Performance of HIV-1 DNA or HIV-1 RNA Tests for Early Diagnosis of Perinatal HIV-1 Infection during Anti-Retroviral Prophylaxis

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Objective To compare performance of testing for human immunodeficiency virus (HIV)-1 DNA and HIV-1 RNA for diagnosis of HIV-1 infection in infants receiving preventive antiretroviral therapy.

Study design This substudy of the French multicenter prospective cohort of neonates born to HIV-infected mothers, included 1567 infants tested for HIV with polymerase chain reaction (PCR) in a single laboratory, receiving post-natal prophylaxis, not breastfed, and having simultaneous HIV-1 DNA and RNA results before 45 days. The performance of PCR was assessed in reference to the 6-month HIV-1 RNA result.

Results Specificity of both HIV-1 RNA and HIV-1 DNA PCR was 100% at all ages (except 99.8% for DNA at birth); sensitivity was 58% (RNA) and 55% (DNA) at birth, and 89% at 1 month, 100% at 3 months for both, and 100% at 6 months (DNA). Concordance between HIV-1 DNA and RNA results was 0.78 and 0.81 (Kappa) at birth and 1 month and 100% at 3 and 6 months. Type of maternal and neonatal prophylaxis had no effect on sensitivity, but influenced viral load.

Conclusion The performances of testing for HIV-1 DNA and RNA were similar with 100% sensitivity at 3 months. At 1 month during prophylaxis, 11% of infected children had negative PCR results. *(J Pediatr 2012;160:60-6)*.

Early diagnosis of human immunodeficiency virus (HIV)-1 infection in babies born to seropositive mothers is essential for preventing AIDS and death by allowing early initiation of appropriate antiretroviral therapy (ART).[1,2]

Recently, the World Health Organization (WHO) recommended systematic HIV-1 diagnosis for all exposed infants, at 4 to 6 weeks of age, with tests having a sensitivity of at least 95%, and initiation of triple-drug ART, immediately after the diagnosis of infection.[3,4]

It has been estimated that, in the absence of antiretroviral prophylaxis, 95% of HIV-1 infections could be detected by polymerase chain reaction (PCR) at 2 to 4 weeks of age, with the exception of those cases transmitted by breastfeeding.[5-7] However, postnatal prophylaxis for at least 4 weeks is currently recommended universally by WHO,[8] and the optimal age for HIV-1 diagnosis in such cases is less well documented.

Detection of HIV-1 DNA in peripheral blood mononuclear cells (PBMC) and HIV-1 RNA in plasma both have been used to diagnose HIV-1 infection in neonates.[9,10] Testing for HIV-1 RNA has been reported to have a better sensitivity than testing for HIV-1 DNA for infants <2 months old receiving zidovudine prophylaxis,[11,12] although another study found no difference.[13]

The goal of this study was to compare the diagnostic performance of PCR tests for HIV-1 DNA in PBMCs with that of PCR for HIV-1 RNA in plasma in a large series of infants followed prospectively from birth as part of the Agence Nationale de Recherche sur le SIDA et les Hepatites virales French Perinatal Cohort (EPF-CO1) and receiving prophylaxis for 4 to 6 weeks.[14]

ART	Antiretroviral therapy
EPF-CO1	French Perinatal Cohort
HIV	Human immunodeficiency virus
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
WHO	World Health Organization

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Methods

We included in this study a subgroup of children born to HIV-infected women enrolled in the prospective multicenter Agence Nationale de Recherche sur le SIDA et les Hepatites virales French Perinatal Cohort (EPF-CO1), delivering on mainland France between 1994 and 2006. The study design of the EPF-CO1 has been described elsewhere.[14] No specific recommendation for HIV treatment and obstetric care was made for women in the cohort, and investigators were expected to follow French national guidelines, as regularly updated.[15] These guidelines recommend screening for HIV by PCR at birth and at ages 1, 3, and 6 months. All positive HIV PCR results have to be confirmed on a sequential sample. Informed consent was obtained from all mothers. This cohort study was approved by the ethics committee of Hôpital Cochin (Comité de Protection des personnes) and the French computer database watchdog commission (Commission Nationale de l'Informatique et des Libertés).

The analysis included all children whose HIV testing was performed in the virology laboratory of Necker Hospital. This laboratory received samples from 30 clinical participating centers.

