

Risk Factors for Perinatal Transmission of Hepatitis C Virus (HCV) and the Natural History of HCV Infection Acquired in Infancy

Eric E. Mast,¹ Lu-Yu Hwang,³ Dexter S. Y. Seto,⁴ Frederick S. Nolte,² Omana V. Nainan,^{1,a} Heather Wurtzel,^{1,b} and Miriam J. Alter¹

¹Centers for Disease Control and Prevention and ²Emory University, Atlanta, Georgia; ³University of Texas Houston School of Public Health, Houston; ⁴Kapiolani Medical Center and University of Hawaii School of Medicine, Honolulu

(See the the editorial commentary by Beasley and the article by the European Paediatric Hepatitis C Virus Network, on pages 1865–6 and 1872–9, respectively.)

Background. The goal of the present study was to assess risk factors for perinatal hepatitis C virus (HCV) transmission and the natural history of infection among HCV-infected infants.

Methods. In a cohort study, 244 infants born to HCV-positive mothers were followed from birth until age ≥ 12 months. Maternal serum was collected at enrollment and delivery; infant serum was collected at birth and at 8 well-child visits. Testing included detection of antibody to HCV, detection of HCV RNA (qualitative and quantitative), and genotyping. HCV-infected infants were followed annually until age 5 years.

Results. Overall, 9 of 190 (4.7% [95% confidence interval {CI}, 2.3%–9.1%]) infants born to mothers who were HCV RNA positive at delivery became infected, compared with 0 of 54 infants born to HCV RNA-negative mothers ($P = .10$). Among HCV RNA-positive mothers, the rate of transmission was 3.8% (95% CI, 1.7%–8.1%) from the 182 who were human immunodeficiency virus (HIV) negative, compared with 25.0% (95% CI, 4.5%–64.4%) from the 8 who were HIV positive ($P < .05$). Three infected infants resolved their infection (i.e., became HCV RNA negative). In multivariate analysis restricted to HCV RNA-positive mothers, membrane rupture ≥ 6 h (odds ratio [OR], 9.3 [95% CI, 1.5–179.7]) and internal fetal monitoring (OR, 6.7 [95% CI, 1.1–35.9]) were associated with transmission of HCV to infants.

Conclusion. If duration of membrane rupture and internal fetal monitoring are confirmed to be associated with transmission, interventions may be possible to decrease the risk of transmission.

The prevalence of hepatitis C virus (HCV) infection among women of childbearing age in the United States is $\sim 1\%$ [1], corresponding to an estimated 40,000 births to HCV-positive pregnant women each year. Factors consistently associated with an increased risk of peri-

natal HCV transmission include the presence of maternal HCV RNA at the time of delivery and maternal coinfection with HCV and HIV [2]. The data have been inconsistent regarding the role of other risk factors, including obstetrical factors and maternal serum HCV RNA titers [2–5]. In addition, the timing and natural history of HCV infection among perinatally infected infants have not been fully determined [6–10]. To evaluate these issues, we conducted a prospective cohort study of HCV-infected women and their infants in Houston, Texas, and Honolulu, Hawaii.

SUBJECTS AND METHODS

Study population and data collection. In Houston during November 1993–July 1996, testing for antibody to HCV (anti-HCV) was offered to pregnant women attending public health clinics for prenatal care and to women with no prior prenatal care who presented for delivery at 2 county hospitals. In Honolulu, all pregnant

Received 9 May 2005; accepted 3 August 2005; electronically published 28 October 2005.

Presented in part: American Association for the Study of Liver Diseases 50th Annual Meeting, 5–9 November 1999, Dallas, Texas (abstract 1354).

Potential conflicts of interest: none reported.

Financial support: Centers for Disease Control and Prevention (contract no. 200-93-0688 with Kapiolani Medical Center for Women and Children, contract no. 200-93-0607 with the University of Texas Health Science Center at Houston, and an interagency personnel agreement with the Emory University School of Medicine).

^a Deceased.

^b Present affiliation: Avondale Estates, Georgia.

Reprints or correspondence: Dr. Eric E. Mast, Div. of Viral Hepatitis, Mailstop G37, 1600 Clifton Rd., Atlanta, GA 30333 (emast@cdc.gov).

The Journal of Infectious Diseases 2005;192:1880–9

© 2005 by the Infectious Diseases Society of America. All rights reserved.
0022-1899/2005/19211-0005\$15.00

women who received prenatal testing on Oahu during November 1994–April 1998 were offered testing. Women with positive or indeterminate anti-HCV test results were counseled regarding their test results, referred for medical evaluation, and invited to enroll in the study.

