (J.P.); the Department of Obstetrics and Gynecology, State University of New York Health Science Center, Brooklyn (H.M.); Johns Hopkins University, Baltimore (T.C.Q., B.J.); the National Institute of Allergy and Infectious Diseases, Bethesda, Md. (T.C.Q., J.F.L.); the Department of Pediatrics, Harvard Medical School, Boston (S.K.B.); Roche Molecular Systems, Alameda, Calif. (J.K.); the National Institute of Child Health and Human Development, Bethesda, Md. (J.M.); the Section of Allergy and Immunology, Baylor College of Medicine, Houston (C.H.); and the Department of Obstetrics and Gynecology, University of Puerto Rico, San Juan (C.Z.). Address reprint requests to Dr. Garcia at Rm. 410, Prentice Women's Hospital, 333 E. Superior St., Chicago, IL 60611, or at p-garcia@nwu.edu.

\*Other participants in the study are listed in the Appendix.

gletton infants between January 14, 1990, and October 15, 1995, and whose infants' HIV-1 infection status was determined before December 1, 1996. In addition, plasma HIV-1 RNA levels had to have been measured at least once in the women during pregnancy or at delivery. Among 800 mother-infant pairs potentially eligible for the analysis, 248 pairs were excluded because of the unavailability of data on HIV-1 RNA levels. The women in the excluded pairs had been enrolled more recently than the women in the included pairs, and the rate of transmission was therefore lower in this group (14 of 248 infants; 6 percent), but the excluded pairs were otherwise similar to the included pairs with respect to maternal and obstetrical covariates. Thus, a total of 552 pairs were studied.

### Study Protocol and Definitions

The women were evaluated three times during pregnancy and once at delivery. At each visit, detailed medical and behavioral questionnaires were administered, a physical examination was performed, and venous blood was obtained. Obstetrical data were abstracted from medical records.

Antiviral-drug therapy was left to the discretion of the clinicians caring for the women and the women themselves. The prevalence of zidovudine therapy increased and the rates of perinatal transmission declined in the cohort over time.<sup>3</sup> Women were classified as treated if they received any zidovudine therapy during pregnancy or labor and delivery. We further classified the women according to whether they were in the active-treatment group of the AIDS Clinical Trials Group (ACTG) Protocol 076 or were treated with zidovudine before the March 1994 release of the results of that study. Women who received zidovudine after March 1994 or before March 1994 if they were enrolled in the treated group of ACTG Protocol 076 were classified separately from women who reported receiving zidovudine therapy for specific indications during pregnancy but who delivered their babies before March 1994 and who were not in the treated group of ACTG Protocol 076.

Maternal use of illicit drugs during pregnancy — such as heroin, opiates, and cocaine — was based on self-report or toxicologic confirmation on testing of urine samples.<sup>4</sup> All mothers were counseled regarding the risk of transmission associated with breastfeeding, and only four infants (none of whom became infected with HIV-1) were breast-fed.

The infants were examined and peripheral-blood mononuclear cells were obtained for culture during the first 6 days of life; at the ages of 1, 2, 4, 6, 9, 12, and 18 months; and every 6 months thereafter. After April 1994, blood was obtained within the first 48 hours after birth and on days 6 to 10 after birth. Infants with no positive cultures and at least two negative cultures at or after the age of one month (with one negative culture obtained when the child was at least six months of age) were classified as uninfected. Infants with two or more positive cultures were defined as infected (there were no infants with only one positive culture). Infection was further classified as early or late according to the timing of the positive culture.<sup>5</sup> If the result of a culture of blood obtained within the first 48 hours after birth was positive, the infant was considered to have acquired the infection early (presumably in utero). If cultures of blood obtained within the first seven days of life were negative, but later cultures were positive, the infant was considered to have acquired the infection late (presumably during labor and delivery).

### Laboratory Studies

Heparin-treated or EDTA-treated blood samples were separated into plasma and peripheral-blood mononuclear cells within 24 hours after collection at each center. CD4 counts were determined in fresh whole blood by flow cytometry. The laboratories followed the cytometry protocols of the Division of AIDS of the National Institute of Allergy and Infectious Diseases and participated in the division's flow-cytometric quality-assurance program.<sup>6</sup> A qualitative approach was used for cocultures of peripheral-blood mononuclear cells until October 1991; thereafter, a quantitative

approach was used, according to the ACTG consensus protocols, with standard modifications for samples from infants.<sup>7</sup>

Plasma HIV-1 RNA was measured in five laboratories from repository samples (stored at  $-70^{\circ}\text{C}$ ) according to the recommendations of the Virology Quality Assurance Program of the Division of AIDS.<sup>8</sup> RNA was extracted from the plasma samples with the use of silica particles.<sup>9</sup> The number of HIV-1 RNA copies per milliliter of maternal plasma was measured by a quantitative RNA polymerase-chain-reaction assay (Amplicor HIV-1 Monitor Test, Roche Diagnostic Systems, Branchburg, N.J.) as previously described.<sup>10</sup> Samples below the limit of quantification of the assay (400 copies per milliliter) were assigned a value of 400 copies per milliliter for the purpose of statistical analysis.

