CLINICAL ARTICLES

Natural History of Perinatal Hepatitis C Virus Infection

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In order to outline the natural course of perinatal hepatitis C virus (HCV) infection, we prospectively followed seven HCV-positive children for a mean period of 65.1 months (range, 26–90 months). Physical examination findings, growth, and bilirubin and immunoglobulin levels were constantly normal. All children were still viremic at last analysis. HCV-RNA was almost constantly detected throughout follow-up, with the exception of the first days of life. All children had initial increases (of variable duration) in alanine aminotransferase values; four children subsequently had normal or borderline values for years, with exacerbation of inflammatory activity in two cases. IgM antibodies to HCV were found in three of the seven patients. Autoantibodies developed in two children. Liver biopsy, performed on five patients, documented different degrees of chronic persistent hepatitis. Thus, recovery from perinatal HCV infection seems unlikely, and chronic hepatitis develops in most infected children, including those with prolonged intervals of remission of inflammatory activity.

Hepatitis C virus (HCV) has been identified as the major cause of parenterally transmitted non-A, non-B hepatitis [1]. Sexual and intrafamiliar transmission have also been suggested as routes of infection, although the data are still controversial. The acute phase of disease tends to be mild, but chronic liver damage develops in more than one-half of all cases, and 20% of these cases progress to cirrhosis [2]. HCV is also heavily implicated as a cause of autoimmune disorders [3, 4] and hepatocellular carcinoma [5, 6]. To prevent these negative outcomes, patients with chronic hepatitis C are treated with interferon, resulting in persistent virus clearance in one-quarter of such cases [7].

Mother-to-child transmission of HCV has been documented, although the risk of infection has varied greatly in different investigations [8–13]. Timing and correlates of perinatal transmission have not been defined, and since the natural history of perinatal HCV infection is unknown, doubts arise about whether and when interferon therapy is to be used for these children.

Women with HIV type 1 (HIV-1) infection are often coinfected with HCV, particularly when they have a history of intravenous drug use. Their children are thus frequently exposed to HCV. In addition, maternal HIV-1 coinfection may increase the likelihood of HCV transmission to the fetus and newborn [12]. From a cohort of infants at risk for HIV-1 and HCV infection, we were able to identify a group who acquired HCV infection alone. The data gathered with regard to these children allowed the natural history of perinatal HCV infection to be studied.

Patients and Methods

Patients. One hundred and thirty-five children who were born to HIV-1-seropositive mothers and regularly followed at the Department of Pediatrics at the University of Turin (Turin, Italy) were tested both retrospectively and prospectively (starting in September 1991) for HCV seropositivity.

Diagnosis of HCV infection. Serum samples were analyzed by a second-generation EIA (Abbott Laboratories Diagnostics Division, Chicago). Positive findings were confirmed by an immunoblotting assay (RIBA-II; Ortho Diagnostic Systems, Raritan, NJ), detecting antibodies to four HCV recombinant proteins: 5-1-1, C100-3 and C33-c (from nonstructural regions of the virus), and C22-3 (from the core region). Sera reacting with two or more antigens were considered to be positive. Since passively acquired maternal antibodies to HIV-1 and HCV may persist in the infant's blood for prolonged periods [11], the diagnosis of HIV-1 and/or HCV infection was made on the basis of the persistence of specific antibodies, beyond 18 months of age. IgM antibodies to HCV were measured by an EIA (Abbott).

Transaminase, bilirubin, and immunoglobulin levels. As the children had been enrolled in a study of perinatal exposure to HIV-1, the transaminase and bilirubin levels as well as the IgG, IgA, and IgM concentrations were determined every 3

Received 6 September 1995; revised 1 March 1996.

Financial support: Ministero dell'Universita' e della Ricerca Scientifica e Tecnologica (60% grant), Italy.

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Clinical Infectious Diseases 1996;23:47–50

1058–4838/96/2301–0005$02.00

months over the first 18 months of life (thereafter, every 4–8 months). In addition, several sequentially stored serum samples were available to assay for other parameters considered in this investigation.

HCV-RNA analysis. Serum HCV-RNA was measured by reverse-transcriptase PCR, as described elsewhere [11], with use of primers of the 5′ noncoding region of the viral genome (Amplicor PCR Diagnostics; Roche Diagnostic Systems, Hoffman-La Roche, Basel, Switzerland).