At enrollment, a questionnaire was administered to pregnant women, and a venous serum sample was collected. During hospital admission for delivery, an additional venous serum sample was collected from all mothers; colostrum samples were collected from mothers intending to breast-feed, and information regarding the pregnancy and intrapartum period was abstracted from the medical records.

Infant serum samples were collected at birth from the umbilical cord and from a peripheral venous site, as well as during well-child visits at ages 1–2, 4, 6, 9, 12, 15, 18, and 24 months. At each well-child visit, breast milk samples were collected from breast-feeding mothers, and information was obtained on breast-feeding, illnesses, and physical examination findings. Among infants with evidence of HCV infection, serum samples were obtained at follow-up visits every 6 months, from ages 2 to 5 years. The study protocol was approved by the institutional review board at each participating institution and the Centers for Disease Control and Prevention, and written informed consent was obtained from all mothers before entry into the study.

Laboratory testing. Serum was tested for anti-HCV by EIA (Abbott HCV EIA version 2.0 [Abbott Laboratories] or ORTHO HCV version 3.0 ELISA [Ortho-Clinical Diagnostics]), and samples with repeatedly reactive results were tested by RIBA (version 3.0; Chiron). Samples with RIBA-positive or -indeterminate results were tested for HCV RNA by modified reverse-transcriptase polymerase chain reaction (RT-PCR) (AMPLICOR HCV Test [version 2.0; Roche Molecular Systems]), by use of standard methods. Women with indeterminate anti-HCV results were enrolled in the study until confirmation of HCV infection status. Maternal serum with detectable levels of HCV RNA was tested for viral levels by use of a branched DNA assay (Quantiplex HCV RNA Assay; Chiron).

Serum from HCV-infected infants that was found to be anti-HCV negative by EIA 2.0 was retested by EIA 3.0. For uninfected infants tested by EIA 2.0, the first anti-HCV-negative sample after loss of maternal anti-HCV was retested by EIA 3.0. Serum from infected infants and peripheral venous serum collected at birth from uninfected infants that was found to be HCV RNA negative by AMPLICOR was retested by nested RT-PCR [1].

HCV genotype was determined by direct sequencing of the NS5b region in serum from all mothers who transmitted HCV and a random selection of approximately half of HCV RNA-positive mothers who did not transmit HCV [1]. Testing for serum alanine aminotransferase (ALT) levels was performed within 24 h of sample collection, using standard methods.

Testing for antibody to HIV (anti-HIV) was performed, in accordance with local standards, by EIA (HIVAB HIV-1 EIA; Abbott Laboratories) on serum from all mothers at enrollment and from infants born to HIV-infected mothers at age ≥ 15 months. Results indicating EIA-reactive serum were confirmed by Western blot (Cambridge Biotech HIV-1 Western Blot Kit; Calypte Biomedical).

HCV RNA was extracted from breast milk, using the MasterPure complete DNA and RNA purification kit (Epicentre Technologies). Briefly, 100 μ L of breast milk samples and 50 μ L of RNase-free water were mixed with 300 μ L of 2 \times T and C lysis buffer containing 100 μ g of proteinase K. After lysis, proteins were removed using protein precipitation reagent, the supernatant containing total nucleic acid was precipitated using isopropanol, and the pellet was washed with 75% ethanol.

Case definitions Mothers were classified as HCV positive if their serum was found to be positive for anti-HCV by RIBA or for HCV RNA. Mothers with serum testing as RIBA indeterminate and HCV RNA negative were excluded from the analysis.

Infants were classified as HCV infected if their serum was found to be positive for HCV RNA on at least 2 follow-up visits or was found to be anti-HCV positive at age ≥ 24 months. Infants who were persistently HCV RNA negative and who seroconverted from anti-HCV positive to anti-HCV negative during follow-up were considered to be uninfected.

Statistical analysis. Data analysis was conducted using SAS for Windows (version 6.12; SAS Institute). For univariate analyses, rates of HCV transmission were compared using Fisher's exact test. Statistical significance of relative risk (RR) estimates was determined by calculating *P* values and exact 95% confidence intervals (CIs). Multivariate analysis was conducted using logistic regression.

RESULTS

Maternal characteristics. Overall, 75,909 pregnant women were tested for anti-HCV, and 567 (0.75%) were confirmed as being HCV positive. The proportions of EIA-positive results and confirmed positive results were similar at both sites. Of the 567 HCV-positive women, 332 agreed to enroll; the final analysis included 242 women and their 244 live-born infants who completed ≥ 12 months of follow-up. Of the 242 women, 126 (52.3%) reported a history of injection drug use, 44 (19.8%) reported blood transfusion before donor screening, and 149 (61.6%) reported having been incarcerated. Frequencies of demographic characteristics, pregnancy history, and HCV risk factors among these women were similar to those among women who did not complete follow-up (table 1).

