Rational Testing of the HIV-Exposed Infant

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ABSTRACT. Objectives. The objectives of this study were 1) to evaluate testing regimens of human immunodeficiency virus (HIV)-exposed infants and 2) to determine optimal methods of follow-up by enzyme-linked immunosorbent assay (ELISA) testing.

Methods. We reviewed the results from 742 HIV-exposed infants in the state of North Carolina; 2474 samples were tested for HIV by DNA polymerase chain reaction (PCR) at the University of North Carolina Retrovirology Core Laboratory. We then reviewed the utility and costs of ELISA testing of all HIV-exposed infants who were seen at the Duke University Pediatric Infectious Disease Clinic between January 1, 1993, and May 5, 1998. We used likelihood ratios to model probability of HIV infection given 3 negative DNA (PCR) tests and to provide recommendations on the use of ELISA follow-up.

Results. The overall sensitivity of the DNA PCR was 87.1%, and its specificity was 99.9%. We evaluated 224 HIV-exposed infants who were seen at Duke University and who had at least 3 negative diagnostic tests using either DNA PCR tests or HIV blood cultures. All 178 infants who subsequently underwent ELISA testing ultimately demonstrated seroreversion. The Duke University Pediatric Infectious Disease Clinic transferred the care of 65 patients to primary care physicians before ELISA testing and retained the care of the remaining 159 patients. Children who remained in Duke’s care were more likely to have documentation of seroreversion (158 of 159 vs 20 of 65). We reviewed costs of travel, physician appointment, and HIV antibody testing in a tertiary care setting. Given 3 negative PCR tests, the expected cost per case of HIV detected by a positive ELISA assay is $23.8 million.

Conclusions. Documentation of seroreversion in this cohort was nearly complete in the multidisciplinary subspecialty clinic but not when such responsibility was left to the primary care physician. Given the low probability of disease in patients who have had 3 negative PCR tests, documentation of a negative ELISA may not be an appropriate use of medical resources. Pediatrics 2001;108(1). URL: http://www.pediatrics.org/cgi/content/full/108/1/e3; pretest probability, posttest, likelihood ratio, cost analysis, HIV.

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ABBREVIATIONS. HIV, human immunodeficiency virus; PCR, polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; PCP, primary care physician; LR, likelihood ratio.

Mother-to-child transmission is the leading cause of human immunodeficiency virus (HIV) infection in childhood. If the mother is given optimal antiretroviral therapy during pregnancy, the transmission rate is now estimated to be 2.5% or even less (PACTG Protocol 247 Study team, personal communication, January 2001). Uninfected infants who are exposed to HIV in utero have transiently acquired HIV antibody that may be detected for as long as 15 months after birth. Early diagnosis, therefore, relies on the use of other diagnostic modalities: HIV blood culture and HIV-specific polymerase chain reaction (PCR).

The sensitivity and the specificity of an HIV DNA PCR at birth have been estimated to be 50% and 99%, respectively. The sensitivity of the test improves dramatically in the first weeks of life. PCR used in infants who are older than 30 days has a sensitivity of 90% or better.

The American Academy of Pediatrics recommends that physicians document 3 negative PCR by DNA followed by serologic reversion by enzyme-linked immunosorbent assay (ELISA) of children who are exposed to HIV in utero. It is unknown whether such follow-up to seroreversion is completed best by the infectious diseases specialist or by the primary care physician. The cost of diagnosing HIV with the use of serologic follow-up given the high quality of PCR also is unknown. Our hypothesis is that documentation of seroreversion is not warranted.

Using simple mathematical modeling, we attempted to determine 1) the probability of HIV infection in children with 3 negative PCR tests, 2) the utility of the ELISA and Western blot in children with 3 negative PCR tests, 3) the costs associated with tertiary care documentation of seroreversion, and 4) whether documentation of seroreversion is a worthwhile use of medical resources. We also sought to determine in our own patient population whether documentation of the ELISA is likely to occur if it is the responsibility of the general pediatrician.

METHODS

University of North Carolina Retrovirology Core Laboratory and DNA PCR Test Performance

The laboratory uses the Roche Amplicor DNA PCR techniques described previously and accepts samples from throughout the state of North Carolina. We reviewed at the laboratory the records
of all samples tested between 1993 and 1999 and determined the sensitivity and specificity as follows: Children were considered infected if either HIV DNA PCR or HIV culture was positive in at least 2 samples of blood. Children who had at least 1 positive test and clinical evidence of HIV infection also were considered to be infected. Children were considered not to be infected if they 1) had only negative results at 3 or more separate time points—with at least 1 time point at >2 months of age; and 2) did not have clinical evidence of HIV. Children whose test was positive at the laboratory and was followed by 3 subsequent negative tests and documented serologic reversion constituted false-positive tests. Children whose test was negative at the laboratory and was followed by 2 positive tests (by DNA PCR or HIV culture) constituted false-negative results. If children had only 1 blood sample or if all blood samples were obtained at <2 months of age, then they were considered to be indeterminate (and excluded from analysis) provided that there was no clinical evidence of HIV infection.

