

# The New England Journal of Medicine

© Copyright, 1997, by the Massachusetts Medical Society

VOLUME 336

MAY 8, 1997

NUMBER 19



## VIRAL LOAD AND DISEASE PROGRESSION IN INFANTS INFECTED WITH HUMAN IMMUNODEFICIENCY VIRUS TYPE 1

WILLIAM T. SHEARER, M.D., PH.D., THOMAS C. QUINN, M.D., PHILIP LARUSSA, M.D., JUDY F. LEW, M.D.,  
LYNNE MOFENSON, M.D., SUSAN ALMY, M.S., KENNETH RICH, M.D., EDWARD HANDELSMAN, M.D., CLEMENTE DIAZ, M.D.,  
MARCELLO PAGANO, PH.D., VINCENT SMERIGLIO, PH.D., AND LESLIE A. KALISH, D.Sc.,  
FOR THE WOMEN AND INFANTS TRANSMISSION STUDY GROUP\*

### ABSTRACT

**Background** There are only limited data on human immunodeficiency virus type 1 (HIV-1) RNA in perinatally infected infants. Understanding the dynamics of HIV-1 infection and its relation to disease progression may help identify opportunities for effective antiviral treatment in infected infants.

**Methods** We obtained plasma samples from 106 HIV-infected infants at birth; at 1, 2, 4, 6, 9, 12, 15, and 18 months of age; and subsequently every 6 months. HIV-1 RNA was assayed by means of a reverse-transcription polymerase chain reaction. The infants were born between 1990 and 1993, and only 21 percent of the infants' mothers received any treatment with zidovudine during pregnancy.

**Results** Plasma HIV-1 RNA levels increased rapidly after birth, peaked at 1 to 2 months of age (median values at 1 and 2 months, 318,000 and 256,000 copies per milliliter, respectively), and then slowly declined to a median of 34,000 copies per milliliter at 24 months. Newborns with a first positive HIV-1 culture within 48 hours after birth had significantly higher HIV-1 RNA levels, although only during the first two months of life, than those with a first positive culture seven or more days after birth. Infants with a rapid progression of disease had higher peak HIV-1 RNA levels in the first two months of life than those without rapid progression (median value, 724,000 vs. 219,000 copies per milliliter;  $P=0.006$ ), as well as a higher geometric mean value during the first year of life (median value, 330,000 vs. 158,000 copies per milliliter;  $P=0.001$ ).

**Conclusions** In perinatally infected infants, HIV-1 RNA levels are high and decline only slowly during the first two years of life. Infants with very high viral loads in the first months of life are at increased risk for a rapid progression of disease, which suggests that early treatment with antiretroviral agents may be indicated for these infants. (N Engl J Med 1997; 336:1337-42.)

©1997, Massachusetts Medical Society.

IN perinatally acquired human immunodeficiency virus type 1 (HIV-1) infection, it is possible to make precise estimates of the time of infection and observe the subsequent changes in plasma viremia. Measurement of the viral load may be of considerable importance not only in understanding the pathogenesis of HIV-1 infection in infants and children but also in managing the infection.

The level of the plasma HIV-1 load appears to predict the progression of disease in children,<sup>1-8</sup> but studies of children have been limited. There is now considerable evidence that in adults the plasma HIV-1 RNA load can be used to predict the progression of disease and is useful in assessing antiretroviral therapies.<sup>9-12</sup> The plasma HIV-1 RNA level in adults reaches a steady state within 6 to 12 months after the initial infection; however, in many adults, the RNA level has already reached a steady state at the time of the initial diagnosis.<sup>13</sup>

We used prospective data from the Women and Infants Transmission Study (WITS) to examine the relation between the viral load and the clinical outcome in infants and children with HIV-1 infection.