HCV RNA was detected at enrollment or delivery in 194 (79.5%) of 242 women; the mean time from enrollment to delivery was 93 days. Of 232 women tested at both enrollment and delivery, HCV RNA was detected at both times in 179

Table 1. Characteristics of enrolled women, according to whether they were included in the final analysis (>12 months of follow-up).

Characteristic	Women included (<i>n</i> = 242 ^a)	Women not included (<i>n</i> = 90 ^b)
Age at enrollment, years		
<20	7 (2.9)	3 (3.3)
20–29	103 (42.6)	35 (38.9)
30–39	120 (49.6)	43 (47.8)
≥40	12 (4.9)	9 (10.0)
Race/ethnicity		
White, non-Hispanic	79 (32.6)	29 (32.6)
Black	77 (31.8)	21 (23.6)
Hispanic	49 (20.3)	18 (20.2)
Other	37 (15.3)	21 (23.3)
Pregnancies, mean, no.	4.5	5.2 ^c
Prior live births, mean, no.	2.1	1.9 ^c
Nulliparous	49 (20.3)	9 (25.0) ^c
History of injection drug use	126 (52.3)	41 (47.1)
History of blood transfusion before 1992	44 (19.8)	12 (15.2)
HIV positive	11 (4.6)	0 (0.0)

NOTE. Data are no. (%) of women, unless otherwise indicated.

^a Two women gave birth to twins.

^b One woman gave birth to twins.

^c *n* = 36; this information was collected at delivery, and some women were lost to follow-up before delivery.

(77.2%), only at delivery in 5 (2.2%), and only at enrollment in 4 (1.7%). Eleven (4.5%) of the women included in the final analysis were anti-HIV positive; 7 (63.6%) of these women were HCV RNA positive.

Among HCV RNA-positive women, geometric mean HCV RNA levels at delivery were higher in the 7 who were HCV/HIV coinfectd (1.48×10^7 genome copies/mL) than in the 187 who were HIV negative (2.38×10^6 genome copies/mL) (Wilcoxon $P < .001$). Independently of HIV status, geometric mean HCV RNA levels increased between enrollment and delivery. Mean HCV RNA levels increased from 7.28×10^6 to 1.48×10^7 genome copies/mL ($P = .05$) in HIV-positive women and from 1.63×10^6 to 2.38×10^6 genome copies/mL ($P < .001$) in HIV-negative women.

Rate of perinatal HCV transmission. Overall, 3.7% (95% CI, 1.8%–7.1%; 9/244) infants became infected with HCV. Infection developed in 0% (0/54) of infants born to mothers without detectable HCV RNA at delivery, compared with 4.6% (9/190) of infants born to HCV RNA-positive mothers (RR, undefined $P = 0.12$). Among HCV RNA-positive mothers, the rate of transmission from those who were HIV negative was 3.8% (7/182), compared with 25.0% (2/8) from those who were HIV positive (RR, 6.5 [95% CI, 1.6–26.4]); 1 of the 2 HCV-infected infants born to an HCV/HIV-coinfectd mother was coinfectd with HIV.

Of the 9 transmitting mothers, 8 (88.9%) were infectd with genotype 1a, and 1 (11.1%) was infectd with genotype 3a. Of the 107 nontransmitting HCV RNA-positive mothers tested

for genotype, 68 (63.6%) had genotype 1a, 16 (15.0%) had genotype 1b, 10 (9.3%) had genotype 2b, 12 (11.2%) had genotype 3a, and 1 (0.9%) had genotype 4a. The distributions were not significantly different between the 2 groups.

Risk factors for transmission. Analysis of risk factors was restricted to the 190 infants born to mothers with detectable HCV RNA at delivery. Maternal HCV RNA levels among HCV-transmitting and -nontransmitting mothers, stratified by maternal HIV status, are presented in figure 1. Among HIV-negative mothers, geometric mean HCV RNA levels were higher in those who transmitted HCV (8.9×10^6 genome copies/mL) than in those who did not transmit HCV (2.2×10^6 genome copies/mL) (Wilcoxon $P = .02$), and the risk increased with increasing level ($P = .03$, Cochran-Armitage test for trend) (table 2). Among HIV-infected mothers, geometric mean HCV RNA levels were lower in those who transmitted HCV (5.6×10^6 genome copies/mL) than in those who did not transmit HCV (2.0×10^7 genome copies/mL), but this finding was not statistically significant ($P = .11$) (figure 1).