### Statistical Analysis

The primary quantitative viral measure used in analyses for each woman was the geometric mean of all available plasma HIV-1 RNA values for that woman. A priori categories of these levels were determined on the basis of clinically relevant cutoff points. For women with at least two RNA values, the within-person rate of change in plasma HIV-1 RNA levels (measured on a logarithmic [base 10] scale) per week was calculated by linear least-squares regression analysis. Cutoff points were chosen to represent a change of 0.0175 log copies per week (corresponding to a fivefold increase during a 40-week pregnancy). The remaining women were categorized as having stable plasma HIV-1 RNA levels. Women who were taking zidovudine during pregnancy whose plasma HIV-1 RNA level declined by at least 0.0175 log copies per week were classified as having a response to zidovudine.

The primary association of interest was between maternal plasma HIV-1 RNA levels at various points during pregnancy and the overall risk of transmission. The Mantel extension test for trend was used to analyze transmission rates in ordered categories of variables, and a stratified version of the test for trend was used to control for categorical covariates.<sup>11</sup> The Wilcoxon or Kruskal-Wallis test was used for univariate comparisons of the association between plasma HIV-1 RNA levels and covariates.<sup>12</sup> Logistic-regression analysis was used to adjust for covariates and determine which factors independently predicted the risk of perinatal transmission.<sup>13</sup> All statistical tests were two-sided.

## RESULTS

### Characteristics of the Women

The 552 women included in the analysis were demographically similar to the entire cohort as well as to all childbearing women with HIV-1 infection in the United States. The mean age at delivery was 28 years, 81 percent of the women were black or Hispanic, and 18 percent had used injection drugs during pregnancy. The overall rate of perinatal transmission of HIV-1 infection in the cohort was 20.6 percent, which is similar to rates reported for other cohorts in North America. The rate of transmission declined during the period of data collection. Before March 1994 it was 24 percent; thereafter it was 9 percent. Univariate analysis was performed with selected measures of obstetrical outcome, disease status, and use of zidovudine therapy in order to identify covariates that were related to the risk of transmission of HIV-1 (Table 1). The resulting covariates were used in a logistic-regression model.

Among the 552 pregnant women, plasma HIV-1 RNA was measured a total of 1396 times at four visits during pregnancy and delivery (Fig. 1). The number

**TABLE 1.** UNIVARIATE ANALYSIS OF THE RELATION OF VARIOUS MATERNAL AND OBSTETRICAL COVARIATES TO THE RISK OF PERINATAL TRANSMISSION OF HIV-1.

CHARACTERISTIC	NO. OF WOMEN	NO. OF INFANTS INFECTED (%)	P VALUE	CHARACTERISTIC	NO. OF WOMEN	NO. OF INFANTS INFECTED (%)	P VALUE	
Maternal age				Mean percentage of CD4 cells during pregnancy				
<30 yr	352	59 (16.8)	0.003	<29%	289	77 (26.6)	<0.001	
≥30 yr	200	55 (27.5)		≥29%	259	37 (14.3)		
Race or ethnic group				Unknown	4			
White	87	21 (24.1)	0.34	Positive HIV-1 cultures during pregnancy				
Black	234	44 (18.8)		Not always	224	33 (14.7)	0.004	
Hispanic	210	43 (20.5)		Always	326	81 (24.8)		
Other	17	6 (35.3)		Unknown	2			
Unknown	4				Hepatitis C coinfection			
Site of enrollment				Yes	120	38 (31.7)	0.009	
Illinois	113	31 (27.4)	No	275	52 (18.9)			
Massachusetts	126	24 (19.0)	Unknown	157				
New York	165	34 (20.6)	0.23	Zidovudine therapy during pregnancy				
Puerto Rico	126	23 (18.3)		Any	230	35 (15.2)	0.01	
Texas	22	2 (9.1)		According to ACTG Protocol 076†	129	11 (8.5)		
Gestational age of infant					Treatment for specific indications	101	24 (23.8)	<0.001‡
≥37 wk	453	84 (18.5)		None	321	79 (24.6)		
<37 wk	99	30 (30.3)	Unknown	1				
Birth weight of infant				Cigarette use during pregnancy				
≥2500 g	430	73 (17.0)	<0.001	Yes	288	70 (24.3)	0.03	
<2500 g	91	34 (37.4)		No	264	44 (16.7)		
Unknown	31			Hard-drug use during pregnancy				
Mode of delivery				Yes	212	59 (27.8)	0.002	
Cesarean section	103	21 (20.4)	0.89	No	339	55 (16.2)		
Elective cesarean section	21	2 (9.5)		Unknown	1			
Vaginal	437	87 (19.9)	0.28*	Total	552	114 (20.7)		
Unknown	12							
Time from rupture of membranes to delivery								
≤4 hr	298	43 (14.4)	<0.001					
>4 hr	217	58 (26.7)						
Unknown	37							

\*The P value is for the comparison with all other modes of delivery.

†ACTG denotes AIDS Clinical Trials Group.

‡The P value is for the comparison with the other three groups.

of measurements was similar in the group of women with perinatal transmission of HIV-1 infection and those without perinatal transmission (data not shown). The individual maternal plasma HIV-1 RNA values ranged from less than 400 copies per milliliter to 3,101,258 copies per milliliter. Only 16 of 279 women (5.7 percent) with at least three values had persistently undetectable levels. The median plasma HIV-1 RNA values for each visit were similar and varied by less than 0.2 log, and the values were also relatively stable during pregnancy in individual women, as reflected by the median SD of 0.2 log within persons. For this reason, except where noted, the geometric mean of all available values from all visits was used in subsequent analyses.