From the Department of Pediatrics, Section of Allergy and Immunology, Baylor College of Medicine, Houston (W.T.S.); Johns Hopkins University, Baltimore (T.C.Q.); the National Institute of Allergy and Infectious Diseases, Bethesda, Md. (T.C.Q., J.E.L.); the Virology Department, Columbia-Presbyterian Hospital, New York (P.L.); the National Institute of Child Health and Human Development, Bethesda, Md. (L.M.); the New England Research Institutes, Watertown, Mass. (S.A., L.A.K.); the University of Illinois at Chicago, Chicago (K.R.); the State University of New York Health Science Center at Brooklyn, Brooklyn (E.H.); the University of Puerto Rico, San Juan (C.D.); the Department of Biostatistics, Harvard School of Public Health, Boston (M.P.); and the National Institute on Drug Abuse, Rockville, Md. (V.S.). Address reprint requests to Dr. Shearer at Texas Children's Hospital, 6621 Fannin St., MC 1-3291, Houston, TX 77030.

\*The members of the Women and Infants Transmission Study Group are listed in the Appendix.

The results of such analyses should help clinicians decide when to administer antiretroviral therapies.

## METHODS

### Patient Population

WITS is a multicenter, longitudinal study of the natural history of HIV-1 infection in pregnant women and their infants. The enrollment of participants began in 1989. At present, pregnant women infected with HIV-1 and their infants are recruited for enrollment at study centers in Massachusetts (Boston and Worcester), Illinois (Chicago), New York (two centers in New York City), Texas (Houston), and Puerto Rico (San Juan). According to the study protocol, no attempt is made to control the use of antiviral treatment in mothers or infants. All mothers are advised not to breast-feed their infants. Since March 1994, pregnant women have been counseled about the use of zidovudine to prevent the transmission of HIV to their infants.

For the present analysis, the population consisted of perinatally infected singleton infants born to HIV-1-positive mothers between January 8, 1990, and December 3, 1993. Plasma HIV-1 RNA levels were measured in 106 of these infants, with a total of 673 samples available for analysis. At the time of plasma-sample selection, all 106 infants had at least 12 months of follow-up or had died before 12 months of age.

The study period predated the completion of AIDS Clinical Trials Group (ACTG) protocol 076, which showed that the administration of zidovudine to HIV-infected women during pregnancy and labor and to their infants during the first six weeks of life resulted in a significantly decreased risk of HIV transmission. Thus, only 22 mothers (21 percent) in our study received zidovudine during pregnancy, and it was administered primarily as treatment for the mothers.

### Sample Collection and Definitions

Blood specimens were collected between February 1990 and July 1995. Whole blood was collected by venipuncture in Vacutainer tubes containing heparin. Peripheral-blood specimens were routinely collected during the first 7 days of life (one specimen); at 1, 2, 4, 6, 9, 12, and 18 months of age; and every 6 months thereafter. In addition, specimens were obtained from some infants at 15 months of age.

The infection status of each infant was determined according to a working definition of HIV-1 infection.<sup>14,15</sup> A child was considered to be infected if peripheral-blood mononuclear-cell cultures for HIV-1 were positive at two or more visits.

Early (in utero) infection was defined as a positive HIV-1 culture in the first 48 hours of life. Late (intrapartum) infection was defined as at least one negative but no positive cultures during the first seven days of life and positive cultures thereafter (modified from Bryson et al.<sup>16</sup>).

Rapid progression of disease was defined as a class C clinical event, according to the Centers for Disease Control and Prevention's (CDC's) 1994 revised classification system for HIV infection in children,<sup>17</sup> or death by 18 months of age. Nonrapid progression of disease was defined as the absence of these end points by 18 months.

### Laboratory Analysis

Heparinized blood samples were transported to the local laboratory, and the peripheral-blood mononuclear cells were separated with Ficoll-Hypaque centrifugation and subsequently cultured for HIV-1. The remaining plasma was centrifuged, divided into aliquots, and stored at  $-70^{\circ}\text{C}$ . The recommended time from collection to freezing at  $-70^{\circ}\text{C}$  was less than six hours. The HIV-1 serologic status of the mothers was determined with the use of a commercially available enzyme-linked immunosorbent assay and confirmed with the Western blot assay.

