Diagnosis of Perinatal Human Immunodeficiency Virus Infection by Polymerase Chain Reaction and p24 Antigen Detection after Immune Complex Dissociation in an Urban Community Hospital

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Results of polymerase chain reaction (PCR) and p24 antigen detection after immune complex dissociation (p24-ICD) were compared with antibody results after 18 months of age for human immunodeficiency virus (HIV) diagnosis in 345 prospectively followed, perinatally exposed infants. Of 59 infected and 286 uninfected infants tested at 1–6 months of age, sensitivity and specificity were, respectively, 100% and >97% for PCR and 90% and >97% for p24-ICD. Testing was done on ≥2 occasions in the first 6 months of life in 43 infected infants; 77% had ≥2 positive results with the same test. Of these infants, 68% had 2 positive p24-ICD tests. In uninfected infants, 96% had only negative tests; none had >1 positive. By 6 months, all uninfected infants with ≥2 PCR results could have been diagnosed. HIV status can be determined by PCR by age 6 months in most HIV-exposed infants. p24-ICD should not be used alone, because of its lower sensitivity, but may be useful in areas without advanced laboratory support.

The use of standard antibody assays for diagnosis of perinatally acquired human immunodeficiency virus (HIV) infection in infants is complicated by transplacentally acquired maternal IgG antibody [1]. To overcome this limitation, a variety of alternative approaches, both virologic (culture [2–6], p24 antigen detection [2, 3], and polymerase chain reaction [PCR] [2, 3, 5]) and immunologic (IgA antibody detection [5, 7, 8], enzyme-linked immunospot [9], and in vitro antibody production [10]), have been used, and high degrees of sensitivity and specificity have been observed with HIV culture and PCR [3, 5, 6]. Though standard p24 antigen detection techniques have shown relatively low sensitivity [3, 11], a modification in which the antigen-antibody immune complex is dissociated before testing (p24-ICD) has shown a dramatic increase in sensitivity when used in neonates [12]. We present here a series in which p24-ICD was used in combination with PCR for diagnosis of infection in a prospectively followed cohort of HIV-exposed infants.

Methods

The study population consisted of all infants born to women known to be HIV-seropositive at Grady Memorial Hospital (GMH) in Atlanta since July 1987 whose mother or other guardian consented to testing. In 1987, the Department of Gynecology and Obstetrics at GMH began to offer HIV counseling and testing to all pregnant women receiving prenatal care and to all women of unknown HIV status presenting in labor. Nearly 95% of women have consented to testing [13, 14]. Since initiation of the obstetric testing program, 416 infants born (dates of birth, 1 July 1987 to 15 March 1994) to these HIV-seropositive women have been followed in a special pediatric infectious disease clinic, according to the schedule for routine child care recommended by the American Academy of Pediatrics. At these visits (at 2 weeks and 2, 4, 6, 9, 12, 15, and 18 months), histories are taken and physical examination (with emphasis on HIV manifestations), routine immunizations, and HIV diagnostic testing are done. For the present study, only infants born before 15 March 1994 (i.e., ≥18 months of age at the time of analysis) were considered.

For this analysis, only infants meeting the following definitions were considered: A child was considered infected if a specimen drawn at ≥18 months of age tested positive for HIV antibody and uninfected if ≥2 specimens after 6 months of age tested negative for HIV antibody [15]. The performance of PCR and p24-ICD, alone and in combination, was evaluated with respect to these definitions.

Schedule of testing. The tests used for HIV diagnostic testing have varied over time, according to their availability. Specimens collected from 1987 to 1990 were tested retrospectively at the Centers for Disease Control and Prevention by PCR for HIV proviral sequences. During this period, we attempted to obtain at least 2 specimens per infant during the first year of life. Peripheral blood mononuclear cells (PBMC) and serum or plasma from these specimens were frozen and stored. After 1990, the schedule of specimen collection was routinized, to include testing by both PCR and p24-ICD at 0–2 weeks and 2, 4, 6, and 15 months of age. In 1992, the number of PCR tests was reduced to include the neonatal test and 2 additional PCR tests obtained at 2–6 months of age. No further PCR assays were performed if these tests were negative;
any positive test was verified with testing on a new specimen. p24-ICD was performed on specimens obtained at every visit in the first year of life, unless 2 positive tests had already been obtained.

