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Article

Broad Spectrum of Time of Detection, Primary Symptoms and Disease Progression in Infants with HIV-1 Infection

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Abstract The relationship between time of HIV-1 detection, appearance of symptoms and disease progression was studied in all 24 HIV-1-infected infants from a cohort of 117 children who were born to HIV-1-infected mothers and monitored from birth. HIV isolation from plasma and mononuclear cells, HIV-1 DNA PCR (polymerase chain reaction) and, retrospectively, a quantitative assay for HIV-1 RNA were used for virus detection. Two infants possibly exhibited a symptomatic primary HIV infection. More children with than without symptoms during the first year of life progressed to immunological class 3 ($P=0.013$) and to AIDS or death ($P=0.003$) during follow-up. HIV-1 was detected within 4 days of age in 4 of 16 infants: 3 of them became symptomatic within 1 year, as did 6 of the remaining 12 infants (not statistically significant). All four infants in whom virus was detected within 4 days of age progressed to severe immunosuppression, compared to 6 of 14 in whom the virus detection test was initially negative prior to the first positive result (n.s.). Two children with previous repeatedly negative HIV detection tests were diagnosed with HIV-1 infection at 8 and 9 months, respectively. Repeated blood sampling is needed for the diagnosis of HIV-1 infection in perinatally exposed infants, and virus detection tests for exclusion of HIV-1 infection must be used with caution.

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Introduction

Early diagnosis of congenitally or perinatally acquired HIV infection is important. Children identified can thereby be monitored carefully for immunological, virological and clinical abnormalities, and prophylactic and therapeutic measures can be instituted. Clinical symptoms of HIV infection in infants are seldom diagnostic, often nonspecific and may be temporary [1]. HIV-infected adults may exhibit a febrile primary infection with sore throat, rash and enlargement of lymph nodes [2]. This has not been reported in vertically infected infants. Testing for HIV antibodies is of little diagnostic value in early infancy, as maternal IgG antibodies are transferred across the placenta. The median age for their disappearance is 10 months [3]. Only infected children are persistently HIV antibody-positive beyond the age of 18 months. Methods for detecting p24 antigenemia were initially insensitive, although immune complex dissociation by acid or heat have increased the sensitivity markedly [4, 5].

Virus culture and polymerase chain reaction (PCR) for detection of HIV-1 DNA are reliable methods for early diagnosis of HIV infection. Quantitative measurement of HIV-1 RNA has been shown to be as reliable as DNA PCR and virus culture [6]. Approximately 40% of infected children have a positive culture or PCR at birth [7], an indicator that infection has occurred in utero. However, a positive PCR test at birth might be due to contamination from maternal blood [8]. A small number of HIV-exposed seroreverting infants have been reported to have positive cultures and/or PCR tests without being infected [9–11]. Thus, both positive and negative virus detection tests performed early in life might be associated with uncertainty in the diagnosis of HIV-1 infection.

A positive virus culture from blood sampled within 48 h of birth has been suggested to indicate transmission in utero [12] and expected to be associated with early symptoms and a rapid progression of the disease. A negative culture at birth in children who later turn out to be HIV-infected might indicate infection in late pregnancy or during delivery. This has been suggested to be associated with a later onset of clinical symptoms and a slower disease progression [13].

The incubation period for detection of HIV-1 by PCR after infection occurring just before or at birth is not completely known. The best indication is given by Dunn et al. [7], who studied 271 non-breast-fed perinatally HIV-1-infected children of HIV-1-infected mothers who were tested by PCR during the first week of life and who were followed prospectively. The frequency of PCR-positive results was stable during the first week of life, after which it rose rapidly. This indicates that the incubation period is around 1 week after infection that occurs at or close to delivery.

The aim of the present study was to evaluate the time of virus detection and the appearance of symptoms in relation to disease progression in vertically HIV-1-infected infants. Furthermore, we were interested in the possible occurrence of symptoms compatible with a primary HIV infection. Therefore, we aimed to describe any symptom occurring at the time of HIV-1 detection that could possibly be associated with the onset of viremia or immunological reactions to the presence of virus. For these purposes, we analyzed prospectively collected data from children of HIV-infected mothers who had thereby been perinatally exposed to HIV-1.