Qualitative peripheral-blood mononuclear-cell cultures and

quantitative peripheral-blood mononuclear-cell microcultures were performed according to the ACTG consensus protocol, with standard modifications for samples from children, and all laboratories participated in the ACTG quality-control program.<sup>14,18</sup>

Plasma HIV-1 RNA was measured in two laboratories according to ACTG quality-assurance recommendations. The quantitative HIV-1 RNA polymerase chain reaction (PCR) was performed with an HIV-1 assay according to the manufacturer's instructions (Amplicor HIV-1 Monitor Test, Roche Diagnostic Systems, Branchburg, N.J.). RNA was extracted from heparinized samples with the use of a modification of the method of Boom et al.<sup>19</sup> The use of silica to extract RNA from heparinized plasma has been found to give results similar to those with the recommended use of EDTA-treated plasma.<sup>20</sup> Briefly, plasma samples (200  $\mu\text{l}$ ) were added to 0.90 ml of lysis buffer containing guanidinium thiocyanate, an internal quantitation standard, and silica particles. After a short incubation, the silica particles and bound nucleic acid were concentrated by centrifugation and washed twice with a buffer containing guanidinium thiocyanate, twice with 70 percent ethanol, and once with acetone. A low-ionic-strength diluent (400  $\mu\text{l}$ ) was added to the dried silica to elute bound nucleic acid.

Fifty microliters of each prepared RNA sample was used for the PCR assay. After amplification and detection of the PCR product, the initial HIV-1 RNA load in each sample was calculated by comparing it with the internal quantitation standard, and the results were expressed as HIV-1 RNA copies per milliliter of plasma. Samples in which HIV-1 RNA was not detected were assigned a value of 400 HIV-1 RNA copies per milliliter for the purpose of the statistical analysis.

### Statistical Analysis

The HIV-1 RNA load was analyzed on a logarithmic (base 10) scale, with the results converted to copies per milliliter. The mean or average number of copies is actually a geometric mean. Three summary measures were used to characterize the pattern of the HIV-1 RNA load during the first year of life. The early peak was defined as the maximal value during the period from birth to two months of age, among infants with at least one available measurement at one or two months of age. The average viral burden and the slope of the decline in the viral burden between months 1 and 12 were calculated from all available values during this period, among infants with at least two available values. The within-person slopes were calculated by least-squares regression. Ninety-five percent confidence intervals were calculated for median values.<sup>21</sup> Two-sample comparisons of viral load were made with the Mann-Whitney-Wilcoxon test.<sup>22</sup> The cumulative probabilities of a rapid progression of disease by certain ages were calculated by the Kaplan-Meier method and compared with the use of the log-rank test.<sup>23,24</sup> All P values are two-sided.

## RESULTS

HIV-1 RNA was measured in a total of 673 plasma samples from 106 HIV-infected infants (born by December 3, 1993), with a mean of 6.35 samples per infant. Most of the specimens (92 percent) were acquired before 30 months of age.

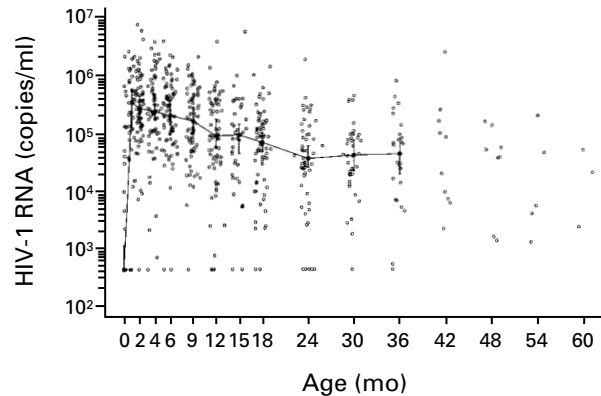
Data from study visits before December 1, 1995, were analyzed. On the basis of these data, 140 children were classified as infected with HIV-1. The mothers of the 106 children included in the study did not differ significantly from the mothers of the 34 excluded infants, with respect to race or ethnic group, age at delivery, education, results of HIV-1 cultures during pregnancy and at delivery, use of hard drugs (heroin, cocaine, methadone, or injec-

tion drugs) during pregnancy, duration of rupture of membranes, percentage of CD4+ cells, infant's gestational age, and mode of delivery. However, more mothers of excluded infants used zidovudine according to the ACTG 076 protocol (47 percent, vs. 2 percent of the mothers whose infants were included;  $P < 0.001$ ), reflecting the fact that most of the excluded infants were born after the results of trial ACTG 076 were made public.