**Maternal Plasma HIV-1 RNA Levels and the Risk of Transmission**

The median plasma HIV-1 RNA levels were higher among women who transmitted the infection to their infants than among those who did not transmit

the infection (geometric mean, 29,235 vs. 10,049 copies per milliliter) (Fig. 1). The women who transmitted the infection had higher values throughout pregnancy, by 0.3 to 0.4 log at each visit. Transmission of HIV-1 infection occurred in a broad range of maternal plasma HIV-1 RNA levels (Fig. 1 and Table 2). In general, the risk of transmission increased with increasing maternal viral loads. However, no threshold could be defined above which the risk of transmission was 100 percent. Women who were not treated with zidovudine and whose plasma HIV-1 RNA levels exceeded 100,000 copies per milliliter had the highest risk of transmission (19 of 30 infants infected; 63.3 percent;  $P < 0.001$ ). Regardless of whether they received zidovudine therapy during pregnancy, none of the 57 women with RNA levels below 1000 copies per milliliter transmitted HIV-1 to their offspring (upper limit of the 95 percent confidence interval, 5.1 percent).

To examine the pattern of change in plasma HIV-1 RNA levels, we stratified the women according to

whether they had increasing, decreasing, or stable levels throughout pregnancy. Although women whose plasma HIV-1 RNA levels increased throughout pregnancy had the highest rate of transmission overall, there was no statistically significant difference in the risk of transmission among these three groups, even after adjustment for the use of zidovudine therapy.

#### Potential Cofactors

The association between maternal plasma HIV-1 RNA levels and the risk of perinatal transmission of HIV-1 infection was assessed for confounding by other potential cofactors. The level was not associated with the time from the rupture of membranes to delivery, birth weight of the infant, illicit drug use or smoking during pregnancy, site of enrollment, or date of delivery. As expected, the level was strongly inversely associated with the percentage of CD4 cells and the absolute CD4 count (data not shown). After adjustment for these two covariates (Table 3), the association between maternal plasma HIV-1 RNA levels and the risk of perinatal transmission remained significant ( $P < 0.001$ ).

#### Maternal Zidovudine Therapy

Treatment with zidovudine was not associated with maternal plasma HIV-1 RNA levels ( $P = 0.26$ ), but the receipt of any type of zidovudine therapy in general was significantly associated with a lower rate of transmission (15.2 percent, as compared with 24.6 percent among women who did not receive zidovudine therapy;  $P < 0.001$ ). Among the women who were treated with zidovudine, plasma HIV-1 RNA levels were significantly higher in those who transmitted the infection than in those who did not (Table 4). However, when the women were stratified according to the type of zidovudine therapy they received, plasma HIV-1 RNA levels were not significantly different between the women who transmitted the infection and those who did not in the subgroup treated for specific indications rather than as part of ACTG Protocol 076. This group of women tended to have higher plasma HIV-1 RNA levels despite treatment with zidovudine, and the rate of perinatal transmission in this group was essentially the same as the rate in the untreated women and significantly higher than the rate in the women who were treated as part of or according to ACTG Protocol 076.

#### Multivariate Analysis

Logistic-regression analysis confirmed that maternal plasma HIV-1 RNA levels were strongly associated with the risk of transmission even after adjustment for other factors (Table 5). There was a progressively higher risk of transmission with higher plasma HIV-1 RNA levels, even after the exclusion of the group of women with values below 1000 copies per milliliter, for which the transmission rate was zero. Ma-

ternal plasma HIV-1 RNA values of more than 50,000 copies per milliliter were associated with the greatest risk of transmission. The absence of zidovudine therapy during pregnancy, an interval of more than four hours between the rupture of membranes and delivery, and delivery of an infant who weighed less than 2500 g at birth were also independently associated with a higher risk of transmission in this analysis.

