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Determining an Optimal Testing Strategy for Infants at Risk for Mother-to-child Transmission of HIV-1 During the Late Postnatal Period

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Abstract

Objectives—To determine the optimal time for a second HIV-1 nucleic acid amplification assay to detect late postnatal transmission of HIV-1 (first negative test at 4–8 weeks of age) in resource limited settings.

Design—A longitudinal analysis of data from HPTN 024

Methods—Children born to HIV-1 infected mothers enrolled in the HIV Prevention Trial Network trial 024 (HPTN 024) were tested for HIV-1 infection at six intervals within the first year of life. Mothers and infants received nevirapine prophylaxis. We estimated the probability of being alive and having a positive test in each interval after 4–8 weeks and at 30 days post-weaning, conditional on having acquired HIV during the late postnatal period. The interval with the highest probability was taken to be the optimal visit interval.

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Author contributions

Authors: Elizabeth Brown developed the model and performed all of the statistical analyses and, in collaboration with Susan Fiscus, designed the study and wrote the first drafts and revisions to the manuscript. Taha Taha and Robert Goldenberg were Protocol Chairs of the original HPTN 024 study and edited this manuscript. Jennifer Read and Usha Sharma represent the agencies that funded HPTN 024 (NICHD and NIAID), provided critical reviews of the manuscript during the drafting stages, and approved each submitted version. Benjamin Chi and Robert Goldenberg (Lusaka), Irving Hoffman (Lilongwe), Taha Taha (Blantyre) and Cheryl Pikora (Dar es Salaam) represent each of the HPTN 024 clinical sites, provided critical reviews of the MS during the drafting stages, and approved each submitted version.

Results—A total of 1609 infants from HPTN 024 had at least one HIV-1 diagnostic test and were included in the analysis. We found that testing at one month after weaning or 12 months of age (whichever comes first), identified 81% of those infected during the late postnatal period (after 4–8 weeks) through breastfeeding. In total, 93% (95% CI: 89,98) of all infected infants would be detected if tests were performed at these two time points.

Conclusions—In resource-limited settings, HIV-1 PCR testing at 4–8 weeks followed by a second test at one month after weaning or at one year of age (whichever comes first), led to the identification of the vast majority of HIV-1 infected infants.

Keywords

HIV infant diagnosis; late postnatal transmission; breast feeding

Introduction

HIV-1 serology cannot be used to diagnose perinatal HIV-1 infection among children under 18 months born to HIV-1 infected women due to the presence of maternal, HIV-1 specific antibodies which cross the placenta during gestation. Infants in resource rich settings are tested repeatedly to establish their HIV infection status during the first few weeks and months of life using polymerase chain reaction (PCR)-based assays that detect either proviral HIV DNA or viral RNA in blood. However, in resource-limited settings, repeated PCR testing is usually not possible due to budgetary and infrastructure constraints. Routine PCR testing at 4–8 weeks of age, which detects 95%–99% of *in utero* and peripartum infections (1–3), may be feasible because it coincides with scheduled visits in many national health plans.

However, if an infant is breastfed, the risk of transmission continues. In resource limited settings, clinicians commonly wait until 15–18 months of age, when complete cessation of breastfeeding has frequently occurred, to conduct HIV-1 diagnostic testing using a less expensive and more accessible antibody test. Relying on serological testing at 15–18 months delays life saving initiation of antiretroviral therapy (ART) (4) and the infant may die or become lost to clinical care prior to 15–18 months of age. Early infection has been associated with increased mortality (5–13). Therefore, determination of a single additional HIV testing timepoint for children after 4–8 weeks of age would have great practical value for global HIV/AIDS programs and could provide a cost-effective method for infant HIV-1 diagnosis.

Using data from the HIV Prevention Trials Network (HPTN) 024 trial, we estimated the proportion of HIV-1 infected infants who were still alive (and therefore who could have ART initiated) if they were tested at one of a set of time points. We sought to establish an optimal visit time for a second HIV-1 virologic test after 4–8 weeks of age to detect late postnatal transmission (transmission between 4–8 weeks and one year) in breastfed infants. Our definition of the optimal time for HIV-1 testing after 4–8 weeks was when the majority of infants who were infected in the late postnatal period would be captured. If there were no deaths due to HIV-1 infection, this visit could be when the child had completely weaned. However, because HIV-1 related deaths often occur prior to this (4–13), the optimal visit time we propose represents a window period late enough that most HIV-1 infections have occurred, but early enough that few of these HIV-1 infected infants have died.