The infants included in the study did not differ significantly from those who were excluded, with respect to the results of HIV-1 cultures at birth, 48 hours, and seven days; the percentage of CD4+ cells at birth, one month, and one year; birth weight; or sex. However, the infants included in the study differed from those not included in terms of the study site ( $P = 0.03$ ) and receipt or nonreceipt of zidovudine according to the ACTG 076 regimen ( $P < 0.001$ ). Sixty-six percent of the cohort received therapeutic zidovudine (not the ACTG 076 regimen) sometime during the study. However, most analyses and conclusions rely primarily on samples obtained before the use of zidovudine (94 percent of the samples obtained during the first two months and 74 percent of those obtained during the first year).

**Plasma HIV-1 RNA Load in Infants**

There was a wide range of plasma HIV-1 RNA values in the 106 infected children at all time points (Fig. 1). The median RNA load was below the cut-off level at birth ( $< 400$ ); rose rapidly to 318,000 and 256,000 copies per milliliter at 1 and 2 months, respectively; and gradually declined to 34,000 copies per milliliter (approximately a decrease of 1 in the  $\log_{10}$  of the number of copies per milliliter) by 24 months of age. When samples obtained after the ad-



**Figure 1.** Plasma HIV-1 RNA in Multiple Samples from 106 Infants with HIV-1, According to Age.

The solid line connects the median values of the individual data points. The vertical bars represent the 95 percent confidence intervals.

ministration of zidovudine were excluded from the analysis, the pattern was quite similar.

Since the predominant pattern was a rapid rise in the viral load followed by a slow decline, we examined three summary measures to characterize this pattern: the early peak value (from birth to 2 months), the average viral burden in each infant during months 1 through 12, and the slope of the decline during months 1 through 12 (Table 1). The median early peak value was 299,000 RNA copies per milliliter; the median value for the average viral burden was 185,000 copies per milliliter, and the slope was  $-0.048 \log_{10}$  copies per milliliter per month (Table 1).

On the basis of the WITS definition of infection

**TABLE 1.** MEDIAN HIV-1 RNA LEVELS IN 106 INFANTS WITH HIV-1 INFECTION.\*

GROUP	EARLY PEAK VALUE			AVERAGE VIRAL BURDEN			SLOPE		
	NO. OF INFANTS	RNA COPIES/ml	P VALUE	NO. OF INFANTS	RNA COPIES/ml	P VALUE	NO. OF INFANTS	$\log_{10}$ RNA COPIES/ml	P VALUE
Overall	78	299,000		100	185,000		100	-0.048	
Early infection	7	780,000	0.04†	11	238,000	0.40†	11	-0.063	0.79†
Late infection	43	243,000		57	169,000		57	-0.024	
Rapid disease progression	17	724,000	0.006‡	21	330,000	0.001‡	21	-0.056	0.79‡
Nonrapid disease progression	48	219,000		63	158,000		63	-0.045	

\*The early peak value is the maximal number of HIV-1 RNA copies during the first two months of life, for infants with at least one available value at one or two months. The average viral burden is the geometric mean for the number of HIV-1 RNA copies between months 1 and 12 of life. The slope is the change in the  $\log_{10}$  value per month between months 1 and 12 of life.