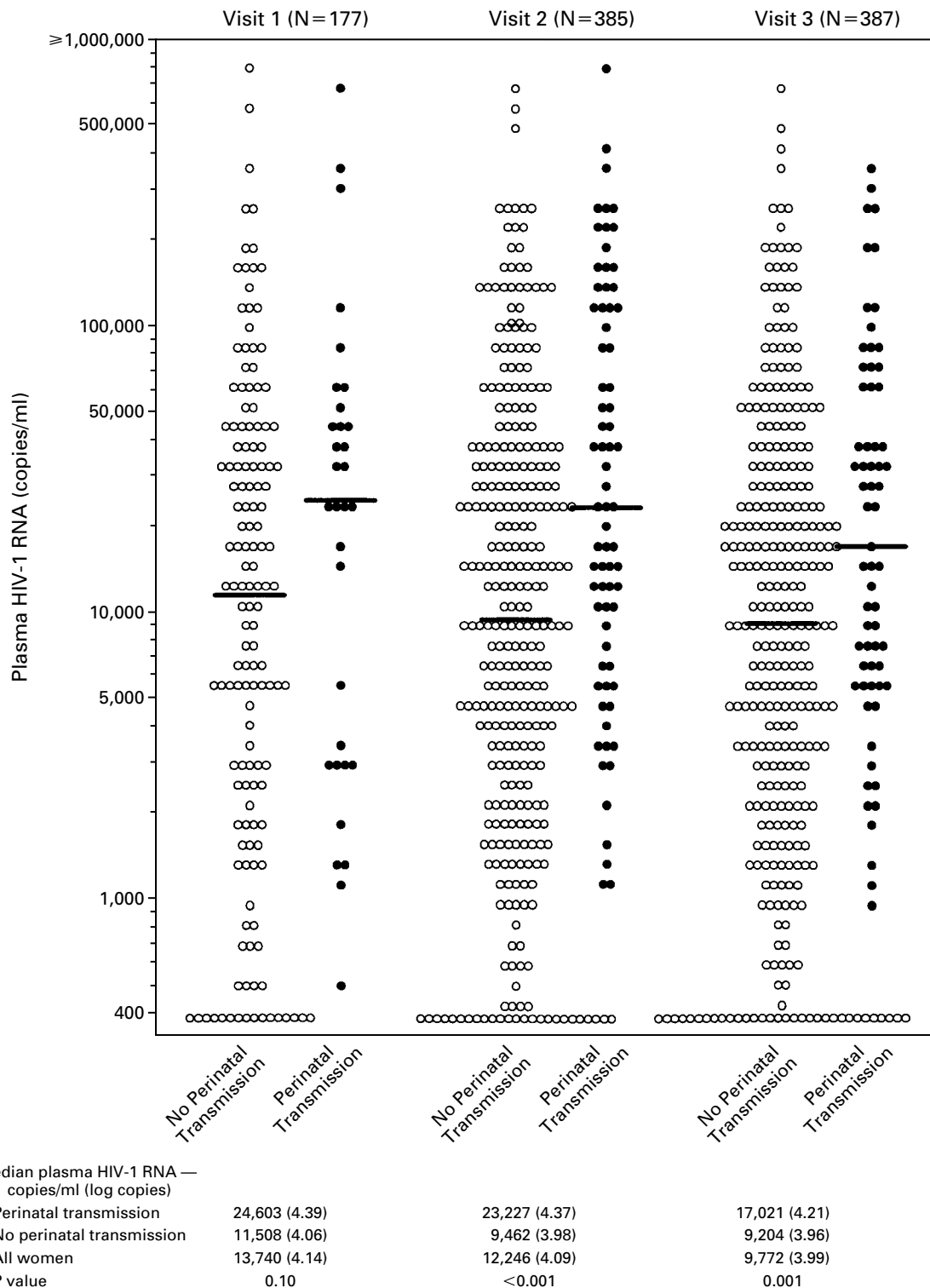
#### Maternal Plasma HIV-1 RNA Levels and the Timing of Transmission

In 88 of the 114 infected infants, the time at which the infection was diagnosed could be determined on the basis of HIV-1 culture results obtained during the first week of life. Sixty-three of the 88 infants (72 percent) were considered to have late, or peripartum, infection. Twenty-five infants had a positive HIV-1 culture within the first week of life. Of these 25, 16 had a culture during the first 48 hours, all of which were positive. These 16 infants were classified as having early — presumably in utero — infection. Despite the strong correlation between maternal plasma HIV-1 RNA levels and the risk of transmission overall, there was no significant difference in the median levels between the mothers of infants with early infection and the mothers of infants with late infection. There was no significant relation between the rates of early or late transmission and maternal plasma HIV-1 RNA levels measured throughout the pregnancy or in early or late pregnancy. Stratification of the groups according to the use of zidovudine therapy during pregnancy did not change the results. These results indicate that there was no relation between maternal plasma HIV-1 RNA levels at any time during pregnancy and the risk of transmission early or late in pregnancy.