Materials and Methods

Subjects. All children in Sweden whose mothers were identified to be HIV-1-infected before or at the time of delivery were included in the study and monitored from birth. From 1987 the children were also enrolled in the European Collaborative Study [1, 3, 14]. Clinical examinations were performed at birth and thereafter every 3 months and, from 1991, also at 3 and 6 weeks and 4.5 months of age [1]. The mothers were strongly advised not to breast-feed. Since 1994 all mothers and their infants have been offered zidovudine according to the ACTG 076 protocol [15, 16].

Blood Samples. Cord blood was not used. Peripheral venous blood, 5 ml if possible, was collected at all clinical examinations, except at 3 weeks and 4.5 months of age. Defibrinated blood (heparin before and EDTA after 1992) was used for virus culture from plasma and peripheral blood mononuclear cells (PBMC) as well as for HIV-1 DNA PCR in PBMC.

Blood samples were collected on Sundays through Wednesdays, and in Stockholm through Thursdays, to make certain that laboratory processing could be initiated within 24 h. Thus, children were up to 4 days old when the first sample was taken. The blood samples were kept at room temperature until processed. All samples but one were received and the process initiated within 24 h. One sample did not arrive until a week after sampling, but the culture was positive. In the smallest children, the volume of blood obtained was often less than 5 ml, but the number of PBMC was usually sufficient. Small amounts of plasma usually gave a positive result early during the infection. Serum was used for serological assays.

Detection of HIV-1. Virus isolation was carried out as described previously [17, 18]. Whenever the volume of blood allowed, virus isolation from PBMC and plasma was performed separately. Testing of the specimens from the children was preceded and followed by testing of PBMC from blood donors as extra negative controls since 1991. HIV-1 DNA PCR was run in a seminested fashion, employing at least two different sets of primers from the *gag*, *pol* and/or *env* region of the HIV-1 gene [18, 19]. The PCR product was visualized after electrophoresis in an agarose gel with ethidium-bromide and photographed using a Polaroid camera. In those cases the test was defined as positive only if it was positive by at least two primer pairs. However, in retrospective HIV-1 DNA PCR analysis in children whose infectious status was already known, often only primers for the *gag* gene were used. This occurred in 29% of tests from uninfected children and in 15% of tests from infected children. A capture enzyme immunosorbent assay (EIA) (Abbott, USA) was used for detection of HIV p24 antigen in serum and in culture supernatants. The specificity was confirmed by an Abbott neutralization assay in the first positive sample.

Quantification of HIV-1. Saved sera stored at –20 °C were analyzed using the commercial nucleic acid sequence-based amplification NASBA HIV-1 RNA QT kit (Organon Teknika, The Netherlands) [20] according to the manufacturer's instructions. The lowest amount detected was 500 copies/ml.

Serological Investigations for HIV. HIV-1 IgG antibodies were analyzed by routine commercial EIA tests. In one child IgA and IgM antibodies against HIV-1 were also investigated. Briefly, the majority of the IgG antibodies were removed by absorption onto protein A sepharose beads, followed by a class-specific immunoblot assay [21]. Affinity-purified goat anti-IgM and -IgG peroxidase conjugated sera were purchased from Sigma, USA. Commercial electrophoretic immunoblots (Diagnostic Biotechnology, Singapore) were used with patient serum diluted 1/50 and with the above-mentioned conjugates diluted 1/500 in the dilution fluids provided by the immunoblot manufacturer.

Diagnostic Criteria. A child was defined as HIV-infected on the basis of either (i) an AIDS-defining diagnosis, (ii) at least two reliably positive virus detection tests (culture, PCR or antigen) on separate sampling occasions, or (iii) persisting HIV antibodies beyond the age of 18 months. Children were defined as uninfected on the basis of (i) two consecutive negative HIV antibody tests or a negative HIV antibody test after the age of 18 months, (ii) negative results in repeated virus detection tests, if performed, and (iii) an absence of any AIDS-defining condition. Conditions regarded as symptoms associated with HIV infection were classified according to the Centers for Disease Control and Prevention (CDC) classification [22].