**Duke University Clinic Population**

We evaluated infants who were exposed to HIV in utero and who were seen at the Duke University Pediatric Infectious Disease Clinic between January 1, 1993, and May 5, 1998. HIV-exposed infants were included in the ELISA follow-up component of the study if they had been determined to be HIV negative at Duke University by 12 months of age. We required negative results on a combination of at least 3 PCR tests and/or HIV blood cultures—at least 2 tests had to have been completed after day of life 30, and at least 1 test had to have been completed after 4 months of age—to determine that an HIV-exposed infant was HIV negative. We defined false-positive ELISA results as a positive ELISA followed by a negative or indeterminate Western blot.

Routine clinical care of a child who is discharged from the Duke clinic consists of documentation of complete evaluation and identification of a primary care physician (PCP). The clinic policy is that the family has to identify the PCP as the child’s doctor and has made at least 2 visits to the PCP. When care is transferred to the PCP, the clinic requests serologic follow-up and written documentation of serologic reversion.

**Data Collection**

We reviewed the charts of all HIV-exposed children. We documented DNA PCR test results, HIV blood culture test results, seroreversion documentation, PCR follow-up, and whether the Pediatric Infectious Disease clinic mailed to the PCP a letter requesting evidence of seroreversion.

For the cost analysis, we determined the rate of reimbursement dispensed by Medicaid to each patient’s family for travel to the Duke University Pediatric Infectious Disease Clinic. We also recorded the cost of a routine follow-up visit to the Duke clinic for Medicaid patients, the cost of an ELISA, and the cost of a Western blot test for HIV.

**RESULTS**

A total of 2474 samples were obtained from 742 children and were tested at the University of North Carolina laboratory; 19 of 2472 were classified as indeterminate. The sensitivity of the test rose rapidly from 54.5% for infants who were younger than 48 hours to 75% for infants who were between 48 hours and 1 week, to greater than 95% for infants who were older than 1 month (Table 1). The specificity was consistently greater than 99%.

Because the 2474 samples were obtained during 3 different maternal antiretroviral standard-of-care treatment time frames—no antiretroviral therapy for pregnant women, single-drug therapy, and highly active antiretroviral therapy—we stratified the data across 3 different time frames: 1) 1993 to March 1994, 2) April 1994 to December 1995, and 3) 1996 to 1999.

**Statistics**

Statistical analyses were performed with the use of Stata (Version 6.0; College Station, TX). Comparisons between categorical variables were based on Fisher’s exact test, and all reported P values are 2-tailed. For the evaluation of posttest probabilities of HIV infection, we used likelihood ratios (LRs). An LR is equal to the probability of the observed test result among the diseased divided by the probability of the same test result among the nondiseased (see “Appendix”). For a negative test, the negative LR may be expressed mathematically as (1 − sensitivity) divided by the specificity. So a negative LR with 95% sensitivity and 99% specificity (much like the DNA PCR for an infant older than 30 days) is equal to 1 − 0.95/0.99 = 0.0505.

We evaluated various vertical transmission, or pretest, probabilities of perinatal HIV in our modeling—from 1% to 30%. We based these pretest probabilities on AIDS Clinical Trial Group data. We derived the sensitivity and specificity of the DNA PCR from analyzing the results of samples tested at the Retrovirology Core Laboratory of Dr Susan Fiscus at the University of North Carolina, Chapel Hill. In the analysis and presentation of recommendations, we used the Duke University Pediatric Infectious Disease Clinic’s requirements to diagnose an HIV-exposed infant as HIV negative:

1. At least 3 negative PCR tests
2. At least 2 PCR tests completed after day of life 30
3. At least 1 PCR test completed after 4 months of age
4. No positive PCR tests