#### DISCUSSION

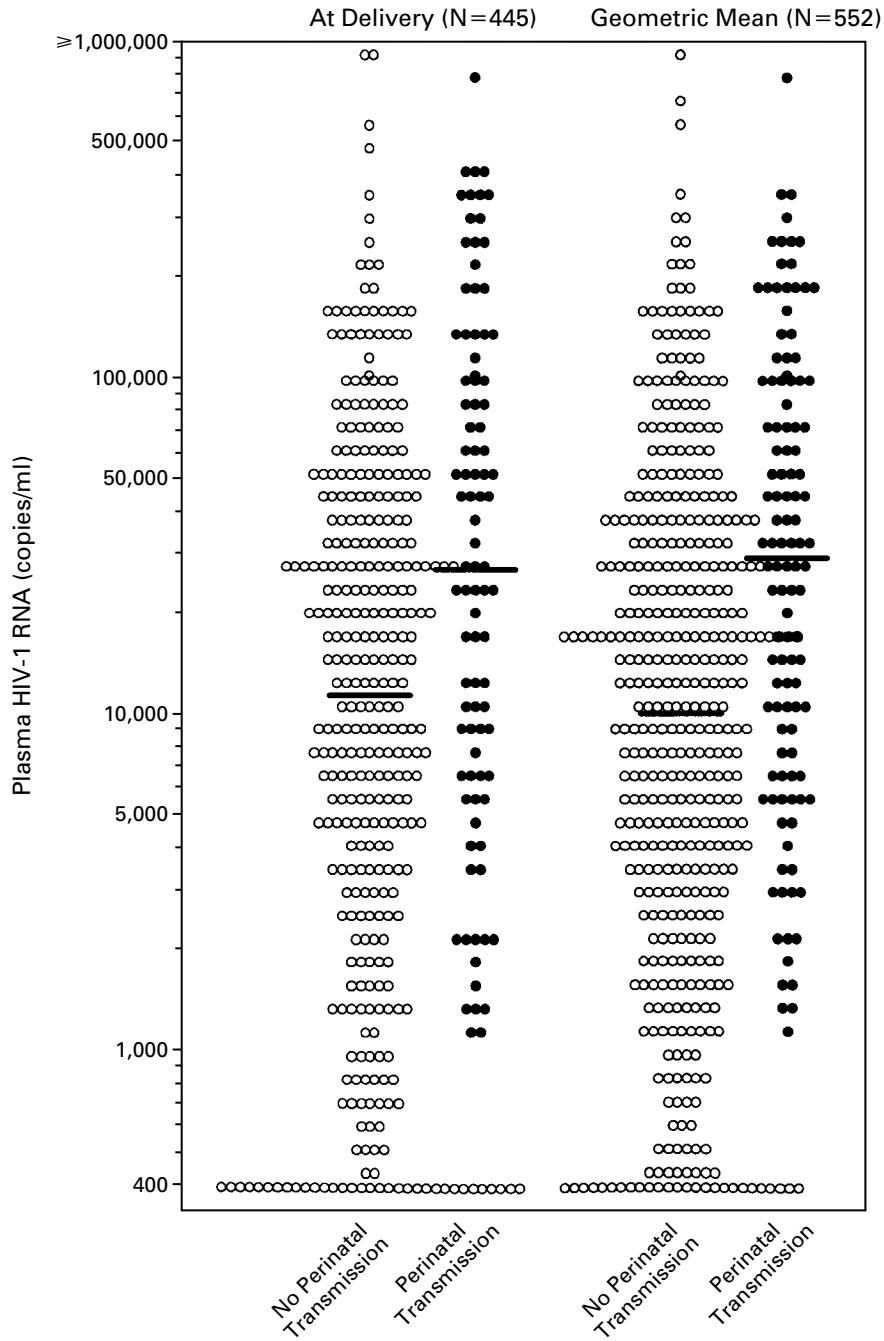
Measurements of plasma HIV-1 RNA levels have considerable value as a marker of the response to antiretroviral therapy as well as for predicting the risk of disease progression and death.<sup>14</sup> Our findings demonstrate the importance of maternal plasma HIV-1 RNA measurements in predicting the risk of perinatal transmission of the infection. Higher plasma HIV-1 RNA values were associated with a significant risk of transmitting HIV-1 from mother to infant.

A very strong correlation between maternal plasma HIV-1 RNA levels and the risk of perinatal transmission has been found in several small studies,<sup>15,16</sup> whereas larger studies have found little or no association.<sup>17-19</sup> Although the levels were not measured in all the women in our study at all four times, the number of measurements and the median plasma HIV-1 RNA levels at each visit were similar in the group of women with perinatal transmission of infection and the group without perinatal transmission. The re-



**Figure 1.** Maternal Plasma HIV-1 RNA Levels at Each Visit and Geometric Mean Values during Pregnancy, According to Transmission Status.

The limit of detection of the assay was 400 HIV-1 RNA copies per milliliter. Visit 1 occurred during the first 18 weeks of gestation, visit 2 between 19 and 31 weeks of gestation, and visit 3 from 32 weeks of gestation to delivery. The geometric mean values include the values obtained throughout pregnancy and at delivery. Bars indicate the median plasma HIV-1 RNA levels. P values are for the comparison between women who transmitted the infection to their infants and those who did not transmit the infection.



Median plasma HIV-1 RNA — copies/ml (log copies)	At Delivery (N=445)	Geometric Mean (N=552)
Perinatal transmission	26,997 (4.43)	29,235 (4.47)
No perinatal transmission	11,350 (4.06)	10,049 (4.0)
All women	14,488 (4.16)	12,971 (4.11)
P value	<0.001	<0.001

**TABLE 2.** RATES OF PERINATAL TRANSMISSION OF HIV-1 ACCORDING TO MATERNAL PLASMA HIV-1 RNA LEVELS AND THE USE OF ZIDOVUDINE THERAPY DURING PREGNANCY.\*

ZIDOVUDINE THERAPY	MATERNAL PLASMA HIV-1 RNA COPIES/ml					P VALUE†
	<1000	1000–10,000	>10,000–50,000	>50,000–100,000	>100,000	
	no. of infants infected/total no. (%)					
Yes	0/22	10/83 (12.0)	13/75 (17.3)	5/16 (31.2)	7/34 (20.6)	0.02
No	0/35	22/110 (20.0)	26/108 (24.1)	12/38 (31.6)	19/30 (63.3)	<0.001
Total	0/57	32/193 (16.6)	39/183 (21.3)	17/54 (30.9)	26/64 (40.6)	<0.001

\*Values are the geometric means of measurements obtained throughout pregnancy. For each woman, levels were measured up to three times during pregnancy and once at delivery. The treatment status of one woman was not known.

