Early Detection of Perinatal Human Immunodeficiency Virus (HIV) Type 1 Infection Using HIV RNA Amplification and Detection


Early diagnosis of perinatally transmitted human immunodeficiency virus type 1 (HIV) infection can guide early interventions. HIV coculture and DNA polymerase chain reaction (DNA-PCR) detect few HIV-infected infants at birth and 90%--100% by age 3 months. Because extracellular HIV RNA may appear soon after infection, a plasma HIV RNA assay was compared with DNA-PCR for early detection of perinatally infected infants. Blood-draw specimens (108) obtained at the same time from 49 HIV-infected infants and 10 specimens from 8 uninfected infants were tested. HIV RNA and DNA-PCR positivity rates were 56% and 33%, respectively, in 36 specimens from 36 infants <28 days of age (binomial test, \( P = .001 \)). Among 81 specimens obtained after age 14 days, 79 (98%) were positive by HIV RNA testing. No HIV-infected infant specimens were DNA-PCR–positive and HIV RNA–negative. All specimens from 8 uninfected infants were HIV RNA–negative. These results suggest that plasma HIV RNA was detectable earlier and more reliably than HIV DNA in perinatal infection.

In the United States, nearly all new human immunodeficiency virus (HIV) infections in children are acquired through perinatal transmission. The accurate and timely detection of HIV in early infancy has become increasingly important because strategies for the medical management of children born to HIV-infected mothers [1] (prevention of opportunistic infections [2], treatment strategies, and inclusion in clinical trials) rely on knowledge of HIV infection status. However, because maternal antibodies are passively transferred to the infant, HIV antibody testing cannot be used to diagnose infection until the second year of life [3]. Efforts to overcome this problem have included evaluation of new bands on serial Western blots [4], monitoring of antibody titer to detect increases indicative of infant infection [5], and evaluation of infant-specific (IgM and IgA) antibodies to HIV [4, 6]. Other efforts have focused on testing for virus (culture) [7], viral antigen (p24) [8], or proviral DNA using polymerase chain reaction (DNA-PCR) [9]. Culture and DNA-PCR testing are highly sensitive [7, 9] and specific [7, 10, 11] in infants by age 3–6 months and possibly earlier (1–2 months) [10].

Because extracellular HIV RNA increases to high levels soon after infection [12], the sensitivity of tests detecting HIV RNA may be adequate for early diagnosis of perinatal infection. We used diagnostic DNA-PCR to detect infant infection and...
evaluated plasma HIV RNA in samples obtained early in life from HIV-infected infants to determine relative sensitivities (compared with DNA-PCR) for detection of HIV infection. We also tested specimens from a small sample of perinatally exposed infants who seroreverted to look for early HIV RNA replication in children who were ultimately found to be uninfected.

Materials and Methods

Study population. Plasma viral RNA testing (see below) for early detection of HIV infection was analyzed among infants born to HIV-infected women who were enrolled from April 1990 to August 1994 in the New York City (NYC) Perinatal HIV Transmission Collaborative Study, a longitudinal study of perinatal HIV transmission and pediatric HIV-related disease progression at seven NYC health care institutions. Enrollment criteria and protocol design for this study have been described [13]. Women were enrolled before or within 2 weeks after giving birth. Both the HIV-infected women and their infants were examined at regular intervals. For the infants, a medical history, physical examination, and phlebotomy were done at birth and during scheduled follow-up visits.

Sample inclusion criteria. A previous study [14] identified all HIV-infected women in this NYC cohort whose infants were perinatally infected and who had available maternal plasma specimens near the time of delivery. For the current investigation, we identified all repository plasma samples obtained from these infants from birth to their first birthday. For this analysis, a child was considered HIV-infected if any of the following conditions were met: PCR was positive for HIV proviral DNA on specimens obtained at ≥2 visits; the child met Centers for Disease Control and Prevention (CDC) criteria for P2 classification and had either an AIDS-defining illness or ≥1 positive PCR assay; or the child tested HIV antibody–positive after age 15 months. All plasma specimens obtained from infants at the same time as specimens that had been tested for HIV by DNA-PCR were tested for quantitative plasma HIV RNA (see below).

In addition, specimens obtained from 8 seroreverting infants (EIA-negative for HIV on ≥2 occasions) born to HIV-infected mothers were examined by HIV RNA quantitation and DNA-PCR. Two of these seroreverting infants had a single positive or indeterminate DNA-PCR result in the first 3 months of life followed by consistently negative DNA-PCR results in subsequent specimens. For each infant, initial and subsequent specimens were HLA-concordant (i.e., there was no evidence of specimen mix-up). The other 6 infants were born to HIV-infected mothers who were considered at high risk for transmitting HIV to their infants because they had HIV RNA >10,000 viral copies/mL plasma (median, 32,000) and had AIDS or HIV-associated symptoms and low CD4 T lymphocyte counts near the time of delivery (range, 29–252 cells/mL). Sample collection. Heparinized blood samples from infants were collected and shipped at ambient temperature by overnight mail to the CDC for separation of plasma and peripheral blood mononuclear cells (PBMC) with ficoll-hypaque. The specimens were stored at −70°C until DNA-PCR testing, typically within 1 week of specimen collection. Total time from phlebotomy to freezing was ~24–30 h for each specimen. Plasma samples were stored frozen in a separate CDC facility.