All cases fulfilling these criteria were included: birth to an HIV-1 infected mother; post-natal prophylaxis; at least one blood sample taken before 45 days of age with PCR results for both HIV-1 DNA and HIV-1 RNA for the same sample; HIV-1 status established at 6 months on the basis of PCR testing for HIV-1 RNA at 6 months (day 183 ± 61), the most widely used criterion to date[16] or of death from unambiguous AIDS before 6 months; absence of breastfeeding. We excluded 4 infected infants receiving fully suppressive ART at 6 months and 3 additional infected children with horizontal, late post-natal infection as previously described.[17]

Virologic Methods

HIV-1 diagnostic tests were performed prospectively, by testing for HIV-1 DNA in PBMCs on the basis of PCR detection of two different genes until 2001, and with PCR tests for both HIV-1 DNA in PBMCs and HIV-1 RNA in plasma from 2002 to 2006.

PCR tests for plasma HIV-1 RNA were used prospectively and systematically for HIV-1 diagnosis in all infants since 2002, and in infected infants after diagnosis of infection since 1996. All earlier samples were retrospectively tested with PCR for HIV-1 RNA. Blood samples were obtained in tubes with venipuncture. Plasma HIV-1 RNA was assayed with Roche tests (Amplicor HIV-1 Monitor version 1 with add-in primers, version 1.5 or Cobas Taqman HIV-1 tests according to availability, Branchburg, New Jersey). High-speed centrifugation of plasma was used to pellet the virus and eliminate heparin from some plasma samples collected in heparinized tubes before the year 2000, and internal controls were used to verify the absence of inhibition. Ultrasensitive tests were used whenever possible. In case of insufficient plasma volume, the entirety of plasma available was used to be as close as possible to an ultrasensitive test. The limit of detection depended on the volume of plasma available (50-1000 μL), but never exceeded 1000 copies/mL. The median detection limit for tests giving negative results for plasma samples collected from infected infants at birth was 50 copies/mL (IQR, 50-200), and at 1 month was 50 copies/mL (IQR, 50-400).

PCR testing for HIV-1 DNA was performed with purified PBMCs until April 2005, and then on whole blood[18] with 3 different methods used successively: first, amplification of *pol*, long-terminal-repeat or *gag* gene sequences from PBMC lysate with radioactive Southern blotting as already described[19]; second, modified Roche Monitor 1.5 HIV-1 RNA kits with DNA internal standard (Roche Diagnostic Systems, Branchburg, New Jersey) as already described[20]; and third, since the year 2000, real-time PCR in the LTR region as already described.[21,22] All these methods were optimized carefully so that their limit of detection was 5 copies per PCR (ie, 35 copies/10^6 PBMCs), with 8E5 cells for reference. One microgram of DNA from PBMCs was tested in each assay. Samples giving positive PCR test results before the year 2000 were re-tested with real-time PCR when possible to get quantitative results, as described[22] (HIV-1 DNA quantification was available for 67% of HIV-1 DNA-positive samples).

Statistical Analysis

We first evaluated the sensitivity, specificity and predictive values of PCR tests for HIV-1 DNA and of PCR tests for HIV1-RNA performed at birth (day 1 to day 7), 1 month (day 30 ± 15), and 3 months (day 91 ± 30), with PCR tests for HIV-1 RNA at 6 months (day 183 ± 61) as the reference. We then estimated the Kappa concordance between tests for HIV-1 DNA and HIV-1 RNA for each period.

Specificity, predictive values, and kappa value were estimated for the period 2002 to 2006 because PCR testing for HIV-1 RNA in plasma was not performed for HIV-1 diagnosis before 2002, except for children scoring positive with PCR for HIV-1 DNA. We evaluated sensitivity in the whole period of the study (1994-2006). Exact 95% CIs were calculated for sensitivity, specificity, and positive- and negative-predictive values. Sensitivity was compared according to the type of maternal antiretroviral therapy, the type of post-natal prophylaxis, and geographic origin.

The χ^2 or 2-sided Fisher exact tests were used to compare categorical variables, and Student *t* test or the Wilcoxon test were used for continuous variables. Statistical analyses were performed with SAS, version 9.1 (SAS Institute, Cary, North Carolina).

Results

A total of 1293 children fulfilled the inclusion criteria: 65 were infected (HIV-1 RNA positive at 6 months in 62, death from unambiguous AIDS before 6 months in 3), and 1228 were uninfected (HIV-1 RNA negative at 6 months; **Table I**).