**TABLE 1. Sensitivity and Specificity of the DNA PCR**

<table>
<thead>
<tr>
<th>Age and Test Result</th>
<th>Infection Status</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIV</td>
<td>No HIV</td>
<td></td>
</tr>
<tr>
<td>&lt;48 H</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test +</td>
<td>12</td>
<td>0</td>
<td>0.55 (0.32, 0.76)</td>
</tr>
<tr>
<td>Test –</td>
<td>10</td>
<td>371</td>
<td></td>
</tr>
<tr>
<td>48 H–7 d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test +</td>
<td>6</td>
<td>0</td>
<td>0.75 (0.36, 1)</td>
</tr>
<tr>
<td>Test –</td>
<td>2</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>8 D–1 mo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test +</td>
<td>22</td>
<td>1</td>
<td>0.81 (0.62, 0.95)</td>
</tr>
<tr>
<td>Test –</td>
<td>5</td>
<td>304</td>
<td></td>
</tr>
<tr>
<td>1–4 Mo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test +</td>
<td>41</td>
<td>1</td>
<td>0.98 (0.87, 1)</td>
</tr>
<tr>
<td>Test –</td>
<td>1</td>
<td>705</td>
<td></td>
</tr>
<tr>
<td>4–6 Mo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test +</td>
<td>36</td>
<td>0</td>
<td>0.95 (0.82, 0.99)</td>
</tr>
<tr>
<td>Test –</td>
<td>2</td>
<td>576</td>
<td></td>
</tr>
<tr>
<td>&gt;6 Mo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test +</td>
<td>38</td>
<td>0</td>
<td>0.93 (0.80, 0.98)</td>
</tr>
<tr>
<td>Test –</td>
<td>3</td>
<td>215</td>
<td></td>
</tr>
</tbody>
</table>

CI indicates confidence interval.
The sensitivity and the specificity of the time frames did not differ substantively (data not shown).

We modeled the probability of infection for children with 3 negative PCR assays under varying conditions. We varied the vertical transmission rate on the basis of the rates that were observed during the HIV epidemic and the performance characteristics of the PCR assays on the basis of the data that we obtained from testing children at various ages (see Fig 1).

The pretest probability is the probability of disease in a patient before testing. Before any testing, the pretest probability of disease for an infant who was exposed to HIV is the same as the prevalence (vertical transmission rate) of the disease. Given the low vertical transmission rate of infection in the children with perinatal prophylaxis, the probability of HIV infection with a single negative PCR assay is very low—ranging from 0.5% to 2.6% in our simulations. Multiple negative tests are even more reassuring (Fig 1). We considered the vertical transmission rate to a formula-fed infant of <5% to be low risk (this is an easily achievable rate if a mother is on effective antiretroviral therapy). Given the sensitivity and the specificity of the PCR test (Table 1), the probability of HIV infection in a low-risk child is <.001% after 3 negative HIV DNA PCR assays. Even under high-risk conditions, with an HIV vertical transmission rate of 30%, the probability of infection with 3 negative HIV DNA PCR assays is only 0.01% (1/10 000).

The probability that an infant who was exposed to HIV indeed has HIV after 3 negative DNA PCR tests is so low that a positive ELISA and Western blot test will most often represent a false-positive test. Given a sensitivity and a specificity of 99% for the ELISA and Western blot, to make a diagnosis of HIV with greater than 99% certainty in an exposed infant who has had 3 negative PCR tests requires at least 2 consecutive positive ELISA and Western blot tests after 15 months of age.

Of the HIV-exposed infants who had samples sent to the laboratory for PCR testing, 224 had at least 3 negative tests and were followed by the Duke University Pediatric Infectious Disease Clinic. Documentation of seroreversion was the responsibility of the pediatric infectious disease service for 159 children. Sixty-five children were discharged from the Duke clinic into the care of their PCP before the documentation of seroreversion. Children who were under the care of the infectious disease service were more likely to be tested for seroreversion (158 [99%] of 159) than children who were under the care of their PCP (20 [31%] of 65; \( P < .0001 \)). At the time of our study, the whereabouts of 34 (76%) of the 46 untested children were unknown. The ELISA test was positive for 19% of the children tested; all 41 children had negative or indeterminate Western blot tests.

Because documentation of seroreversion was so low in the local practice setting, we tried to evaluate the utility and costs associated with complete serologic follow-up of the low-risk infant. The probability that a child has HIV after 3 consecutive negative PCR tests is 1/100 000 (Fig 1). The cost of diagnosing that 1 child in 100 000 is extreme (Table 2). The cost per child of conducting both an ELISA and a Western blot test is approximately $351.30, including the cost of the office visit, the test procedures, and the transportation for parent and child. Given the posttest probability of 3 negative PCR tests, to diagnose 1 new HIV-infected child, we must expect to perform 1 set of ELISA tests on 100 000 children.

Our results indicate a specificity of 81% in an HIV-exposed infant who 15 to 24 months old, so of those 100 000 children, 19 000 require a Western blot test.