†The P values were calculated with use of the Mantel extension test for trend.

**TABLE 3.** RATES OF PERINATAL TRANSMISSION OF HIV-1 ACCORDING TO MATERNAL PLASMA HIV-1 RNA LEVELS, PERCENTAGE OF CD4 CELLS, AND CD4 CELL COUNT.

VARIABLE	MATERNAL PLASMA HIV-1 RNA COPIES/ml*			
	<1000	1000–10,000	>10,000–50,000	>50,000
	no. of infants infected/total no. (%)			
Percentage of CD4 cells†				
<14%	0/1	2/4 (50.0)	2/21 (9.5)	7/21 (33.3)
14–28%	0/7	11/71 (15.5)	25/96 (26.0)	30/68 (44.1)
≥29%	0/48	19/116 (16.4)	12/66 (18.2)	6/29 (20.7)
CD4 cell count‡				
<200/mm <sup>3</sup>	0/1	2/10 (20.0)	4/26 (15.4)	12/33 (36.4)
200–499/mm <sup>3</sup>	0/12	11/71 (15.5)	18/90 (20.0)	26/64 (40.6)
≥500/mm <sup>3</sup>	0/43	17/108 (15.7)	17/67 (25.4)	5/21 (23.8)

\*The median values for all visits are shown. P<0.001 by tests for trend for rates of transmission and maternal plasma HIV-1 RNA levels, with adjustment for the percentage of CD4 cells and CD4 cell count.

†Data were missing for four women.

‡Data were missing for six women.

sults of our analyses were unchanged whether geometric mean values for all visits or values from each of the four visits were used. In addition, the risk of transmission was more closely related to the absolute maternal plasma HIV-1 RNA level than to a change in the level.

Previous studies have suggested that there is an upper threshold of maternal viremia, above which transmission is unavoidable, and a lower threshold, below which transmission is rare.<sup>15,20</sup> Despite the high rate of transmission (63.3 percent) among women who had not received zidovudine and whose plasma HIV-1 RNA levels exceeded 100,000 copies per milliliter, we found no threshold level above which the rate of transmission was 100 percent. Perinatal trans-

mission did not occur among women with plasma HIV-1 RNA levels of less than 1000 copies per milliliter, but there are at least three reports of transmission occurring at low levels, including one in which 16 of 132 women (12 percent) who had HIV-1 RNA levels of less than 1000 copies per milliliter transmitted HIV-1 to their infants.<sup>17,18,21</sup>

Treatment with zidovudine during pregnancy reduces the risk of perinatal transmission.<sup>1,3,22,23</sup> In our study, treatment with zidovudine during pregnancy was associated with a lower rate of transmission among women with plasma HIV-1 RNA levels of 1000 copies per milliliter or more, especially among those who were treated according to ACTG Protocol 076. There was no significant difference in trans-

**TABLE 4.** MATERNAL PLASMA HIV-1 RNA LEVELS THROUGHOUT PREGNANCY, ACCORDING TO TRANSMISSION STATUS AND THE TYPE OF ZIDOVUDINE THERAPY.

ZIDOVUDINE THERAPY*	PERINATAL TRANSMISSION			NO PERINATAL TRANSMISSION		P VALUE‡
	NO. OF WOMEN	MEDIAN HIV-1 RNA LEVEL†	TRANSMISSION RATE	NO. OF WOMEN	MEDIAN HIV-1 RNA LEVEL†	
		copies/ml	%		copies/ml	
None	79	28,242	24.6	242	9,691	<0.001
Any	35	30,227	15.2	195	11,625	0.007
According to ACTG Protocol 076	11	31,352	8.5	118	7,587	0.02
Treatment for specific indications	24	26,071	23.8	77	18,266	0.40
Total	114	29,235	20.7	438	10,049	<0.001

\*Data were missing for one woman. ACTG denotes AIDS Clinical Trials Group.

†Values are the geometric means of all measurements obtained throughout pregnancy and at delivery.

‡P values were calculated by the two-sample Wilcoxon test for the comparison of women who transmitted HIV-1 infection with those who did not.

mission rates between the women with a response to zidovudine therapy and those with no response. Taken together, these data support the conclusion of Sperling et al.<sup>17</sup> that treatment with zidovudine during pregnancy reduces the risk of perinatal transmission, but not solely as a result of a reduction in maternal plasma HIV-1 RNA levels. The same may not be true for combinations of potent antiretroviral drugs that are capable of reducing maternal plasma HIV-1 RNA to undetectable levels.

In our study, perinatal transmission occurred predominantly in the peripartum period. Although maternal plasma HIV-1 RNA levels were closely associated with the risk of perinatal transmission of HIV-1 infection overall, there was no discernible relation between the levels and the timing of neonatal infection. The relation between maternal plasma HIV-1 RNA levels and the risk of perinatal transmission may relate more to the mechanism of transmission (i.e., transplacental transfer of virus or exposure to virus in the genital tract) than to the timing of transmission.