Other analyses. Specific tests for discovering other hepatotropic viruses—such as for hepatitis B virus surface antigen and antibody, IgG antibody to hepatitis A virus, and antibodies to cytomegalovirus, Epstein-Barr virus, and herpes simplex virus type 1—were performed in coincidence with transaminase analyses, as previously described [14]. Patients were also tested to exclude metabolic and immunologic causes of chronic liver disease (e.g., α_1-antitrypsin, ceruloplasmin, and autoantibodies).

Liver echography and biopsy. Ultrasonographic evaluations of the liver were carried out for all patients. Five children underwent percutaneous liver biopsy after their parents gave informed consent; in the other two cases the parents refused biopsy.

Results

Study population. At the last follow-up visit, among 108 children older than 18 months of age, 10 (9.2%) were HCV seropositive: 3 had concurrent HIV-1 infection, while 7 (4 females and 3 males) had acquired only HCV. The latter were the subjects of the present study. Of these, 5 were followed-up from birth, 1 from the first month of age, and 1 from the third month of age. Their mean age at the last follow-up visit was 65.1 months (range, 26–90 months).

Six mothers had a history of intravenous drug use, and one had had sexual contact with an HIV-1-infected partner. Five children were born by vaginal delivery and two (patients 3 and 5) by cesarean section. No infants were breast-fed or received blood transfusion. Three children (patients 2, 3, and 5) were abandoned at birth by their drug-addicted mothers and live with HCV-negative foster parents.

Clinical features and bilirubin levels. Physical examination findings, growth, and serum bilirubin concentrations were normal for every child at all times.

Transaminase values. Alanine aminotransferase (ALT) levels in the individual patients varied widely during the follow-up (figure 1). Two of five infants tested within the first week of life (patients 5 and 7) had a mild increase in ALT activity. This enhancement appeared in all seven children between 3 and 6 months of age, was more pronounced in the first period of life, and persisted (to different extents) for variable intervals (figure 1). In particular, four children's ALT values were normal or borderline for prolonged periods, but exacerbations of ALT activity were observed in two cases (patients 4 and 5).

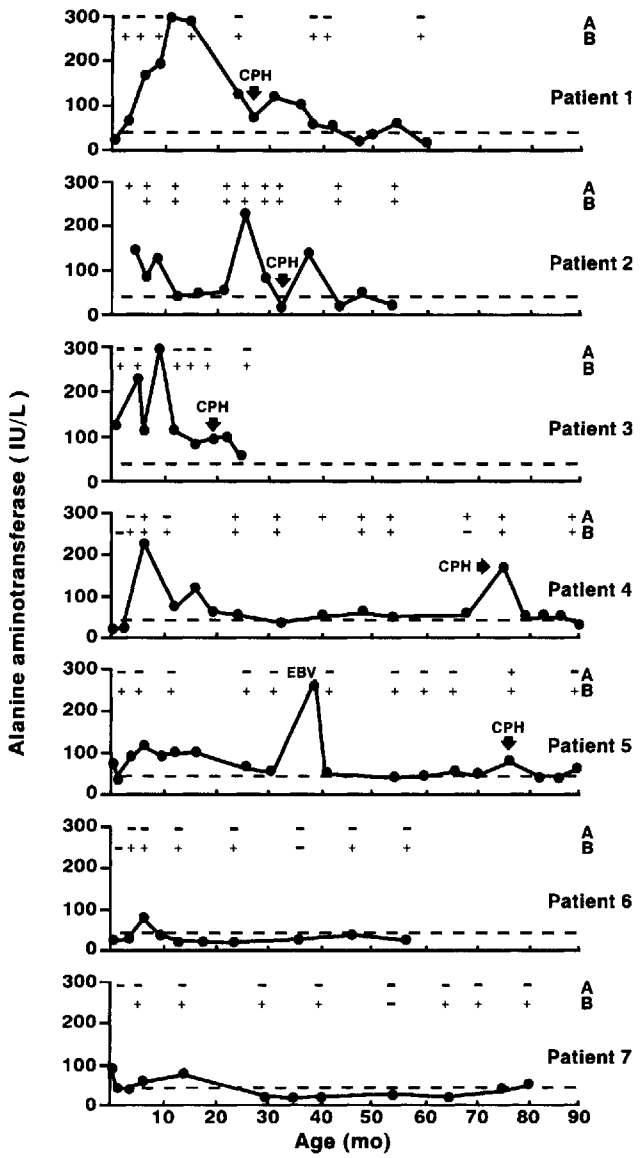

Figure 1. Levels of alanine aminotransferase (*solid lines*) in seven children with perinatal HCV infection. The presence (+) or absence (−) of IgM antibody to HCV (*line A*) and of HCV-RNA (*line B*) is indicated for each patient. The dashed lines indicate the upper limit of the normal level of ALT. (EBV = Epstein-Barr virus infection; CPH = chronic persistent hepatitis.)