p24-ICD. This assay procedure consisted of pretreatment of plasma specimens by acid hydrolysis and detection of free p24 antigen in the pH-neutralized product. Plasma (70 µL) was mixed with an equal volume of 1 M glycine buffer (pH 1.85) and 21 µL of 5% Triton X-100 solution in clustered minitubes. The tubes were incubated in a water bath at 37°C for 1 h. The samples were then restored to neutral pH by the addition of 70 µL of 1 M TRIS buffer (pH 9.0). Each treated sample (200 µL) was then tested for p24 antigen using an HIV p24 antigen–capture ELISA kit (Coulter, Hialeah, FL). Samples were incubated overnight on antibody-coated plates at 37°C in a moist chamber; then the wells were washed and probed for p24 antigen following the manufacturer’s instructions. The optical density (OD) of the reaction product was read at 450 nm. The cutoff value for a positive reaction was the sum of a predetermined factor that equals 0.10 for neonatal specimens (<1 month of age) or 0.055 for all other specimens and the mean OD of the 3 negative controls.

PCR. PBMC from patients were split into 2 aliquots and washed with PBS. The DNA was extracted by the detergent–proteinase K procedure. Diagnostic DNA PCR was performed using two gag region primer pairs, SK38/SK39 and SK145/SK150, as described [16]. Detection for HIV DNA was performed using the Accusearch Chemiluminescent DNA Probe and Detection System (Gen-Probe, San Diego) with HIV-1 gag-1/gag-2 and gag-3/gag-4 probes.

Statistical analysis. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were determined. McNemar’s test was used to compare the results of PCR and p24-ICD for specimens for which both tests were performed [17].

Results

Data on 59 HIV-infected and 286 uninfected infants were available for analysis. Overall, among infected infants, p24-ICD was positive at some time in specimens from 53 (90%) of 59 infants, and PCR was positive in 54 (96%) of 56 infants tested. Among all specimens from these 59 children, 120 (69%) of 175 tested positive for p24-ICD and 89 (90%) of 99 tested positive by PCR. Sensitivity, specificity, PPV, and NPV of the individual tests at various ages are shown in table 1 (p24-ICD) and table 2 (PCR).

PCR or p24-ICD testing was performed on ≥2 occasions in the first 6 months of life in 43 infected infants (table 3), 33 (77%) of whom had ≥2 positive tests on separate specimens by that time (median age at second positive test, 62 days). The second positive test was done by the ages of 2 and 4 months in 58% and 73% of children, respectively. If the diagnosis of HIV infection were allowed with only 2 positive tests on a single specimen, a diagnosis could have been established in 36 (84%) of 43 infants tested on ≥2 occasions by 6 months of age (mean age, 67 days).

Considering only infected infants with ≥2 specimens obtained between 1 and 6 months of age (i.e., omitting tests done during the first month, when sensitivity is lower), the percentages with either p24-ICD or PCR positive on ≥2 occasions were 84% and 88% at 4 and 6 months of age, respectively. The positive predictive value of 2 positive p24-ICD results in these infants was 100% for both p24-ICD and PCR, considering tests drawn at 1–6 months of age.

The proportion of infected children with ≥2 positive p24-ICD tests among the 40 children tested ≥2 times in the first 6 months of life never exceeds 63% (table 3), even if samples from the first 6 months of life are excluded from consideration (data not shown). On the other hand, among 27 infected children tested ≥2 times by PCR, 24 (89%) had ≥2 positive tests. Testing was done on ≥2 occasions in the first 6 months in 195 uninfected infants. By 6 months of age, all uninfected infants who had ≥2 tests had at least 2 negative tests (median age at second negative test, 159 days), 187 (96%) of whom had only negative tests. Eight infants (4%) had 1 false-positive test each: 6 were p24-ICD and 2 were PCR. No uninfected children tested positive ≥1 time.

Overall, among 285 uninfected infants tested at least once, positive tests occurred once each in 13 infants (10 p24-ICD, 3 PCR). These false-positive tests occurred between the ages of 0 and 187 days for p24-ICD (median, 11) and 10 and 116 days for PCR (median, 102).