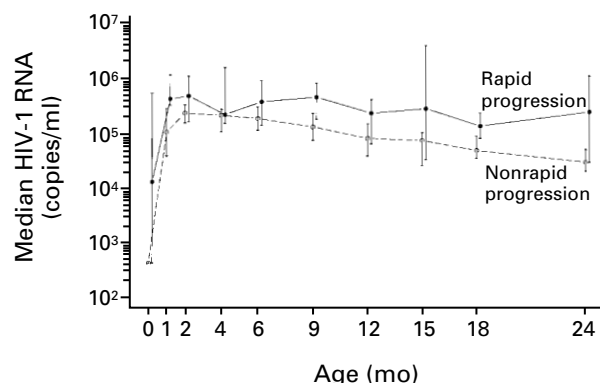
†P values are for the comparison with the late-infection group.

‡P values are for the comparison with the nonrapid-progression group.

status, it was possible to estimate the sensitivity of the HIV-1 RNA PCR assay by determining the number of plasma specimens with detectable RNA. The sensitivity was 34 percent (13 of 38 specimens) at birth and ranged from 95 to 99 percent at one, two, four, and six months.

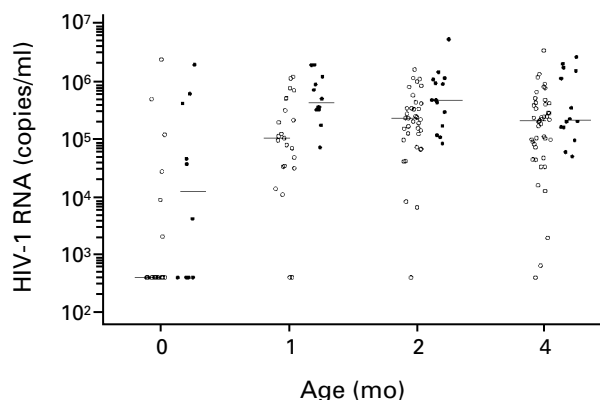
**Viral Load in Relation to Early or Late Infection**

Infants with early HIV-1 infection (in utero transmission) and those with late infection (peripartum transmission) had significantly different median



**Figure 2.** Median HIV-1 RNA Levels According to Whether the Infants Had Rapid or Nonrapid Progression of Disease.

Rapid progression was defined as CDC class C HIV-1 disease or death by 18 months of age, and nonrapid progression as the absence of these end points by 18 months of age. The vertical bars represent the 95 percent confidence intervals. The number of RNA copies differed significantly between the two groups of infants at 1, 2, 6, 9, 15, 18, and 24 months ( $P < 0.05$ , by the Mann-Whitney-Wilcoxon test).



**Figure 3.** HIV-1 RNA Levels during the First Four Months of Life in the Infants with Rapid Progression of Disease (Solid Circles) and Those with Nonrapid Progression (Open Circles).

The horizontal lines represent the median values. Not all infants had viral loads measured at every time point.

HIV-1 RNA values in the early months of life. At birth, the median HIV-1 RNA values were 10,800 copies per milliliter in the early-infection group and less than 400 copies per milliliter in the late-infection group ( $P = 0.002$ ), and at one month, the median values were 716,000 and 100,000 copies per milliliter, respectively ( $P = 0.02$ ). The median peak values were 780,000 copies per milliliter in the early-infection group and 243,000 copies per milliliter in the late-infection group ( $P = 0.04$ ) (Table 1). After 1 month, the median RNA values in the two groups were similar, and in both groups, the values slowly declined over a period of 24 months to 101,000 and 28,900 copies per milliliter ( $P = 0.54$ ). The average viral burden and the slope of the decline during the first year were similar in the infants with early infection and those with late infection.