Analysis of Data. Data from this study were analyzed retrospectively. The comparisons between groups regarding progression to immunological class 3 and to AIDS or death were made by Fisher's exact test. The between-group comparisons of follow-up time were made with the Wilcoxon two-sample test. All probability (P) values were two-tailed.

Results

Between June 1985 and March 1998, 117 children born to HIV-1-infected women in Sweden were monitored prospectively from birth for 18 months or more. Twenty-four (21%) infants were found to be infected. Two children with HIV-1-related symptoms at the time of appearance of viremia as well as two children in whom virus detection tests were positive but who did not fulfill the criteria for HIV infection are described separately. Two infected children and their mothers had been treated with zidovudine in order to reduce the risk of transmission [15, 16].

Detection of HIV after Birth. An HIV culture was performed on blood from 16 infected infants within the first 4 days of life, and in 13 of these cases an analysis of HIV-1 DNA by PCR was performed as well. Culture was positive in 4 of 16 (25%) infants, and, in these four infants, but in no other infant at this age, HIV-1 DNA was demonstrable by PCR. In eight children no blood test for virus detection was performed during the neonatal period. Therefore, no information regarding when HIV was first detectable is available for these patients.

Children with Demonstrable HIV-1 Within the First Four Days of Life (Group 1). Three of the four children with demonstrable virus by culture and PCR within the first 4 days of life (group 1) presented with clinical symptoms before the age of 12 months. The fourth child remained asymptomatic without treatment during a follow-up period of 5.5 years. The follow-up time of the children in group 1 was 4.6 ± 1.3 years (median 5 years). At the end of the follow-up period, the infection in all four children had reached the CDC immunological class 3. Two children progressed to AIDS, one of whom died.

Children with a Negative HIV-1 Detection Test Result Preceding the First Positive Result (Group 2). Among the 14 children with at least one negative virus culture and/or negative HIV-1 DNA PCR preceding the first positive virus detection test, six became symptomatic before 12 months of age. Two infants presented with symptoms suggestive of a primary HIV infection. The clinical and laboratory findings for these infants are described in detail below. Eight children remained asymptomatic without treatment during the follow-up period between 3 and 11 years of age, with the exception of a temporarily swollen parotid gland in one child.

The mother of one of these children possibly had a primary HIV infection 1 month before delivery, but cord blood was negative for HIV antibodies (EIA and Western blot). The child was temporarily lost to follow-up until 15 months of age, when the child was found to be HIV antibody-positive by EIA and Western blot. This child was the only one in the study who was breast-fed.

Two children in this group had been exposed to zidovudine, but not completely in accordance with the ACTG 076 protocol. In both cases, HIV was not detected at birth. In one case HIV isolation in both PBMC and in plasma was positive at 6 weeks of age. In the other, the second sample was obtained at 9 months, when HIV isolation from both PBMC and plasma was negative, while PCR was positive. The same result was obtained at 11 months.

The mean follow-up time in group 2 was 6.1 ± 3.2 years (median 6.1 years), not significantly different from that in group 1. In 6 of the 14 children, HIV infection reached immunological class 3, and 5 of the 14 contracted AIDS or died during follow-up. Group 2 did not differ significantly from group 1 regarding progression to immunological class 3 or to AIDS or death.

Two Patients with Symptoms Suggestive of Primary HIV-1 Infection. In two patients clinical symptoms that could possibly be associated with the onset of viremia occurred close to the time of HIV-1 detection. These two patients are described in detail below.

The first child was born after an elective caesarean section at term. Neither the mother nor the child received antiretroviral prophylaxis, since the child was born before 1994 [15]. At the age of 3 weeks, he had mild signs of upper respiratory infection and cried and seemed dissatisfied. On the hard palate there were small vesicles on red areas that increased in size and turned into seemingly painful ulcerations within a couple of weeks. Bacterial culture yielded growth of *Klebsiella pneumoniae*, and the child was treated with trimethoprim-sulfamethoxazole and phenoxymethylpenicillin for 10 days, with no obvious effect on the condition. He failed to thrive and had feeding difficulties. The symptoms lasted for 5 weeks.