Materials and Methods

Patient Population

We analyzed data from subjects enrolled in HPTN 024. This was a multi-site, double-blinded, placebo-controlled randomized trial of antibiotics to prevent chorioamnionitis-associated

mother-to-child transmission (MTCT) of HIV-1 and preterm birth (14). All mothers and infants received single dose nevirapine prophylaxis following the HIVNET 012 protocol (15). Infants underwent HIV-1 diagnostic testing using HIV-1 RNA assays at birth, at 4–8 weeks, and at three, six, nine, and 12 months of age. Of the four sites in HPTN 024, one counseled women to wean at six months (Dar es Salaam, Tanzania), and these data were not used in the analysis. Data from the three other sites in Lilongwe and Blantyre, Malawi, and Lusaka, Zambia, were analyzed. Breast feeding duration at these three sites was similar with 93–97% still breastfeeding at six months, 86–93% at nine months and 77–87% at 12 months with details previously published (16).

Statistical Methods

In the model we define s to be the imprecisely measured time an infant would first test positive for HIV-1 and u to be the time of death. Because s is only known within an interval (e.g. some time between the last negative and the first positive PCR result), we used a multiple imputation procedure specifically designed for MTCT of HIV-1 (17). Briefly, the imputation procedure was implemented 10 times resulting in 10 data sets with imputed values for s when the event time was interval or right censored. The analysis was run on each of the data sets. Rubin's rules (18) for combining results from imputed data analyses were used to obtain the final results.

We estimated the optimal visit time as follows: The variables $t_1, \dots, t_J$ denote the times of the J visits under consideration for HIV-1 testing beyond 4–8 weeks. At the j th, $j = 1, \dots, J$, visit, occurring at t_j , the number of infants who are alive and would test positive is denoted by n_j (the total number of infants with $s \leq t_j$ & $u > t_j$) which follows a binomial distribution with mean $p_j \times m$ where m is the total number of infants who acquired HIV-1 infection through late postnatal transmission and p_j probability of being alive and testing positive at t_j conditional on acquiring HIV-1 infection through late postnatal transmission. An unbiased estimate of p_j is given by n_j/m . We defined the optimal visit time as the visit for which p_j is greatest.

Figure 1 illustrates an example of this procedure using hypothetical data with the same visit structure as HPTN 024. In this example, subjects 1 and 6 are not included because their HIV-1 infection was detected at the 4–8 week visit. There are $J=4$ visit times. Of the $m=7$ infants who test positive for HIV-1 infection within the first year, four (57%) are positive and alive at $t_1=$ three months (subjects 2, 3, 4, and 9). Therefore, if testing occurred at that point, only 57% of the infections would be detected. At $t_2=6$ months, subjects 2, 3, 5, 8, and 9 (71%) would be infected and detected. However, at $t_3=9$ and $t_4=12$ months, just four of the seven ($p_3=p_4=57\%$) infections would be detected. Because the largest p_j is p_2 , this hypothetical example indicates that six months would be the optimal additional testing point. To determine the proportion of infants who would be captured if testing was only performed at six weeks and three, six, nine or 12 months, we used a similar procedure as above with n_j equaling the number of infants who would first test positive at six weeks or the j th month and m equaling the total number of infants infected before their first birthday (including cases of *in utero* and intrapartum infection).

Because duration of breastfeeding could influence the results, we also performed an alternate analysis that defined infant-specific testing times at one month after weaning or at one year of age, whichever comes first. The procedure for calculating the proportion detected under this algorithm is identical to the one described above except for adding a t_j that varies over infants.

Results

Of 1671 infants born to HIV-1 infected women enrolled in HPTN 024 at the three African sites, 1609 (96.3%) had at least one HIV-1 diagnostic test. Figure 2 summarizes the HIV-1 infection status of these infants at 12 months of age, if known, or, if unknown, the timing of

their last negative test. At 12 months of age, 336 infants (20.8%) had at least one positive HIV-1 diagnostic test result, 830 (51.5%) had only negative results, and 443 (27.5%) were of uncertain HIV-1 infection status and therefore had their HIV-1 infection status imputed as part of the multiple imputation procedure.

