The Amount of Early p24 Antigenemia and Not the Time of First Detection of Virus Predicts the Clinical Outcome of Infants Vertically Infected with Human Immunodeficiency Virus


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Twenty-three children vertically infected with human immunodeficiency virus type 1 (HIV-1) were studied for viremia during the first days of life. Nine had HIV-1 infection within the first week (early); 14 had HIV-1 first detected by day 11–90 (late). The groups had similar incidence and time of onset of symptomatic HIV-1 infection and survival. CD4 T cell percentages, rates of CD4 T cell attrition, quantitative cell-associated viremia, and p24 antigen concentrations were comparable. Children with peak antigen concentrations >100 pg/mL during the first 6 months (5 early, 6 late) fared worse than those with lower p24 levels. Thus, HIV-1–infected infants with detectable virus in the first few days of life do not have a worse prognosis than infants whose virus is detectable only later. Elevated p24 antigenemia during the first 6 months of life correlates strongly with poor clinical outcome and is independent of the time that virus was first detected.

Vertical transmission of human immunodeficiency virus (HIV-1) represents the primary mode of pediatric infection [1]. Prospective studies have estimated that 14%–50% of infants born to HIV-1–positive women will be infected [1–3]. In utero, intrapartum, and postpartum transmission of HIV-1 infection have been reported [4–7]. Recent data suggest that most transmission (67%) occurs either late in pregnancy or during the peripartum period [7–13]. Determining when transmission occurs has been of major interest, as some investigators have suggested that the time at which transmission occurs may be a critical determinant of outcome in infants [14, 15].

Using sensitive laboratory techniques (virus isolation, polymerase chain reaction [PCR], plasma p24 antigen assays), virus can be detected in 30%–60% of HIV-1–infected newborns during the first days of life [10–18]. It has been proposed that the detection of viremia in the first 48 h of life be considered in utero infection, while detection of virus after the first week of life be considered intrapartum infection [19]. In a previous study [20], we found that concentrations of p24 antigen >100 pg/mL occurring in early infancy correlated with more rapid disease progression and worse clinical outcome. Insufficient early virologic data prevented examination of the relationship between antigenemia and first detection of viremia.

In this study, we analyzed data from a cohort of 23 children vertically infected with HIV-1 who were followed prospectively from birth to examine whether detectable virus in the first days of life is associated with subsequent elevated plasma p24 antigen and rapid disease progression.

Patients and Methods

Population and collection of specimens. This is a retrospective study of all HIV-1–infected children born and followed prospectively at Bellevue Hospital Center who had blood obtained within the first week of life and tested for the presence of HIV-1. About one-fourth of the children born and prospectively followed in our institution during the study period (1987–1994) had blood samples obtained during the first week of life and were therefore eligible to be included in this study.

Children were considered HIV-1–infected if HIV-1 was detected in at least 2 separate blood specimens by either virus isolation from peripheral blood mononuclear cell (PBMC) culture, PCR, or presence of p24 antigen in plasma. Early viremia was defined as detection of HIV in blood specimens collected within the first 7 days of life. Late viremia was defined as detection of virus from specimens collected after the first week of life in infants whose initial first-week specimen was negative.

All children were followed in our clinic monthly during the first 3 months and every 1–3 months thereafter. Children were classified by Centers for Disease Control and Prevention criteria as P-1 (infected, asymptomatic) or P-2 (infected, symptomatic). Trimethoprim-sulfamethoxazole was prescribed for children born after 1991 for the prophylaxis of Pneumocystis carinii pneumonia (PCP). Antiretroviral therapy was also prescribed for symptomatic
children. Two infants who died in early infancy were neither prescribed prophylaxis for PCP nor treated with antiretroviral therapy. Blood was collected into EDTA-containing tubes and processed within 2 h. PBMC were isolated by density gradient centrifugation using ficoll-hypaque. Cell pellets were stored at −20°C. Plasma samples were stored at −70°C.

**Lymphocyte phenotyping.** Before 1990, lymphocyte phenotyping was done on freshly collected PBMC using fluorescence microscopy as described [21]. Beginning in 1990, phenotyping was done using an Epics Profile II flow cytometer and the whole blood lysis method (Q Prep; Coulter Electronics, Hialeah, FL).

**Plasma p24 antigen assay.** HIV-1 p24 antigen was measured by a standard (not immune complex–dissociated) EIA (Coulter Immunology, Hialeah, FL) in batch assays.