None of the seven children was perinatally infected with other hepatotropic viruses, such as cytomegalovirus, Epstein-Barr virus, herpes simplex virus 1, or hepatitis A or B virus; the role of such viruses in causing an increase in ALT levels was ruled out in all but one case, in which primary Epstein-Barr virus infection was diagnosed (figure 1). Nonviral causes of hepatocellular injury were not found in any child.

HCV viremia. HCV-RNA was detected by PCR in every child in all or almost all determinations; in particular, all children were still viremic at the last analysis (figure 1). Four infants were tested for HCV-RNA during the first 3 weeks of life. PCR was negative for the two children tested within 3

days after birth (patients 4 and 6), whereas it was positive for two other children (patients 1 and 5) who were tested for the first time on days 9 and 20, respectively.

Total and specific immunoglobulins. IgG, IgA, and IgM levels were within the normal range for age in all but one child (patient 2), who had a constant increase in both total immunoglobulin levels and level of IgM antibody to HCV. IgG antibody to HCV persisted in all children during the entire observation period. At the last analysis, five children were antibody-positive to all four antigens in tests with RIBA II, while two were reactive only to C33-c and C22-3. These two children (patients 1 and 7) never had antibodies to the 5-1-1 and C100-3 antigens, including at the first determination (at 20 and 48 days of life, respectively), when the antibody pattern was still of maternal origin.

IgM antibody to HCV was detected in three (42.8%) of the seven children. In two cases (patients 2 and 4) the antibody appeared at 4 and 6 months of life, in coincidence with the enhancement of ALT levels (figure 1); then, specific IgM persisted during the follow-up. In a third child (patient 5), IgM to HCV was seen only at 76 months of age; in the other four children, specific IgM was never detected.

Autoimmune reactions. Autoantibodies developed in two children. One (patient 5) had antiphospholipid antibody with prolonged prothrombin time; the other (patient 4) had antinucleus and anti–smooth muscle antibody, in coincidence with the appearance of insulin-dependent diabetes mellitus at 6 years of age.

Liver echography and biopsy. Findings of ultrasonographic evaluation of the liver were normal for all but one child (patient 1), whose evaluation revealed a mild, diffuse increase in hepatic echogenicity. Percutaneous liver biopsy showed a histologic picture of chronic persistent hepatitis in all five children studied. Hepatitis was mild in one case (patient 5) and intermediate in the remaining four. It was associated with signs of fibrosis in one patient (number 2) and with mild (patient 4) or marked (patient 1) steatosis in the other two children.

Discussion

The risk of mother-to-child transmission of HCV is still unclear [8–13]. In a prospective cohort study, we found that none of the 27 exposed infants whose mothers were HIV-1-negative acquired HCV infection [11]. The present analysis permits only an estimate of the minimum risk of HCV transmission from women coinfected with HIV-1. However, the percentage of HCV-infected children was higher (9.2%) than that found in children born to HIV-1-uninfected women, confirming that the mother's HIV-1 status can favor HCV transmission to the offspring [12].

The timing of transmission is unknown. Being at risk for HIV-1 infection, all children in our cohort were bottle-fed, thus excluding the possibility of postnatal HCV infection through breast-feeding. Furthermore, some infants were abandoned at birth, and their subsequent caregivers (foster parents) were HCV-negative. Therefore, they must have contracted the infection in utero or at the time of delivery.

PCR analysis is a very sensitive technique for detecting HCV-RNA in serum samples from infected subjects. For those who acquired infection parenterally, PCR usually is positive within 1 week, while the increase in ALT levels is manifested within 2 months [15]. When assayed for during the first days of life, HCV-RNA was not detected in infants who were ultimately shown to be infected, whereas it was almost constantly found from the second week onward.