**Viral Load in Relation to Disease Progression**

HIV-1 RNA levels were compared in the group of children with rapid progression of disease (CDC class C HIV-1 disease or death by 18 months of age) and in the group without rapid progression (Fig. 2). From birth to 24 months of age, the children with rapid progression had a higher median HIV-1 RNA load than those without rapid progression. This difference was significant at 1 month (431,000 vs. 105,000 HIV-1 RNA copies per milliliter,  $P = 0.01$ ), 2 months (490,000 vs. 236,000,  $P = 0.03$ ), 6 months (377,000 vs. 185,000,  $P = 0.04$ ), 9 months (456,000 vs. 128,000,  $P = 0.003$ ), 15 months (277,000 vs. 70,900,  $P = 0.03$ ), 18 months (130,000 vs. 45,900,  $P = 0.01$ ), and 24 months (233,000 vs. 27,500,  $P = 0.006$ ). The corresponding early peak values were 724,000 copies per milliliter in the group with rapid progression and 219,000 copies per milliliter in the group without rapid progression ( $P = 0.006$ ), and the average viral loads were 330,000 and 158,000 copies per milliliter, respectively ( $P = 0.001$ ) (Table 1).

To highlight the range of the data around the median HIV-1 RNA load in infants with rapid progression and those with nonrapid progression, individual data points at birth and one, two, and four months are shown in Figure 3. At each age and especially at birth, there is considerable overlap in the values for the two groups. No threshold RNA value was identified that could predict rapid progression to advanced HIV-1 disease or death. However, no infant with an HIV-1 RNA value below 70,000 to 80,000 copies per milliliter at one, two, and four months of age had rapidly progressive disease.

Early peak HIV-1 RNA values were analyzed with the use of a Kaplan-Meier plot depicting the cumulative incidence of CDC class C HIV-1 disease or death over time. Children were classified according to whether the early peak RNA load was above or below the median value. Figure 4 shows a striking

difference between these two groups, with an early peak value above the median associated with a 44 percent rate of progression by 24 months and a value below the median associated with a 15 percent rate of progression ( $P=0.008$ ).

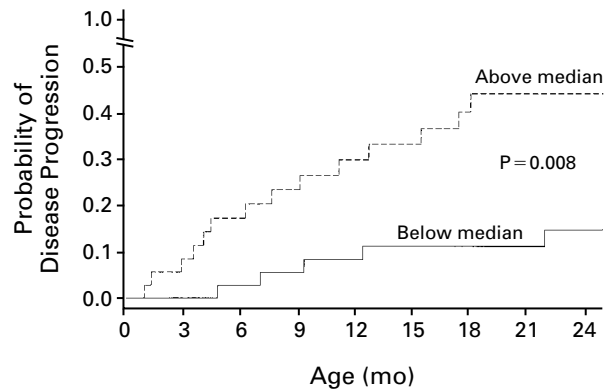
### DISCUSSION

This prospective study of the plasma HIV-1 RNA load in infected infants reveals a unique pattern of HIV-1 replication and plasma viral levels. The mean HIV-1 RNA load rises from generally low values ( $<10,000$  copies per milliliter) at birth to extremely high values ( $>100,000$  copies per milliliter) within the first 2 months of life and falls very slowly until at least the age of 24 months.

The generally low HIV-1 RNA levels at birth suggest that most neonates have acquired their infections very close to the time of birth, probably in the intrapartum period, or that maternal or placental factors inhibit viral replication before delivery. The rapid rise in the HIV-1 RNA load within one to two months, to values of several hundred thousand copies per milliliter, reflects a rapid replication of the virus within a short period of time, but the subsequent decline suggests a process of containment, possibly immune in nature, which prevents a further rise in the viral load. The slow decline in the viral load, which contrasts sharply with the rapid decline after primary HIV-1 infection in adults,<sup>13</sup> suggests that the still immature neonatal immune system has difficulty containing the viral infection. The similarity in the general pattern of plasma and serum HIV-1 RNA loads in children, as measured in smaller prospective studies<sup>4,8</sup> and cross-sectional studies,<sup>2,7,25</sup> is striking. Although the studies of HIV-1 RNA in children are much smaller than those in adults, the collective HIV-1 RNA data in children clearly indicate a unique pattern of viral replication and containment.