Culture and PCR tests for CMV and herpes simplex types 1 and 2 were negative. The CD4+ cell count was 2.10×10^9/l (30%) at week 6 after birth and 1.04×10^9/l (20%) at month 3. Virus cultures, PCR and antigen tests for HIV were all negative at day 4 but positive at week 6 and at months 3 and 5. In the retrospective analysis, HIV-1 RNA was not detected with the NASBA technique. Since the clinical symptoms were suggestive of HIV infection, we reanalyzed the number of HIV-1 RNA copies with another method (Amplicor Monitor; Roche Diagnostic Systems, USA) and performed genetic sequencing [23] of the virus, which was found to belong to subtype G. The number of HIV-1 RNA copies with the Amplicor Monitor test was 81,000/ml at week 6 and 141,000/ml and 571,000/ml at month 3 and 5, respectively.

The second child, who also had not received antiretroviral prophylaxis and was born before 1994, had a virus culture performed at day 1 and month 6 after birth and an HIV-1 DNA PCR test performed on day 1 as well as at months 3 and 6. All these virus detection tests were negative. The child presented at 7 months of age with measles-like exanthema, high fever, lymphadenopathy and tonsillitis. A throat culture for bacteria was negative. Treatment with phenoxymethylpenicillin and, thereafter, oral ampicillin had no effect. The febrile period lasted intermittently for about 1 month, during which time the child developed neutropenia (0.7×10^9/l) and, at the end of the month, thrombocytopenia (5×10^9/l) and varicella. The thrombocytopenia was successfully treated with intravenous immunoglobulin and the varicella was treated with intravenous acyclovir. Ten days later the child presented with pneumonia and parotitis. At 6 and 8 months of age, the CD4+ cell count was 2.79×10^9/l (30%) and 2.56×10^9/l (20%), and the CD4+/CD8+ ratio was 1.55 and 0.51, respectively.

Virus culture and PCR for HIV-1 DNA were positive at month 8 and were confirmed at months 12 and 18. Antigenemia appeared at month 12. The retrospective analysis of samples collected at day 1 and month 3 did not reveal HIV-1 RNA. At the age of 6 months, 500 copies/ml of HIV-1 RNA was present, and at 12 months 57,000 copies/ml. Subtyping using DNA sequencing revealed subtype D [24]. The HIV-1 antibody titers were decreasing at months 3 and 6 but had increased by month 8.

Children Not Tested Within the First Four Days of Life and for Whom No Negative Test Result was Obtained Prior to the First Positive Result (Group 3). Two of the six children in group 3 presented with AIDS before 12 months of age. Neither of them was cultured for HIV prior to the development of AIDS. Four children were asymptomatic during the first 12 months of life, three of whom were first cultured for HIV between week 6 and month 4 after birth, with positive results. For one asymptomatic child no virus detection test was performed during the first year of life.

Patterns of HIV Detection During the First Year of Life. The numbers of positive cultures, PCR tests, HIV-1 RNA quantification tests and antigen tests in relation to the number of tests performed in infected children at different ages during the first year of life are presented in Table 1. In none of the infected children was detection of p24 antigen the first test to be positive. For 4 of 16 (25%) infected children, at least one HIV-1 detection test performed at birth was positive. All virus cultures from plasma, 9 of 10 virus cultures from PBMC, all DNA PCR assays and all RNA quantification tests performed in infected children between weeks 4 and 8 were positive.

For two children, virus detection tests were negative at birth, at month 3 and at month 6, but no tests were performed between weeks 4 and 8. HIV infection was diagnosed in these children at months 8 and 9, respectively. For one child, the first virus detection test was performed at month 6. This test was negative, and HIV infection was diagnosed at 15 months of age.

HIV-1 Detection in Uninfected Children. HIV-1 detection tests were positive in two children eventually identified as uninfected. All virus cultures from PBMC ($n = 362$) and all p24 antigen tests ($n = 342$) performed in uninfected children between day 1 and month 13 were negative. One of 338 (0.3%) plasma cultures and 1 of 316 (0.3%) HIV-1 DNA PCR tests were positive in this group.