Laboratory testing. Routine laboratory testing of infant blood samples included PCR of the earliest available infant PBMC specimens to detect the presence of HIV proviral DNA as previously described [11]. PBMC were processed, and DNA was extracted by the detergent–proteinase K procedure as described [9]. Diagnostic DNA PCR was done using two primer pairs (SK38/SK39 and SK145/SK150) at 96°C for 1 min, 55°C for 30 s, and 60°C for 1.5 min for 35 cycles. Thermocycling was done in a PCR system (GeneAmp 9600; Perkin-Elmer Cetus, Branchburg, NJ) without an oil overlay. HIV DNA was detected using a chemiluminescent DNA probe and detection system (Accusearch; Gen-Probe, San Diego) with HIV-1 gag-1/gag-2 and gag-3/gag-4 probes.

Assay for plasma HIV RNA. Plasma HIV RNA was measured at the CDC using a recently developed isothermal nucleic acid amplification assay (NASBA HIV RNA QT kit; Organon-Teknika, Durham, NC) adapted for use with HIV [15]. The assay consists of three stages: isolation of nucleic acids from patient specimens and the addition of three internal RNA standards, then amplification of all RNAs with an enzyme solution containing avian myeloblastosis virus–reverse transcriptase, RNase H, and T-7 polymerase, followed by detection using the NASBA QR system electrochemiluminescence principle. The NASBA QT system calculates the amount of RNA in the original sample based on data generated from native RNA and the three synthetic internal viral calibrators. The assay threshold of sensitivity is 100 HIV RNA copies per input volume or 1000 HIV RNA copies for this analysis. The assay was done blinded to all other test results according to the manufacturer’s specifications.

To verify that plasma specimens with discordant HIV DNA-PCR results came from the same subject study, HLA DQ PCR amplification and typing using the AmpliType assay (Perkin-Elmer) were done on DNA from successive infant samples.

Statistical analysis. For analysis of test sensitivity, DNA-PCR–positive and “not positive” (negative and indeterminate) results were compared with HIV RNA results above (positive) and below (negative) the limit of detection for the test (1000 copies/mL plasma). Log_{10} transformations of RNA copy number were calculated because of the nonnormal distribution of virus load values. Test measures were compared and evaluated for differences in means (Student’s t test) or proportions (binomial test). All probability values are two-tailed; the threshold of statistical significance was set at P = .05.

Results

From 49 HIV-infected infants, 108 same-draw samples of PBMC for DNA-PCR and plasma for quantitative HIV RNA testing were obtained (range, 1–4 samples/infant). Infant age at sampling ranged from birth to 318 days. Sixteen infants received antiretroviral therapy during their first year of life, but only 2 received an antiretroviral drug before plasma specimens were collected (described below); all specimens (n = 4) from the 2 infants were positive by both HIV RNA and DNA-PCR testing.
Table 1. Viral DNA polymerase chain reaction (PCR) and quantitative HIV RNA measurements by age in HIV-infected infants.

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>No. tested</th>
<th>DNA-PCR-positive (%)</th>
<th>HIV RNA-positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19</td>
<td>3 (16)</td>
<td>6 (32)</td>
</tr>
<tr>
<td>2</td>
<td>8*</td>
<td>3 (38)</td>
<td>5 (63)</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>1 (50)</td>
<td>2 (100)</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>5 (71)</td>
<td>7 (100)</td>
</tr>
<tr>
<td>5</td>
<td>8*</td>
<td>6 (75)</td>
<td>7 (88)</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>7 (88)</td>
<td>8 (100)</td>
</tr>
<tr>
<td>7–8</td>
<td>4</td>
<td>4 (100)</td>
<td>4 (100)</td>
</tr>
<tr>
<td>9–12</td>
<td>16*</td>
<td>11 (69)</td>
<td>15 (94)</td>
</tr>
<tr>
<td>13–16</td>
<td>9</td>
<td>8 (89)</td>
<td>9 (100)</td>
</tr>
<tr>
<td>17–20</td>
<td>16</td>
<td>16 (100)</td>
<td>16 (100)</td>
</tr>
<tr>
<td>≥21</td>
<td>11</td>
<td>10 (91)</td>
<td>11 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>108</td>
<td>74 (69)</td>
<td>90 (83)</td>
</tr>
</tbody>
</table>

* Includes specimens (1 each obtained during weeks 2 and 5 and month 3 of life) from patient A, who was DNA-PCR-negative and viral RNA-negative in all tests during first 5 months of life but DNA-PCR-positive after age 9 months; infant remained HIV antibody-positive in year 2 (infant was not breast-fed and was in foster care after first weeks of life).