In our study, the RNA load differed according to whether the infection was acquired early or late. Infants with positive HIV-1 cultures during the first 48 hours after birth had significantly higher HIV-1 RNA values than those whose cultures were negative during the first seven days. However, the median HIV-1 RNA plasma level in the early-infection group was only 10,800 copies per milliliter at birth. This low value may indicate the presence of a maternal or placental protective mechanism that has not yet been identified. An alternative explanation may be that the infants were infected just before birth. The differences between the early-infection and late-infection groups disappeared very shortly after birth, and the subsequent response to the virus was similar in the two groups.

Although it is difficult to evaluate the effect of zidovudine on the viral load in this observational study, treatment did not have a pronounced effect



**Figure 4.** Kaplan-Meier Estimates of the Probability of Disease Progression, According to the Median Number of HIV-1 RNA Copies during the First Two Months of Life.

The median value of 299,000 RNA copies per milliliter was used as the threshold for predicting a rapid progression of disease.

on the results of the analysis. The pattern of the viral load over time changed very little when the plasma samples obtained after the receipt of therapeutic zidovudine were excluded from the analysis. Most of the samples were obtained before the receipt of therapeutic zidovudine.

The infants whose disease progressed rapidly tended to have high numbers of HIV-1 RNA copies not only at birth but also during most of the first 24 months of life. Although the differences between the median HIV-1 RNA levels in the rapid-progression group and the median levels in the nonrapid-progression group are large, there was considerable overlap in the values between the two groups. There was no threshold value above which rapid progression of HIV-1 disease could be predicted. However, none of the infants with less than 70,000 copies per milliliter at one, two, and four months had rapidly progressive disease. Additional studies are needed to verify this finding.

In summary, in this prospective study of 106 children with HIV-1 infection, there was a rapid rise in the median HIV-1 RNA load in the first 1 or 2 months after birth, followed by a slow decline during the next 22 months. This same pattern was observed in infants with early infection and those with late infection and in infants with a rapid progression of disease and those with a nonrapid progression. The association of rapidly progressive disease with a higher plasma HIV-1 RNA load in the first months of life suggests that early antiretroviral treatment should be of some use in this subgroup of children. We found no threshold viral level above which HIV disease progressed rapidly, but there may be a threshold level below which rapid progression does not occur.

*We are indebted to the investigators, the study staffs, and especially the families who have participated in the Women and Infants Transmission Study and to Roche Molecular Systems for providing the AmpliCor HIV-1 Monitor Test kits.*

## APPENDIX

The Women and Infants Transmission Study Group included the following investigators and centers (with grants from the National Institutes of Health listed in parentheses): W. Shearer, C. Hanson, and N. Cooper — Baylor College of Medicine, Houston (U01 AI 34840, AI 36211, and RR 00188); J. Pitt and A. Higgins — Columbia–Presbyterian Hospital, New York (U01 AI 34842); K. Rich and D. Turpin — University of Illinois at Chicago (U01 AI 34841); S. Landesman, H. Mendez, and G. Moroso — State University of New York, Brooklyn (HD-8-2913 and R0-1-HD-25714); R. Tuomala, E. Cooper, and D. Mesthene — Brigham and Women's Hospital, Boston (U01 AI 34856); C. Diaz and E. Pacheco-Acosta — University of Puerto Rico, San Juan (U01 AI 34858); M.G. Fowler, J. Lew, and E. Matzen — National Institute of Allergy and Infectious Diseases, Bethesda, Md.; A. Willoughby, D. Burns, J. Moye, J. Read, and L. Mofenson — National Institute of Child Health and Human Development, Bethesda, Md.; V. Smeriglio and K. Davenny — National Institute on Drug Abuse, Rockville, Md.; and S. McKinlay, L. Kalish, and K. Sherrieb — New England Research Institutes, Watertown, Mass. (N01 AI 05072 and N01 AI 35161).