How, then, should measurements of viral load be used in the care of HIV-infected pregnant women? One answer is to use antiretroviral therapy to improve the woman's health, as advised in treatment guidelines developed by the Public Health Service.<sup>24</sup> Although there is uncertainty about the degree to which viral replication must be suppressed to prevent disease progression, preserve immune function, and inhibit the development of resistant strains of HIV-1, there is general agreement that decreasing plasma HIV-1 RNA levels to below the limits of detection of currently available assays is the most appropriate treatment goal. Achieving that goal should

**TABLE 5.** RESULTS OF MULTIPLE LOGISTIC-REGRESSION ANALYSIS OF THE RISK OF PERINATAL TRANSMISSION OF HIV-1.\*

COVARIATE	ODDS RATIO (95% CI)	P VALUE
Maternal plasma HIV-1 RNA level†		
1000–10,000 copies/ml	1.0	<0.001‡
>10,000–50,000 copies/ml	1.6 (0.9–2.9)	
>50,000 copies/ml	3.7 (2.0–7.0)	
Zidovudine therapy		
None	1.0	
Any		
Treatment for specific indications	0.7 (0.4–1.2)	0.20
According to ACTG Protocol 076	0.3 (0.1–0.6)	<0.001
Time from rupture of membranes to delivery >4 hr	2.0 (1.2–3.3)	0.005
Low birth weight of infant (<2500 g)	2.8 (1.6–5.0)	<0.001

\*The analysis excluded women with plasma HIV-1 RNA levels of less than 1000 copies per milliliter, since there were no cases of transmission in this group. CI denotes confidence interval, and ACTG AIDS Clinical Trials Group.

†Plasma HIV-1 RNA levels (geometric mean values for all measurements made throughout pregnancy and at delivery) were modeled as a categorical variable (data were available for 437 women). Results were very similar when plasma HIV-1 RNA levels were modeled as a single ordered categorical variable (with equally spaced categories) that included women with values of less than 1000 copies per milliliter (data were available for 491 women).

‡The P value is for the test for trend.

also help prevent perinatal transmission, given the absence of transmission among women with low plasma HIV-1 RNA levels in our study. Validating this treatment goal is important, since a treatment-induced reduction in the viral load may not be equivalent to a spontaneously low level with respect to the risk of perinatal transmission.

Because the causes of perinatal transmission are multifactorial, attention must be paid to other potentially modifiable risk factors. Of all the independent factors related to the risk of transmission in this cohort of women, maternal plasma HIV-1 RNA levels and the use of antiretroviral-drug therapy are perhaps the most easily modifiable risk factors. Yet, women with low or undetectable plasma HIV-1 RNA levels should not be falsely reassured but, instead, should be offered zidovudine therapy because of its demonstrated efficacy in reducing the risk of transmission regardless of the maternal HIV-1 RNA levels. Whether there is an additional benefit of elective cesarean delivery in decreasing the already low risk of perinatal transmission among pregnant women with low levels of plasma HIV-1 RNA has yet to be determined.<sup>25-27</sup>

Supported by grants from the National Institute of Allergy and Infectious Diseases (U01 AI 34840, U01 AI 34841, U01 AI 34842, U01 AI 34856, U01 AI 34858, and N01 AI 35161), the National Institute of Child Health and Human Development (HD-8-2913 and RO-1-HD-25714), and the National Institute on Drug Abuse.

Presented in part at the meeting of the Society of Perinatal Obstetricians, Miami, February 5, 1998.

*We are indebted to the patients and dedicated study personnel (data-entry persons, nurses, coordinators, laboratory technicians, and clinicians) at all sites for their efforts; to Ms. Tracy Antonelli for assistance with statistical analysis; to Dr. Mike Socol for his support and review of the manuscript; and to Roche Molecular Systems for their generous contribution of Amplicor HIV-1 Monitor Test kits.*

## APPENDIX

The study's principal investigators, coordinators, and program officers were as follows: *University of Puerto Rico, San Juan* — C. Diaz, E. Pacheco-Acosta; *Boston-Worcester site, Boston* — R. Tuomala, E. Cooper, D. Messtene; *Columbia-Presbyterian Hospital, New York* — J. Pitt, A. Higgins; *State University of New York, Brooklyn* — S. Landesman, H. Mendez, G. Moroso; *University of Illinois at Chicago, Chicago* — K. Rich, D. Turpin; *Baylor College of Medicine, Houston* — W. Shearer, N. Cooper; *National Institute of Allergy and Infectious Diseases, Bethesda, Md.* — M. Fowler; *National Institute of Child Health and Human Development, Bethesda, Md.* — R. Nugent; *National Institute on Drug Abuse, Rockville, Md.* — V. Smeriglio; *New England Research Institute, Watertown, Mass.* — S. McKinlay, L. Kalish, S. Ellis.