Table 4 categorizes specimens that were tested by both p24-ICD and PCR by the pattern of their results. Among discordant specimens from infected children, PCR was more likely to be positive than p24-ICD ($P = .006$).

Discussion

In a population of >300 HIV-exposed infants prospectively followed from birth, we found >97% specificity and, after the first week of life, 88%–100% sensitivity for PCR and 97%–100% specificity and 65%–85% sensitivity for p24-ICD. Although our assay is being used in studies in Thailand and Ivory Coast, where subtypes E and A predominate, respectively, our study can only address its use in diagnosing subtype B infection, the only subtype identified in our population [18].

By considering both tests in combination, different approaches to analysis are possible. At least 1 test was positive by 6 months in 88% of infected infants; a single positive test was found in 4.5% of uninfected infants. Alternatively, among infants with ≥2 specimens obtained by 6 months, if the criterion for infection is that either 1 specimen be positive for both tests or that 2 tests be positive on separate specimens, 84% were diagnosable; 6 infected infants (14%) had only a single test positive in this group.

Importantly, among uninfected infants, the few with a positive test all had that test soon followed by 2 subsequent specimens for which all tests were negative. If it is required that tests be positive on 2 separate specimens, 77% of infected infants were diagnosable by 6 months. Given the sensitivities of both tests in this study, that only this percentage of infected infants could be diagnosed by 6 months is somewhat surprising.
### Table 1. p24-ICD in HIV-exposed infants: negative predictive value (NPV), positive predictive value (PPV), sensitivity, and specificity by age at time of test.

<table>
<thead>
<tr>
<th>No. tested</th>
<th>Time of test*</th>
<th>Infected</th>
<th>Uninfected</th>
<th>NPV</th>
<th>PPV</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 week</td>
<td>31</td>
<td>74</td>
<td>77.5</td>
<td>68.8</td>
<td>35</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>1 month</td>
<td>17</td>
<td>85</td>
<td>93.3</td>
<td>84.6</td>
<td>64.7</td>
<td>97.6</td>
</tr>
<tr>
<td></td>
<td>2 months</td>
<td>42</td>
<td>127</td>
<td>93.3</td>
<td>97.1</td>
<td>78.6</td>
<td>99.2</td>
</tr>
<tr>
<td></td>
<td>4 months</td>
<td>20</td>
<td>83</td>
<td>91.2</td>
<td>100</td>
<td>65</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>21</td>
<td>115</td>
<td>97.5</td>
<td>100</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>9 months</td>
<td>14</td>
<td>74</td>
<td>97.4</td>
<td>100</td>
<td>93.3</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>9</td>
<td>63</td>
<td>96.9</td>
<td>87.5</td>
<td>80</td>
<td>98.4</td>
</tr>
</tbody>
</table>

*1 week = 0–7 days; 1 month = 8–30 days; 2 months = 31–90 days; 4 months = 91–150 days; 6 months = 151–231 days; 9 months = 232–320 days; 12 months = 321–411 days.

### Table 2. PCR in HIV-exposed infants: negative predictive value (NPV), positive predictive value (PPV), sensitivity, and specificity by age at time of test.

<table>
<thead>
<tr>
<th>No. tested</th>
<th>Time of test*</th>
<th>Infected</th>
<th>Uninfected</th>
<th>NPV</th>
<th>PPV</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 week</td>
<td>7</td>
<td>51</td>
<td>94.4</td>
<td>100</td>
<td>57</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1 month</td>
<td>13</td>
<td>72</td>
<td>100</td>
<td>92.9</td>
<td>100</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>2 months</td>
<td>31</td>
<td>123</td>
<td>97.6</td>
<td>100</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>4 months</td>
<td>16</td>
<td>70</td>
<td>97.1</td>
<td>87.5</td>
<td>88</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>10</td>
<td>89</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>9 months</td>
<td>10</td>
<td>65</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>2</td>
<td>28</td>
<td>96.6</td>
<td>100</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

*1 week = 0–7 days; 1 month = 8–30 days; 2 months = 31–90 days; 4 months = 91–150 days; 6 months = 230–315 days; 9 months = 232–320 days; 12 months = 321–411 days.

This finding can be explained at least partly by the fact that not all infants had testing performed at the indicated time points. It is expected that, if samples are obtained frequently enough and early enough, an even higher proportion of infants should be diagnosable at an earlier age, emphasizing the necessity of ensuring adequate follow-up in these infants. Furthermore, it is recognized that the testing schedule was changed twice over the course of the study.