Our model determined the optimal time point to test for HIV-1 by estimating the proportion of infants who first test positive in HPTN 024, after excluding those who had been diagnosed as infected at birth or 4–8 weeks. At three months only 43% [95% confidence interval (95% CI): 33, 54] of HIV-1 infections would be detected. This increases to 68% (95% CI: 59, 79) at six months, 78% (95% CI: 69, 87) at nine months, 77% (95% CI: 68, 86) at 12 months and 81% (95% CI: 71, 91) at the time of weaning plus one month or 12 months, whichever came first. Although the 9 and 12 month confidence intervals include the highest estimate (81%), the weaning plus one month/12 month algorithm met the criteria defined *a priori* for designating the optimal testing time. When we included HIV-1 PCR results from the six week window, the cumulative proportion of HIV-1-infected infants captured by our testing algorithm was 85% (95% CI: 80,90) at three months, 91% (95% CI: 87, 95) at six months, 93% (95% CI: 90, 96) at nine months, 93% (95% CI: 90, 96) at 12 months, and 93% (95% CI: 89, 98) at the first of one month after weaning or 12 months.

Discussion

Natural history studies have shown that 10–20% and 35–40% of untreated, HIV-1-infected children in resource-rich and resource-poor settings, respectively, die by two years of age (5–13). Several reports have indicated that early HIV-1 infection in infants is associated with early mortality (11,12). Early identification of HIV-1 infection and initiation of appropriate treatment can delay disease progression significantly (4). Thus, diagnostic testing of all HIV-exposed infants should be performed at a younger age (by 4–8 weeks of age), coincident with a routine infant vaccination visit, if possible. Such testing will identify those infants infected *in utero*, peripartum, and by early transmission through breast milk. Overall, in HPTN 024, 78% of all cases of HIV-1 transmission were detected at six weeks of age (16). The remaining 22% of infected infants were infected during the late postnatal period. This is similar to other studies (15,19–25).

Our results indicate that, among breastfeeding, HIV-1-exposed infants, HIV-1 diagnostic testing using a virologic assay should be performed at 4–8 weeks of age to capture early HIV-1 transmission, and at the first of one month after weaning or 12 months of age to capture late postnatal transmission. This does not preclude additional HIV-1 testing in symptomatic infants or testing one month after the complete cessation of breastfeeding, if breastfeeding continues after 12 months. Incorporation of HIV-1 antibody assays beginning at 12 months of age may also be of benefit since a negative result could be definitive among non-breastfeeding infants and may thus reduce the number of virologic assays that need to be performed (26).

Although these results suggest testing at one month post-weaning, this may not in fact be realistic in many resource-poor settings where caretakers must travel long distances for clinical care. Many caretakers may instead wish to wait until a scheduled clinic visit instead of making an extra trip to the clinic. In this case, our results suggest the testing at the 9 or 12 month visit is also an adequate approach, resulting in roughly the same proportion of infections detected.

These results were obtained using data from HPTN 024 where single dose nevirapine given to the mother during labor and to the infant shortly after birth was used for prevention of MTCT of HIV-1 (14,15). If other interventions to prevent MTCT of HIV-1 are used, such as prolonged prophylactic treatment of the infant with nevirapine, or highly active antiretroviral therapy to the lactating mother, the optimal timing for diagnostic testing, and the most sensitive virologic

test used (HIV-1 DNA vs HIV-1 RNA) may be different. As described above, duration of breast feeding also would make a difference in the optimal timing of a second test. For instance, in HPTN 024, mothers at one site stopped breast feeding at 6 months. In this case, the optimal timing for a second test would presumably be a combination of one month after weaning and between six and nine months. Exclusive breastfeeding was not recommended in HPTN 024 which was conducted before the benefits of exclusive breastfeeding were widely accepted. We did not capture data on breast feeding practices, such as mixed or exclusive breast feeding which may have affected transmission rates and potentially change the optimal time for a second HIV diagnostic test. With these caveats in mind, the model used in this study to estimate the most efficient and cost-effective timing of a second diagnostic test should prove useful with regard to other strategies for the prevention of MTCT in resource limited settings.

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Figure 1.

Figure 2.