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**Fig 1.** The posttest probability of infection after 3 negative HIV DNA PCR assays across a range of HIV prevalence in infants. We provide 3 different testing regimens, combined with various (1% to 35%) maternal–child transmission rates.
test. The specificity of the Western blot is high, but not 100%. Given a specificity of 99% on a Western blot test, an additional 190 children will need to return for a clinic visit and an ELISA test (Table 2). Hence, the expected cost per confirmed HIV is $23.8 million. We do not include any measure of the value of the time spent by the parent in getting the child to and from the office. Doing so would raise the estimated costs of follow-up testing.

**DISCUSSION**

We attempted to ascertain the best method of evaluating infants who have been exposed to HIV, costs associated with documentation of seroreversion, and whether seroreversion should be documented. If documentation of seroreversion is warranted, then multidisciplinary support in a subspecialty clinic is warranted. Follow-up by the PCP in this study, despite letters of reminder, was less than 31%. More than half of the children who were not tested could not be contacted for testing.

The posttest probability of 3 negative PCR tests is near 0, provided that the child has not had a conflicting positive HIV DNA PCR test and is low-risk—defined as an infant who met the following 4 conditions: 1) mother received effective antiretroviral therapy around delivery, 2) the child received antiretroviral therapy after delivery, 3) the child was not breastfed, and 4) the child does not have symptoms suggestive of HIV disease (Table 3). Therefore, we believe that serologic documentation is not warranted in a low-risk child.

If the child is not low risk, then the vertical transmission rate may be as high as 30%. If the mother received no prenatal care, then the risk of transmission given 3 negative PCR tests may be as high as 1/10 000. In such cases, our conclusion of not documenting seroreversion has less support; but unless the infant is breastfeeding, we believe that (regardless of prenatal care) 3 negative DNA PCR tests are enough data to determine that an HIV-exposed infant is uninfected. This conclusion, however, depends on at least 2 DNA PCR tests conducted after 4 weeks of age and 1 of those PCR tests taking place after the infant is at least 16 weeks of age (Table 4).

Furthermore, if the infant has had 2 negative DNA PCR tests (at least 1 after day of life 30), then we would not administer prophylactic Trimethoprim-Sulfamethoxazole.

If the infant is breastfed, if 1 of the DNA PCR tests is positive, or if there are clinical manifestations of HIV, then documentation of seroreversion is warranted (Table 4). On the basis of our experience with the lack of documentation provided by the PCP, if documentation of seroreversion is warranted, then multidisciplinary care (social workers, research nurses) in a subspecialty environment should remain involved until seroreversion is verified.

We recommend that the first DNA PCR test be completed after 48 hours of life but before 30 days of life. This recommendation stems from the lower sensitivity (approximately 50%) of the DNA PCR in the first 48 hours of life. This recommendation is of particular utility if the mother has a high viral load at delivery or has had no prenatal care, because if there has been no prenatal care, then the maternal–infant transmission rate approaches 30% and the decreased sensitivity of the DNA PCR test near birth in such cases results in a relatively higher posttest probability (Fig 1).

There were several limitations to our study. We did not estimate the time and cost to the families to document seroreversion, thereby significantly underestimating the cost of follow-up. Our sample size was relatively small, and we used mathematical modeling rather than following a prospective cohort of 100 000 exposed infants to estimate costs of follow-up (224 children cared for at Duke) and rates of

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**TABLE 2. Costs of Diagnosing 1 Child With HIV Using ELISA and Western Blot Follow-up Given 3 Negative DNA PCR**

<table>
<thead>
<tr>
<th>Costs</th>
<th>Average Cost Per Patient</th>
<th>Number of Patients</th>
<th>Estimated Total Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Patient Visit (n = 100 000)</td>
<td>$70.89</td>
<td>100 000</td>
<td>$7 089 000</td>
</tr>
<tr>
<td>Medicaid reimbursement</td>
<td>$45.41</td>
<td>100 000</td>
<td>$4 541 000</td>
</tr>
<tr>
<td>First ELISA test specificity = 81%</td>
<td>$95.00</td>
<td>100 000</td>
<td>$9 500 000</td>
</tr>
<tr>
<td>First Western blot specificity = 99%</td>
<td>$140.00</td>
<td>19 000</td>
<td>$2 660 000</td>
</tr>
<tr>
<td>Second Patient Visit (n = 190)</td>
<td>$70.89</td>
<td>190</td>
<td>$13 469</td>
</tr>
<tr>
<td>Medicaid reimbursement</td>
<td>$45.41</td>
<td>190</td>
<td>$8 628</td>
</tr>
<tr>
<td>Second ELISA test specificity = 81%</td>
<td>$95.00</td>
<td>36</td>
<td>$5 040</td>
</tr>
<tr>
<td>Total cost of follow up by ELISA and Western blot</td>
<td>$140.00</td>
<td></td>
<td>$5 040</td>
</tr>
<tr>
<td>Children diagnosed with HIV</td>
<td></td>
<td></td>
<td>$23,835 187</td>
</tr>
</tbody>
</table>