In addition, the first significant increase in transaminase levels was mostly seen from 3 to 6 months of age. Similar results have been reported by others [10, 13]. Although the number of cases described herein is small, these findings provide evidence that HCV transmission occurs largely in the peripartum period, similar to transmission of hepatitis B virus infection.

One of the most relevant findings in this study was that in none of the infected children was the virus cleared during the observation period; all were still viremic at the last analysis. This strongly suggests that few perinatally infected children recover from HCV infection, whereas most become persistently infected, even in the absence of inflammatory activity in the liver. An increase in ALT levels during the first year of life occurred in all children, frequently persisting beyond 6 months. These infants' conditions consequently progressed toward a chronic form of hepatitis. Some children subsequently had protracted periods in which there were no biochemical signs of hepatitis; however, in certain cases, these periods were followed by exacerbation of inflammatory activity and histologically documented chronic hepatitis.

Marked variations between normal and abnormal ALT concentrations have been observed in adult patients; thus, long-lasting normalization of transaminase activity in some children may be accounted for by the infrequency of blood tests. Overall, our findings point out that the progression to chronic hepatitis cannot be predicted by early clinical or biochemical features. The fact that HCV did not elicit any increase in serum bilurubin or immunoglobulin levels (with the exception of one case in which there was persistent enhancement of levels of total immunoglobulin and specific IgM antibody to HCV) further highlights that HCV induces mild liver damage, but this does not rule out its potential long-term consequences. Given the slow rate of progression, perinatal infection with HCV may remain clinically inapparent in children but cause significant chronic liver disease in adults without known risk factors.

While the majority of children developed a humoral response against all viral antigens, two of them did not have antibodies to the 5-1-1 and C100-3 antigens. The spectrum of antibody response did not change over time in any child, including during the first period of life, when IgG antibodies were still of maternal origin. The mother's antibody pattern consequently was mirrored in her infant. In mice it has been

shown that idiotype suppression is passed on from the mother to offspring [16]. Our findings suggest that human mothers also can make an imprint on the developing immune system of the newborn. No particular evolution of infection was noted in the two children who were not reactive to the 5-1-1 and C100-3 antigens. Therefore, a clonal restriction in the humoral response to HCV does not seem to have a significant impact on disease progression.

The early diagnosis of HCV infection in infants born to seropositive mothers is complicated by the passage of maternal IgG across the placenta. The detection of IgM antibody to HCV, revealing endogenous antibody synthesis, may be a simple means of rapidly discriminating between infection and noninfection in infants. However, its usefulness appears limited by low sensitivity, since only a minority of our infected infants had IgM antibody to HCV.

These antibodies can be found not only during the primary immune response to the virus but also during disease reactivation [17]. It has been proposed that their presence may help identify patients who will benefit from therapy with recombinant IFN-α [18]. The presence of specific IgM was associated with increased ALT levels, but the latter was manifested even without enhancement of the level of IgM antibody to HCV, thus confirming that the IgM levels may constitute an index (albeit a poorly sensitive one) of disease activity.

Most fetal or neonatal viral infections have a longer duration and a worse outcome than those acquired postnatally. The data from our study suggest that this also is true for HCV infection, although the influence of maternal HIV-1 coinfection on HCV persistence and progression in the baby cannot be ruled out. If HCV replicates indefinitively in perinatally infected children, they will then be life-long reservoirs of the virus; this circumstance presents ominous implications for the continuation of the epidemic.

Furthermore, chronic persistent hepatitis developed in most children, and although it was clinically and biochemically silent for prolonged periods of time, it is associated with a prognosis that seems to be worse than initially thought [19]. HCV infection may also trigger autoimmune phenomena [3, 4], and autoantibodies were found in a few of our patients.

Finally, the risk of hepatocellular carcinoma [5, 6] may be especially prominent in patients infected early in life. Therefore, long-term follow-up is needed to assess the full consequences of perinatal HCV infection, as progression to cirrhosis, autoimmune diseases, and liver cancer is unusual in the short term.

Acknowledgments

The authors thank Dr. James Oleske and Dr. Paul Palumbo (Department of Pediatrics, New Jersey Medical School, Newark, NJ) for critical revision of the manuscript and thank Dr. Carlo Scolfaro, Dr. Caterina Riva, and Dr. Luciano Balbo (Department of Pediatrics, University of Turin, Turin, Italy) for help with the study.

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