In one previously described child [11] who was unexposed to zidovudine, blood samples were not taken within the first 4 days of life. Virus culture at week 2 was positive from plasma but negative from PBMC. The p24 antigen tests performed on the virus isolated from plasma became positive after 1 month of culture. However, the culture became contaminated by bacteria and was discarded without storage of remaining material. HIV-1 DNA in PBMC was demonstrable by primers for the *env* gene, but was negative with primers

Table 1 HIV-1 detection in prospectively followed infected children at different time points during the first year of life

Age	No. of positive tests/no. of tests performed (%)				
	Virus culture from plasma	Virus culture from PBMC	HIV-1 DNA PCR	p24 antigen detection	HIV-1 RNA
0–4 days	3/16 (19)	4/16 (25)	4/12 (33)	0/15	1/7 (14)
4–8 weeks	10/10 (100)	9/10 (90)	9/9 (100)	2/9 (22)	7/7 (100)
3–4 months	10/13 (77)	10/12 (83)	10/12 (83)	4/15 (27)	6/7 (86)
5–7 months	12/14 (86)	9/14 (64)	9/11 (82)	5/14 (36)	7/9 (78)
8–10 months	4/5 (80)	3/5 (60)	4/4 (100)	5/13 (38)	3/4 (75)
11–13 months	7/10 (70)	10/12 (83)	7/7 (100)	5/15 (33)	10/11 (91)

specific for the *gag* and *pol* genes. Virus culture from plasma and PBMC was negative at months 3 and 6. At month 6, PCR was negative with all three primers (*gag*, *pol* and *env*). During follow-up, culture from plasma and/or PBMC and PCR for HIV-1 DNA remained negative at months 9, 12, 15, 20, 25, 36, 48 and 60 (PCR was not performed at months 12, 36 or 48). However, IgM antibodies against HIV-1 were also detected at months 6 and 9, and IgA antibodies at month 6. Seroreversion had occurred at 11.5 months of age. By the age of 7 years, the child showed no clinical signs of HIV infection or any immunological abnormalities; he remained HIV antibody-negative and was regarded as uninfected by HIV. When this child was described previously [11], it was reported that PCR had been transiently positive in a sample taken at 5 months of age. Instead, it occurred in the sample taken at 2 weeks of age, as documented above.

In a second child, also unexposed to zidovudine, HIV-1 DNA was detected on day 1 by PCR (2 primers: *gag* and *pol*), but virus cultures were negative. HIV-1 DNA was detected by one primer (*pol*) but was negative by another (*gag*) at 7 weeks of age, when HIV culture was negative. HIV-1 DNA PCR and cultures were negative at months 4.5, 9, 12, 21 and 25. By month 15–21, seroreversion had occurred, the clinical and immunological status was normal, and the child was regarded as uninfected.

The positive PCR test results in these two uninfected children were obtained within 8 weeks after birth. Among 206 PCR tests performed after 8 weeks of age in uninfected children, none was reactive.

Early Symptoms and Disease Progression. Of all 24 HIV-1-infected children, 11 exhibited HIV-related symptoms during the first year of life. Ten of these 11 children progressed to immunological class 3 during follow-up and nine progressed to AIDS or death. This was significantly different from the 13 children who remained asymptomatic during the first year of life, of whom five progressed to immunological class 3 ($P=0.013$) and two to AIDS or death ($P=0.003$). The follow-up time of the children who exhibited symptoms of HIV infection before 1 year of age was 4.6 ± 2.7 years (median 4.9 years), not significantly different from that of children who remained asymptomatic during that time, 6.9 ± 3.0 years (median 5.7 years).

Discussion

The virus detection rate during the first week of life has been reported to be 30–60% in perinatally HIV-infected children [7, 25–27]. This is similar to the 25% in our study and may illustrate the relative distribution between infection in utero and infection at or close to the time of delivery [7, 12, 28]. It has been suggested that children infected at or near birth are those who will have the greatest chance to remain asymptomatic for a long period of time, whereas those children who are infected in utero will develop symptoms early in life [29]. This hypothesis is supported by some studies [13, 30, 31], but others have found no association between the time of the first virus detection and clinical outcome [32, 33]. High levels of HIV-1 RNA at birth and during primary viremia have been associated with early onset of symptoms and rapid disease progression [30]. Others have shown that disease progression in infected infants may be related more to characteristics of the maternal virus [34], or to the maternal state of disease [34–36], than to the time of transmission.