**HIV-1 culture.** Qualitative and quantitative HIV-1 cultures were done using fresh PBMC as reported [16, 22]. Qualitative HIV-1 cultures were done on all patients. A culture result was considered positive if the p24 antigen concentration in successive weekly supernatants was >30 pg/mL. Quantitative HIV-1 cultures were done by limiting dilution as reported [20] after 1991. The greatest dilution of PBMC that resulted in a positive culture was used to determine the clonal frequency of HIV in PBMC, resulting in titers ranging from 1 infectious dose per 10^3 PBMC to 1 infectious dose per 10^6 PBMC. Negative culture results were arbitrarily assigned a value of 1 per 10^6 PBMC. The cell-associated virus load in peripheral blood was calculated for each child and expressed in HIV-1–infected cells per 10^6 PBMC.

**PCR.** HIV-1 DNA was detected in PBMC by PCR as described [17]. Primer pairs specific for the core region (gag SK38/39), the long terminal repeat (LTR SK29/30), and the envelope region (env SK68/69) were used for sample amplification. Amplified DNA was tested for the presence of HIV DNA using liquid hybridization. Samples were considered positive only if amplified HIV-1 DNA from two or more regions of the genome was detected.

**Statistical analysis.** The following clinical, virologic, and immunologic outcome parameters were compared between the group of infants with early viremia and the group of infants with late viremia, using an α of .05.

The mean age of developing symptomatic infection and the duration of follow-up between groups of children were compared using Student’s t test. Survival analysis were done by Kaplan-Meier product limit analysis and compared using the log-rank test.

Immunologic and virologic parameters between groups of children were compared using the Mann-Whitney rank sum test. Median CD4 cell percentages for early and late viremia infant groups were compared cross-sectionally at 6-month intervals from birth to 24 months. The within-subject rate of change in CD4 cell percentage was estimated by computing the individual slopes of percentage of CD4 T cells over time using the least squares method. Only children with three or more T cell determinations were included in this analysis.

The median peak plasma p24 antigen concentrations for the first 6 months of life and for the entire study period were also compared between groups. In addition, we compared the proportion of infants with high and low p24 antigenemia, differentiated by a threshold concentration of 100 pg/mL. This threshold value was chosen on the basis of its prior value in predicting outcome [17]. Finally, for every child with two or more available measurements, the area under the time-concentration curve that was determined by p24 concentration at different points in time was calculated. This was divided by the days of follow-up, to control for different periods of follow-up for each child, and represented the computed average p24 antigen concentration.

**Results**

**Population.** Twenty-three HIV-1–infected children assessed prospectively from birth had initial testing done during the first week of life (median, 3 days; range, 1–6). None of the infants was breast-fed. Nine (39%) had early viremia as determined by positive PCR, HIV-1 culture, or presence of p24 antigen in plasma within 7 days of birth. Fourteen children (61%) had late viremia, since they were negative by at least two of the techniques used (PCR, HIV-1 culture, p24 antigen) in the first week of life but positive at a later time point (median, 30 days of age; range, 11–90). The mean duration of follow-up was 24.9 months (range, 3.1–46.4) for children with early viremia and 29.3 months (range, 2.1–77.9) for children with late viremia (P = .650).

There were 4 deaths, 2 in each group. Among the children with early infection, 1 died at age 3 months secondary to disseminated cytomegalovirus infection and another child, not compliant with PCP prophylaxis, died at 9.8 months of age from PCP. In the group of children with late infection, 1 died at age 8.7 months of liver failure secondary to acute hepatitis of unknown etiology and another developed hepatitis, cardiomyopathy, and *Candida* esophagitis and died at age 20.8 months. The proportional survival of the 2 groups of children was 77.7% versus 85.7% (log rank = .09, P = .77; figure 1).
The incidence of symptomatic disease did not differ between groups (89% vs. 86%). More important, the mean age of developing symptomatic disease was not different between children with early and late viremia (4.9 vs. 5 months, \( P = .969 \)). Only 1 child with early viremia and 2 children with late viremia were still asymptomatic (P-I-A) at the end of the study period (ages 36, 3, and 15.5 months, respectively).

All 6 symptomatic children with early viremia who survived beyond early infancy were given antiretroviral therapy (mean age, 4 months), as were 11 of 12 symptomatic children with late viremia (mean age, 5 months). PCP prophylaxis was prescribed for 7 of 9 and 9 of 14 infants with early and late viremia at a mean age of 3 months (same for both groups).