## REFERENCES

- Connor EM, Sperling RS, Gelber R, et al. Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. *N Engl J Med* 1994;331:1173-80.
- Sheon AR, Fox HE, Rich KC, et al. The Women and Infants Transmission Study (WITS) of maternal-infant HIV transmission: study design, methods, and baseline data. *J Womens Health* 1996;5:69-78.
- Cooper ER, Nugent RP, Diaz C, et al. After AIDS clinical trial 076: the changing pattern of zidovudine use during pregnancy, and the subsequent reduction in the vertical transmission of human immunodeficiency virus in a cohort of infected women and their infants. *J Infect Dis* 1996; 174:1207-11.
- Rodriguez EM, Mofenson LM, Chang B-H, et al. Association of maternal drug use during pregnancy with maternal HIV culture positivity and perinatal HIV transmission. *AIDS* 1996;10:273-82.
- Bryson YJ, Luzuriaga K, Sullivan JL, Wara DW. Proposed definitions for in utero versus intrapartum transmission of HIV-1. *N Engl J Med* 1992;327:1246-7.
- Calvelli T, Denny TN, Paxton H, Gelman R, Kagan J. Guideline for flow cytometric immunophenotyping; a report from the National Institute of Allergy and Infectious Diseases, Division of AIDS. *Cytometry* 1993;14: 702-15.
- Hollinger FB, ed. ACTG virology manual for HIV laboratories: version 2.1. Bethesda, Md.: National Institutes of Allergy and Infectious Diseases, September 1993.
- Hollinger FB, Bremer JW, Myers LE, Gold JWM, McQuay L, NIH/NIAID/DAIDS/ACTG Virology Laboratories. Standardization of sensitive human immunodeficiency virus coculture procedures and establishment of a multicenter quality assurance program for the AIDS Clinical Trials Group. *J Clin Microbiol* 1992;30:1787-94.
- Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim-van Dillen PM, van der Noordaa J. Rapid and simple method for purification of nucleic acids. *J Clin Microbiol* 1990;28:495-503.
- Shearer WT, Quinn TC, LaRussa P, et al. Viral load and disease progression in infants infected with human immunodeficiency virus type 1. *N Engl J Med* 1997;336:1337-42.
- Mantel N. Chi-square tests with one degree of freedom: extensions of the Mantel-Haenszel procedure. *J Am Stat Assoc* 1963;58:690-700.
- Rosner B. Fundamentals of biostatistics. 3rd ed. Boston: PWS-Kent Publishing, 1990.
- Hosmer DW Jr, Lemeshow S. Applied logistic regression. New York: John Wiley, 1989.
- Mellors JW, Munoz A, Giorgi JV, et al. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. *Ann Intern Med* 1997;126:946-54.
- Dickover RE, Garratty EM, Herman SA, et al. Identification of levels of maternal HIV-1 RNA associated with risk of perinatal transmission: effect of maternal zidovudine treatment on viral load. *JAMA* 1996;275:599-605.
- Weiser B, Nachman S, Tropper P, et al. Quantitation of human immunodeficiency virus type 1 during pregnancy: relationship of viral titer to mother-to-child transmission and stability of viral load. *Proc Natl Acad Sci U S A* 1994;91:8037-41.
- Sperling RS, Shapiro DE, Coombs RW, et al. Maternal viral load, zidovudine treatment, and the risk of transmission of human immunodeficiency virus type 1 from mother to infant. *N Engl J Med* 1996;335:1621-9.
- Cao Y, Krogstad P, Korber BT, et al. Maternal HIV-1 viral load and vertical transmission of infection: the Ariel Project for the prevention of HIV transmission from mother to infant. *Nat Med* 1997;3:549-52.
- Jackson JB, Hom D, Piwowar E, et al. Maternal HIV-1 RNA serum levels at delivery and vertical transmission in Uganda. *Pediatr AIDS HIV Infect* 1996;7:37-42.
- Fang G, Burger H, Grimson R, et al. Maternal plasma human immunodeficiency virus type 1 RNA level: a determinant and projected threshold for mother-to-child transmission. *Proc Natl Acad Sci U S A* 1995;92: 12100-4.
- Mayaux MJ, Dussaux E, Isopet J, et al. Maternal virus load during pregnancy and mother-to-child transmission of human immunodeficiency virus type 1: the French perinatal cohort studies. *J Infect Dis* 1997;175:172-5.
- Administration of zidovudine during late pregnancy and delivery to prevent perinatal HIV transmission — Thailand, 1996-1998. *MMWR Morb Mortal Wkly Rep* 1998;47:287, 315.
- Fiscus SA, Adimora AA, Schoenbach VJ, et al. Perinatal HIV infection and the effect of zidovudine therapy on transmission in rural and urban counties. *JAMA* 1996;275:1483-8.
- Public Health Service Task Force recommendations for the use of antiretroviral drugs in pregnant women infected with HIV-1 for maternal health and for reducing perinatal HIV-1 transmission in the United States. *MMWR Morb Mortal Wkly Rep* 1998;47(RR-2):1-30. [Errata, *MMWR Morb Mortal Wkly Rep* 1998;47:287, 315.]
- Mandelbrot L, Le Chenadec J, Berrebi A, et al. Perinatal HIV-1 transmission: interaction between zidovudine prophylaxis and mode of delivery in the French Perinatal Cohort. *JAMA* 1998;280:55-60.
- The European Mode of Delivery Collaboration. Elective caesarean-section versus vaginal delivery in prevention of vertical HIV-1 transmission: a randomised clinical trial. *Lancet* 1999;353:1035-9.
- The International Perinatal HIV Group. The mode of delivery and the risk of vertical transmission of human immunodeficiency virus type 1: a meta-analysis of 15 prospective cohort studies. *N Engl J Med* 1999;340:977-87.