**TABLE 3. History, Physical, and Laboratory Evidence Suggestive of Perinatal HIV During the First 6 Months of Life**

- Bacteremia
- Generalized adenopathy
- Growth failure
- Hepatomegaly
- Hypergammaglobulinemia
- Otitis media, ≥3 episodes
- Persistent thrush unresponsive to therapy
- Recurrent diarrhea
- Splenomegaly
diagnostic failure (742 children tested at UNC Retrovirology Core Laboratory). A substantial percentage of our clinic population that was not tested (34%) could not be contacted; their infection status likely is negative but is unknown.

The method of follow-up that we propose will not guarantee 100% diagnostic accuracy but provides greater than 99.99% accuracy. Undoubtedly, there will be several case reports over the ensuing years of children who have had 3 negative PCR tests but in fact have HIV infection. In such cases, evaluation of possible horizontal transmission is indicated.

We report a specificity (81%) that is much lower than the standard for the ELISA test. Although the ELISA for HIV is an outstanding test with high sensitivity and specificity, our specificity is lower because a significant number of infants will continue to have maternal antibodies even after 12 months of age.

Although it would be ideal to ensure that every HIV-infected child is identified, medical funding is not limitless. Documentation of seroreversion in the low-risk infant is not cost-effective if complete PCR testing has been performed. We believe that the American Academy of Pediatrics recommendation of documentation of seroreversion of all HIV-exposed infants should be reconsidered and that infants without significant indication for testing not be subjected to a test with an extremely low yield.

ACKNOWLEDGMENTS

William C. Miller received support through the Clinical Associate Physician Program of the General Clinical Research Center (RR00046), Division of Research Resources, National Institutes of Health.

We thank Dr Robert H. Smith.

APPENDIX

The physician uses the likelihood ratio in a 3-step process: 1. Convert the pretest probability (prevalence or risk of vertical transmission) to pretest odds. Pretest odds  = probability/(1 − probability). 2. Determine the posttest odd by multiplying the pretest odds by the likelihood ratio. Posttest odds  = pretest odd*likelihood ratio. 3. Convert the posttest odds to posttest probability. Posttest probability  = odds/(1 + odds).

The physician may use multiple rounds of testing in step 2 and multiply the pretest odds by multiple likelihood ratios. For example, if we were to test an exposed infant for HIV 3 times with the DNA PCR at 2 weeks and 6 weeks and between 4 and 6 months of age (Table 1), then our 3 negative likelihood ratios would be 0.25, 0.024, and 0.053. If the pretest probability (vertical transmission) of HIV is 1.5%, then our 3 step process is as follows:

1. Pretest odds  = 0.015/1 − 0.015 = 0.015
2. Posttest odds  = 0.015*0.25*0.024*0.053 = 0.000004
3. Posttest probability  = 0.000004/1.000004 = 0.000004, or 0.0004% chance of the infant being infected with HIV (Fig 1).

REFERENCES


TABLE 4. Testing Algorithm for the HIV-Exposed Infant

| First DNA PCR between day of life 3 and 30 | yes → 1) Repeat DNA PCR |
| Second DNA PCR between weeks 4 and 8* | 2) Trimethoprim-Sulfamethoxazole prophylaxis |
| Third DNA PCR after 4 months of age | 3) Document seroreversion between 12 and 18 months old |
| Are any of the DNA PCR positive? | |
| no ↓ | 1) Repeat DNA PCR |
| Is the infant breast-feeding? | 2) Trimethoprim-Sulfamethoxazole prophylaxis |
| yes → 1) Trimethoprim-Sulfamethoxazole prophylaxis | 3) Document seroreversion 3–6 months after breast-feeding stops |
| no ↓ | 2) Document seroreversion between 12 and 18 months old |
| Are there clinical symptoms consistent with HIV? | 3) Document seroreversion between 12 and 18 months old |

* If first 2 PCR are negative, and the infant is not breastfeeding and does not have symptoms of HIV, posttest probability  = 1/10,000, stop AZT at 6 weeks old and do not give Trimethoprim-Sulfa.
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