**CD4 T cell decline.** The median CD4 cell percentage at each interval of time from birth to 24 months for the group of early and late viremia infants is shown in table 1. No differences were seen between groups at any time point. The absence of differences between groups is corroborated by the results of the longitudinal analysis of the slopes of CD4 cell decline from 8 of 9 infants with early and 13 of 14 with late viremia for whom data were available. The median slopes, expressed as decline in CD4 cell percentage/day, and the interquartile range were \(-.021 (-.040 \text{ to } -.015)\) and \(-.023 (-.198 \text{ to } -.011)\), respectively (\( P = .68 \)).

**p24 antigenemia.** p24 antigen was detected in all infants in this study. The median peak p24 antigen concentrations for infants with early and late viremia during the first 6 months of life were 600 pg/mL (range, 3-11,260) versus 95 pg/mL (range, 0-18,234) (\( P = .62 \)); means, 1494 vs. 1482 pg/mL). For the total study period, the median peak p24 antigen concentrations were 878 pg/mL (range, 67-11,260) versus 152 pg/mL (range, 13-18,234; \( P = .14 \)); means, 2185 vs. 1512 pg/mL; table 2).

Eleven children had high peak concentrations of p24 antigen (>100 pg/mL) during the first 6 months of life: 5 with early viremia and 6 with late viremia. Twelve children had low concentrations of p24 antigen (peak <100 pg/mL) during the first 6 months of life: 4 with early viremia and 8 with late viremia. All four deaths in the study population occurred in children who had peak p24 antigenemia levels >100 pg/mL in the first 6 months of life. The proportional survival of the group of infants with high concentrations of p24 antigen was different from the survival of infants with low concentrations (2-year survival, 63.6% vs. 100%; log rank = 4.85, \( P = .028 \); figure 2).

The average p24 antigen concentration over the entire study period for 8 infants with early and 12 with late viremia with at least two measurements of p24 antigen was calculated to be 160.6 versus 22.7 pg/mL (\( P > .1 \)). The average p24 antigen concentration during the first 6 months of life was estimated to be 126.4 pg/mL for infants with early viremia and 19.4 pg/mL for infants with late viremia (\( P = .7 \); table 2).

**Cell-associated virus load.** Multiple quantitative HIV cultures were done on 7 infants with early viremia (mean, 7.1 infant) and 7 infants with late viremia (mean, 10.1 infant). The median cell-associated virus load in peripheral blood for the total study period for infants with early viremia was 52.5 HIV-1-infected cells per 10° PBMC (range, 19.9-158.5) compared with 25.1 cells per 10° PBMC (range, 0.3-46.5) for infants with late viremia (\( P = .2 \)). In addition, the median cell-associated virus load in peripheral blood for the first 6 months of life was 62.7 versus 46.5 infected cells per 10° PBMC for 6 early and 5 late viremia infants (\( P = .58 \); table 2).

**Table 1.** Cross-sectional CD4 T cell percentages in HIV-1-infected infants.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Early viremia</th>
<th>Late viremia</th>
<th>( P )</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>Median %</td>
<td>n</td>
<td>Median %</td>
</tr>
<tr>
<td>0</td>
<td>9</td>
<td>47</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>30</td>
<td>12</td>
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<td>12</td>
<td>7</td>
<td>24</td>
<td>10</td>
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<td>18</td>
<td>7</td>
<td>21</td>
<td>8</td>
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<td>24</td>
<td>5</td>
<td>17</td>
<td>7</td>
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</tbody>
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**Discussion**

The clinical outcome and the immunologic and virologic measures of HIV-1-infected children were reviewed to examine whether early viremia correlates with disease progression and virus load. No significant differences between early and late viremia groups were found in the rates of survival, the incidence and time of onset of symptoms, the CD4 cell percentage at birth and during the first 2 years of life, the rate of decline in CD4 T cells, the amount of cell-associated viremia, or average and peak p24 antigen. This is in contrast to the report by Dickover et al. [14] involving a smaller cohort followed for a shorter period of time. That study showed that all 4 infants with viremia in the first 48 h had high early virus load and rapid disease progression.