It is reassuring that, among uninfected infants tested at least twice in the first 6 months, all could have their uninfected status determined by that time. Repeatedly positive diagnostic tests in infants eventually found to be uninfected, a phenomenon that has been noted by others and that has been suggested as evidence for clearance of infection [19–21], were not observed in our cohort. Overall, our results in uninfected infants support the present approach to prophylaxis of *Pneumocystis*

### Table 3. Number of infected children testing positive at least twice by p24-ICD, PCR, or either test among children who were tested on ≥2 occasions between 0 and 6 months of age.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>p24-ICD alone</th>
<th>PCR alone</th>
<th>Either test</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>15/29 (52)</td>
<td>8/12 (67)</td>
<td>18/31 (58)</td>
</tr>
<tr>
<td>4</td>
<td>23/36 (61)</td>
<td>19/22 (86)</td>
<td>29/40 (73)</td>
</tr>
<tr>
<td>6</td>
<td>25/40 (63)</td>
<td>24/27 (89)</td>
<td>33/43 (77)</td>
</tr>
</tbody>
</table>

**Note:** Data are no. positive/no. tested (%).

### Table 4. Pattern of test results among specimens tested for both PCR and p24-ICD by infection status of children.

<table>
<thead>
<tr>
<th>Specimens</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>True positive by both assays</td>
<td>56</td>
<td>70</td>
</tr>
<tr>
<td>True positive by PCR only</td>
<td>18</td>
<td>22.5</td>
</tr>
<tr>
<td>True positive by p24-ICD only</td>
<td>4</td>
<td>5.0</td>
</tr>
<tr>
<td>False negative by both assays</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>Uninfected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>True negative by both assays</td>
<td>357</td>
<td>98.1</td>
</tr>
<tr>
<td>True negative by PCR only</td>
<td>5</td>
<td>1.4</td>
</tr>
<tr>
<td>True negative by p24-ICD only</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>False positive by both assays</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
carinii pneumonia, in which prophylaxis is begun in all HIV-exposed infants by 2 months and discontinued when adequate documentation of uninfected status is obtained, since it appears that uninfected status can be established at a very young age.

The high sensitivity and specificity of PCR observed in our study are consistent with results obtained in other large series [3, 5, 11], all of which showed sensitivities >90% between ages 1 and 6 months. As in those series, the sensitivity in neonates is much less (31%–57%), a finding that has been attributed to the possibility that a large proportion of transmissions may occur late in gestation or intrapartum [22]. Our results with p24-ICD differ from those of Miles et al. [12], in which 5 of 8 cord-blood specimens were positive by the assay. In our study, only 35% of 31 infected infants tested positive by p24-ICD using venous blood from the first week. Our results are similar to those presented by Paul et al. [4] in that they showed an increasing sensitivity (64%–90%) and high specificity (97%–100%) from 1 to 6 months of age.

Used alone, p24-ICD was neither as sensitive nor as specific as PCR, and performance of p24-ICD along with PCR did not add sensitivity or specificity to the diagnostic process. Therefore, p24 should not be used as the single diagnostic test in HIV-exposed infants if more sensitive tests, such as PCR, are available. On the other hand, repeated p24-ICD testing can accurately diagnose at least two-thirds of HIV-exposed infants, and it may thus be useful because of its relative technical simplicity and lower cost in areas where advanced laboratory support is unavailable. The test can also be used as an adjunct test if problematic results are found with other tests.

Overall, our data support using PCR alone for diagnosis of HIV-exposed infants. Samples for testing should be obtained on ≥2 occasions between ages 1 and 6 months; any single positive test should be followed by testing on a new specimen. Infants who have 2 specimens positive by PCR can be considered HIV-infected. Infants who have 2 specimens negative by PCR (and no positive specimens) can be considered HIV-uninfected. If test results are discordant, further testing using the same or different test method (e.g., p24-ICD, culture) is recommended. It is prudent to make diagnoses of uninfected status be contingent upon seroconversion to a negative HIV antibody test. Such a testing strategy is only valid in situations without postpartum exposure to HIV, such as breastfeeding.

References