At 4–8 weeks of age, all cultures, HIV-1 DNA PCR assays and quantitative HIV-1 RNA tests performed in infected infants in our study were positive, except one viral culture from lymphocytes. This might indicate a high viral load at this time, which is in accord with other studies [37, 38] and supports studies [6, 39] showing the highest detection rate of HIV-1 by virus culture and/or DNA PCR in perinatally acquired infection at this age. However, three infants with undetectable virus before and after this period were not tested at 4–8 weeks, which, in combination with the small number of patients, could reinforce differences in detection rates and give an impression of a decline in sensitivity of the tests during the first year of life.

We found no relationship between the time of the first virus detection and either disease progression or the appearance of early symptoms. The lack of statistical significance may be due to small sample size. However,

those infants with HIV-related symptoms during the first year of life had a more rapid progression of disease. All children in this study were followed-up prospectively from birth and were monitored closely for clinical symptoms, although not all were sampled from birth. Therefore, in all probability, very few symptoms were overlooked. Four children were treated with zidovudine, but only because of symptomatic disease and deterioration of their immune system, which is why we do not attach any importance to this. The study was performed mainly before 1994, but two mothers of infected children were given zidovudine prophylactically to prevent mother-to-child transmission, which could of course have influenced the time of the first virus detection and the disease progression in their children.

Two infants had symptoms suggestive of primary HIV-1 infection. Virus detection tests were negative at birth in both cases, suggesting transmission late in pregnancy or at delivery. These cases are of interest because symptomatic primary infection in vertically infected children has not been reported. Whether the presence of a symptomatic primary infection prognosticates a more rapid disease progression and a severe clinical outcome in infants, as in adults [40], is not known. One of these two children died at 5 months of age from a cause unrelated to HIV. The other child developed AIDS at the age of 4.2 years. In this child the time interval between birth and the onset of symptoms and virus detection was remarkably long, 7–8 months. Indeed, there was a striking covariance between the appearance of viremia, affection of the immune status and the debut of clinical symptoms. Postpartum transmission through breast-feeding was excluded since the child was bottle-fed and there were no indications that the child was given breast milk as well. It is a weakness that tests for detection of other viral infections, for example EBV infection, were not performed.

In another infant, HIV was first detected a remarkably long time after birth (9 months) despite repeated sampling before that time. This child remained asymptomatic for 6.7 years without treatment. Thus, the two children with similar patterns regarding virus detection and antibodies had different clinical outcomes, indicating that factors other than the time of virus detection are important for the course of the infection. They illustrate that thorough follow-up of infants born to HIV-infected women is important, also beyond the age of 6 months. Two negative HIV cultures between months 1 and 6 have previously been shown to identify uninfected infants with a specificity of more than 99% [39]. There is no indication that the variety of subtypes had any influence on the diagnosis, only on the ability to detect HIV-1 RNA in a few cases.

The mother of one child possibly had a primary HIV infection, which is a known risk factor for transmission [41], 1 month before delivery. It is also possible that transmission occurred through breast-feeding during the first 15 months of life. Breast-feeding has been found to contribute considerably to maternal-infant transmission of HIV-1 [42, 43].

During recent years, the possibility of clearance of HIV has been the subject of debate [9–11]. One explanation might be that the uninfected children with detectable HIV-1 had been exposed to maternal HIV without being truly infected. In our study HIV detection in uninfected children occurred only during the first 2 months of life, which possibly supports this theory, but it could of course be the result of false-positive tests. Frenkel et al. [44] analyzed all the reports in the literature and found no evidence of a true clearance of HIV infection, and Simonds et al. [28] identified mislabelling of samples as the most likely factor of unexplained positive results. The accepted criteria for HIV infection in perinatally exposed infants exclude these children in whom virus detection tests are positive but who are uninfected.

This study supports the necessity for repeated blood sampling for the diagnosis of HIV in infants born to HIV-1-infected mothers. Application of different methods for HIV detection increases the reliability. It also supports previous findings that 4–8 weeks of age is the period when the positive predictive value of different diagnostic tests is the highest for the diagnosis of HIV-1 in perinatally exposed infants. Virus detection tests may, however, be negative in infected children during the first 6 months of life. The broad spectrum of diagnostic and clinical outcome demands careful clinical and virological monitoring throughout the first year of life.

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