Same-sample comparisons by age for HIV RNA testing and DNA-PCR testing are shown in table 1. Although not statistically significant within any individual age group in table 1, the proportion of infant specimens that were positive by HIV RNA testing was consistently the same or greater than the proportion that were positive by DNA-PCR testing in all age groups. Among 36 samples from 36 infants <28 days old, test results were significantly more likely to be positive using HIV RNA testing compared with DNA-PCR (20 [56%] vs. 12 [33%]; binomial test, \( P = .008 \)). Among 81 specimens obtained after age 14 days, 79 (98%) were positive by HIV RNA testing. Two specimens collected in the fifth week and third month of life from 1 infected infant (see footnote, table 1) were HIV RNA- and DNA-PCR-negative. No specimens from HIV-infected infants were DNA-PCR-positive and HIV RNA-negative.

Among samples collected within the first 8 weeks of life, the \( \log_{10} \) mean HIV RNA copy number was lower for 10 specimens that were positive for HIV RNA but negative by DNA-PCR testing compared with 29 dually positive specimens: 0.36 \( \times 10^8 \) vs. 1.07 \( \times 10^8 \) copies/mL, respectively, but this difference was not significant (t test, \( P = .06 \)).

Figure 1 shows the distribution of HIV RNA test results and \( \log_{10} \) mean HIV RNA copy number among the positive specimens by infant age. Mean \( \log_{10} \) HIV RNA levels were highest for specimens obtained between ages 3 and 12 weeks, but levels generally remained high (\( \log_{10} \) mean >500,000 copies/mL) between 13 and 45 weeks of age. Among samples with detectable HIV RNA, all quantitative results were >1 log above the sensitivity threshold of the test; 76 (84%) of 90 were >2 logs above the sensitivity threshold.

Among the 6 seroreverting infants born to mothers who were thought to be at high risk of transmitting HIV perinatally, HIV RNA in plasma collected at a mean age of 38 days (range, 29–61) was below the limit of detection for all infants. Two specimens with the same HLA type but DNA-PCR discordant results (1 DNA-PCR-negative followed by 1 DNA-PCR-positive or indeterminate specimen collected within the first 4 months of life) from each of 2 seroreverting infants were all negative by HIV RNA testing.

Discussion

In this preliminary study of perinatally HIV-infected infants, quantitative plasma HIV RNA testing (NASBA method) was consistently more sensitive for early detection of HIV infection than were other diagnostic tests. Except for 1 infant, all HIV-infected infants tested positive for HIV RNA after day 14 of life.

HIV RNA copy number in these infants was high and stayed high for prolonged periods. This was consistent with previous findings [12] and suggests that HIV RNA may be elevated in HIV-infected infants for most of the first year of life well above the threshold of detection (range, 200–1000 viral RNA copies/mL, depending on the manufacturer) of commercially available HIV RNA assays.

None of the 6 uninfected infants born to HIV-infected women who were thought to be at high risk for transmitting perinatally had detectable HIV RNA. In addition, discordant DNA-PCR-positive samples from 2 seroreverting infants tested negative for HIV RNA. These findings are compatible with a high specificity for HIV RNA testing in early infancy but need to be confirmed with studies of substantially more infants.

All specimens were tested without knowledge of prior test results by the CDC HIV Molecular Biology Laboratory. Because all available specimens were selected for testing from a predefined cohort, we do not believe that patient or plasma specimen selection biased our results. However, this investigation had several limitations. The limited number of specimens from early infancy necessitated a cross-sectional evaluation of samples at each time interval. In addition, the small number of uninfected children tested in this study limited our ability to evaluate the specificity of the HIV RNA assay.

This analysis suggests that HIV RNA is present in plasma very early in perinatally infected infants. If similar findings of high sensitivity and high specificity occur in future studies, the increased sensitivity of HIV RNA assays compared with HIV DNA-PCR assays during the first year of life may enable determination of HIV infection status of perinatally exposed infants earlier than is currently possible and may improve our ability to distinguish the timing of intrauterine versus intrapartum HIV transmission. Earlier detection of perinatal infections will improve care of HIV-exposed children by facilitating earlier antiretroviral or other treatments; earlier exclusion of perinatal HIV infection may shorten the duration of prophylaxis for Pneumocystis carinii pneumonia for uninfected children [2].
Figure 1. Plasma HIV RNA (log copies/mL plasma) by age among perinatally HIV-infected infants, New York City. Each dot represents 1 measurement: O = DNA−polymerase chain reaction−negative (DNA-PCR), • = DNA-PCR−positive specimens. * Log₁₀ mean plasma HIV RNA copies/mL among values above limit of detection. # Specimens (1 from week 5 and 1 from month 3) from 1 infant who was DNA-PCR− and viral RNA−negative in all tests during first 6 months of life, DNA-PCR−positive after age 9 months, and HIV antibody−positive in year 2.

For parents, guardians, other care givers, and social service providers, such early diagnosis will also facilitate the resolution of other family and social factors affected by the child’s infection status.

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References