**Table 2.** Virus load in HIV-1-infected children with early and late viremia.

<table>
<thead>
<tr>
<th>Overall study period</th>
<th>Early viremia</th>
<th>Late viremia</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak p24 antigen (pg/mL)</td>
<td>878</td>
<td>152</td>
<td>.14</td>
</tr>
<tr>
<td>Median virus concentration</td>
<td>160</td>
<td>22</td>
<td>&gt;.1</td>
</tr>
<tr>
<td>HIV-positive cells/10° PBMC</td>
<td>52.5</td>
<td>25.1</td>
<td>.2</td>
</tr>
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First 6 months of life

<table>
<thead>
<tr>
<th>Overall study period</th>
<th>Early viremia</th>
<th>Late viremia</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak p24 antigen (pg/mL)</td>
<td>600</td>
<td>95</td>
<td>.62</td>
</tr>
<tr>
<td>Average virus concentration</td>
<td>120</td>
<td>19</td>
<td>.71</td>
</tr>
<tr>
<td>HIV-positive cells/10° PBMC</td>
<td>62.7</td>
<td>46.5</td>
<td>.58</td>
</tr>
</tbody>
</table>

NOTE. PBMC, peripheral blood mononuclear cells.
This discrepancy cannot be explained by differences in the definition of early viremia (48 h vs. 1 week), as further examination of our data using more stringent criteria for classification of early versus late infection did not affect the conclusions of our study. Four of the 9 children classified with early viremia were tested and found to have been viremic within the first 48 h of life. Using this smaller cohort of 18 children (4 with in utero infection and 14 with intrapartum), we found no correlation between the onset of viremia and survival, CD4 cell decline, p24 antigenemia, or the amount of cell-associated viremia (data not shown).

Other investigators have also reported an absence of correlation between early viremia and rapid disease progression in small cohorts of patients. Kovacs et al. [23] recently reported that time of first detection of virus did not correlate with the amount of circulating viral RNA, CD4 cell counts, or disease progression. Burchett et al. [24] suggested that host humoral response might be a more important factor for the control of viral replication and found that rapid disease progression correlated better with an incomplete humoral response (<5 bands in Western blot) than with timing of transmission. In that study, Burchett et al. did not find a difference in the evolution of CD4 T cells in children with early versus late onset of detectable viremia.

Rich et al. [25] proposed that children infected in utero had a higher proportion of activated CD8 cells at birth compared with children infected intrapartum. Highly activated CD8 lymphocytes at birth correlated with decreased CD4 lymphocytes at birth and at 4 months of age, but there was no correlation of timing of vertical transmission and CD4 cell percentages during the first 6 months of life [25]. Finally, Comeau et al. [26], using PCR on Guthrie cards to detect HIV-1 infection, found no association between PCR positivity of neonatal specimens and clinical outcome: A high proportion of neonatal blood specimens of infants with rapid disease progression were PCR-positive, as were specimens of half of the children with mild disease.

Of our patients, 17% (4/23) had rapid progression leading to death before 2 years of age. Our population does not appear to be atypical in this regard [27]. The fact that infants with rapidly progressive disease were equally divided among those with early and late viremia is the strongest evidence that the time of first detection and, by extension, the mode of transmission (intrauterine versus peripartum) is not the sole explanation of the bimodal progression of disease in this population. It also suggests that our results cannot merely be explained by sample bias or small sample size. Although PCP prophylaxis and antiretroviral treatment may be confounding factors in survival analyses of vertically infected infants, in this cohort the 2 groups of children had similar exposure to therapeutic and prophylactic intervention. Although it appeared that early viremia was associated with increased p24 antigen concentration or cell-associated virus load, our study did not have the statistical power to indicate such a correlation; larger studies will be necessary to determine whether small differences in outcome truly exist.

The present study confirms our prior finding that the amount of p24 antigenemia in the first 6 months of life is closely correlated with outcome. An important new finding is that the time of first detectable viremia is independent of the amount of p24 antigenemia in the first 6 months of life. Similarly, there was no association between early viremia and the amount of cell-associated virus in peripheral blood. Unfortunately, we could not evaluate whether there was an association between the amount of cell-associated virus and outcome because of the small number of infants who had quantitative cultures in the first few months of life. The analysis of virus load by more sensitive techniques, such as the quantification of HIV-1 RNA in plasma, may provide additional information to clarify our understanding of these events.

Although timing of transmission may play a role in the pathogenesis of vertical HIV-1 infection, this may be less important than other factors, such as viral phenotype, infectious dose of virus during transmission, host genetic background, and host defense mechanisms that may affect the vigor of viral replication. Our data suggest that the ability of the infant to control viral replication and limit virus load in early infancy might have a large effect on disease progression and outcome. This could have implications for the primary treatment of perinatal HIV infection. Further studies analyzing the factors that determine why certain infants are more successful at controlling viral replication during the first few months of life will be essential in understanding the pathogenesis of HIV-1 infection in children.

Acknowledgment

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