## REFERENCES

- De Rossi A, Masiero S, Giaquinto C, et al. Dynamics of viral replication in infants with vertically acquired human immunodeficiency virus type 1 infection. *J Clin Invest* 1996;97:323-30.
- Palumbo PE, Kwok S, Waters S, et al. Viral measurement by polymerase chain reaction-based assays in human immunodeficiency virus-infected infants. *J Pediatr* 1995;126:592-5.
- O'Brien TR, Blattner WA, Waters D, et al. Serum HIV-1 RNA levels and time to development of AIDS in the Multicenter Hemophilia Cohort Study. *JAMA* 1996;276:105-10.
- McIntosh K, Shevitz A, Zaknun D, et al. Age- and time-related changes in extracellular viral load in children vertically infected by human immunodeficiency virus. *Pediatr Infect Dis J* 1996;15:1087-91.
- Mofenson LM, Korelitz J, Meyer WA III, et al. The relationship between serum HIV-1 RNA levels, CD4 lymphocyte percent, and long-term mortality risk in HIV-1-infected children. *J Infect Dis* (in press).
- Dickover R, Kwok S, Wei L, Christopherson C, Bryson Y. HIV RNA peak during primary viremia is a major determinant of clinical course in perinatally infected infants. In: Program and abstracts of the 3rd Conference on Retroviruses and Opportunistic Infections, Washington, D.C., January 28–February 1, 1996. Alexandria, Va.: Infectious Diseases Society of America, 1996:93. abstract.
- Tetali S, Romano J, Than S, et al. Virus load as marker of disease progression in HIV-infected children. In: Program and abstracts of the 3rd Conference on Retroviruses and Opportunistic Infections, Washington, D.C., January 28–February 1, 1996. Alexandria, Va.: Infectious Diseases Society of America, 1996:99. abstract.
- Abrams EJ, Weedon JC, Lambert G, Steketeer R. HIV viral load early in life as a predictor of disease progression in HIV-infected infants. In: Vol. 2. Abstracts. XI International Conference on AIDS, Vancouver, B.C., July 7–12, 1996:25. abstract.
- Mellors JW, Rinaldo CR Jr, Gupta P, White RM, Todd JA, Kingsley LA. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science* 1996;272:1167-70.
- Mellors JW, Kingsley LA, Rinaldo CR Jr, et al. Quantitation of HIV-1 RNA in plasma predicts outcome after seroconversion. *Ann Intern Med* 1995;122:573-9.
- Jurriaans S, Van Gemen B, Weverling GJ, et al. The natural history of HIV-1 infection: virus load and virus phenotype independent determinants of clinical course? *Virology* 1994;204:223-33.
- Henrard DR, Phillips JE, Muenz LR, et al. Natural history of HIV-1 cell-free viremia. *JAMA* 1995;274:554-8.
- Ho DD. Viral counts in HIV infection. *Science* 1996;272:1124-5.
- 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.
- Landesman SH, Kalish LA, Burns DN, et al. Obstetrical factors and the transmission of human immunodeficiency virus type 1 from mother to child. *N Engl J Med* 1996;334:1617-23.
- 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.
- 1994 Revised classification system for human immunodeficiency virus infection in children less than 13 years of age. *MMWR Morb Mortal Wkly Rep* 1994;43(RR-12):1-10.
- McIntosh K, Pitt J, Brambilla D, et al. Blood culture in the first 6 months of life for the diagnosis of vertically transmitted human immunodeficiency virus infection. *J Infect Dis* 1994;170:996-1000.
- 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.
- 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.
- Zar JH. Biostatistical analysis. 2nd ed. Englewood Cliffs, N.J.: Prentice-Hall, 1974:390.
- Rosner B. Fundamentals of biostatistics. 3rd ed. Boston: PWS-Kent Publishing, 1990:304-9.
- Kaplan E, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958;53:457-81.
- Peto R, Peto J. Asymptotically efficient rank invariant test procedures. *J R Stat Soc [A]* 1972;135:185-206.
- Conway B, Allen U, Lapointe N, et al. HIV viremia in relation to clinical and laboratory correlates among HIV-infected children. In: Program and abstracts of the 3rd Conference on Retroviruses and Opportunistic Infections, Washington, D.C., January 28–February 1, 1996. Alexandria, Va.: Infectious Diseases Society of America, 1996:102. abstract.