Among the HCV RNA-positive, HIV-negative mothers and their 182 infants, maternal characteristics associated with transmission in the univariate analysis included membrane rupture >6 h before delivery (RR, 9.9 [95% CI, 1.2–81.0]) and use of internal fetal monitoring devices (uterine or fetal scalp) (RR, 7.7 [95% CI, 1.9–32.3]) (table 2). Of the 7 HIV-negative mothers with HCV-infected infants, 6 (H026, H058, H102, H139, K005, and K048) had membrane rupture >6 h before delivery, 3 (H102, K005, and K048) had internal fetal monitoring, and

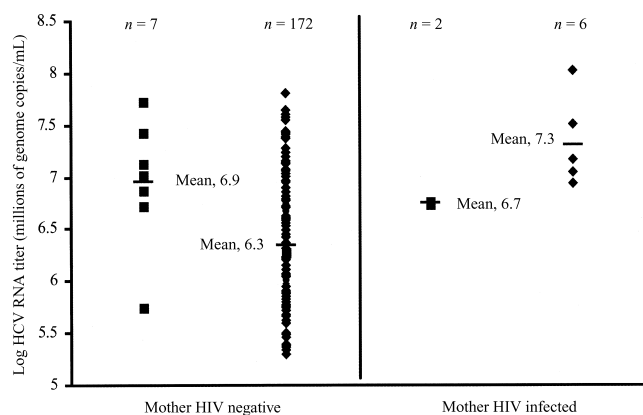


Figure 1. Hepatitis C virus (HCV) RNA levels among mothers who transmitted HCV to their infants (■) and mothers who did not transmit (◆), by maternal HIV infection status.

1 (H007) had neither risk factor. The maternal HCV RNA level for the transmitting mother without other risk factors was 7.6×10^6 genome copies/mL. No infant characteristics were associated with transmission (table 3).

Variables with $P < .1$ from the univariate analysis, along with maternal demographic characteristics, were included in the multivariate analysis. Maternal HCV RNA level was examined using 2 variables: $\geq 10^7$ versus $< 10^7$ genome copies/mL and $> 10^6$ versus $\leq 10^6$ genome copies/mL. In the final model, membrane rupture > 6 h (adjusted OR, 9.3 [95% CI, 1.5–179.7]) and use of internal fetal monitoring (adjusted OR, 6.7 [95% CI, 1.1–35.9]) were the only factors independently associated with transmission.

HCV RNA in colostrum and breast milk. The median duration of breast-feeding for the 63 women who breast-fed their infants was 1 month (mean, 4.3 months [range, 1–24 months]). HCV RNA was detected in at least 1 colostrum or breast milk sample from 19 (51.4%) of 37 HCV RNA-positive mothers who provided samples. None of these mothers transmitted HCV to their infants. Detection of HCV RNA in colostrum or breast milk was not related to HCV RNA level in maternal serum; at least 1 HCV RNA-positive breast milk or colostrum sample was detected in 5 (50%) of 10 mothers with serum HCV RNA levels $\leq 10^6$ genome copies/mL, 8 (47%) of 17 mothers with levels of 10^6 – 10^7 genome copies/mL, and 6 (60%) of 10 mothers with levels $> 10^7$ genome copies/mL ($P = .65$, χ^2 test for trend).

Natural history of HCV infection among infected infants. Diagnostic profile for the 9 HCV-infected infants are shown in figure 2. HCV RNA testing of cord blood or peripheral venous serum samples collected at birth did not distinguish between infected and uninfected infants. HCV RNA was detected by AMPLICOR in cord blood samples from 5 (62.5%) of 8 infected infants and 57 (37.8%) of 151 uninfected infants.

HCV RNA was detected by nested RT-PCR (but not by AMPLICOR) in the peripheral venous serum samples from 4 (66.7%) of 6 infected infants; 2 of these infants were born to HIV-positive mothers, and 2 were born to HIV-negative mothers. Although detection rates were lower in uninfected infants, 6 (7.4%) of 81 peripheral venous serum samples from uninfected infants were found to be HCV RNA positive by nested RT-PCR; none were HCV RNA positive in follow-up samples. All 9 infected infants were HCV RNA positive at age 2 months; in 8 infants, HCV RNA was detected by AMPLICOR, but in 1 infant HCV RNA was detected only by nested RT-PCR.

Anti-HCV profile among the 9 infected infants varied markedly (figure 2). Three infected infants (H026, H071, and K005) were found to be anti-HCV positive by EIA and RIBA during the entire follow-up period. Another 4 infants had serologic evidence indicating a loss of passively transferred maternal antibody, followed by seroconversion to anti-HCV positivity in response to infection. One of these infants (H139) tested anti-HCV positive by EIA during follow-up but tested negative by RIBA at age 9 months; the other 3 infants (H058, H065, and H085) tested anti-HCV negative by EIA on at least 1 follow-up visit. Of the final 2 infants, 1 (K048) seroconverted from anti-HCV indeterminate to anti-HCV negative between ages 15 and 24 months, and 1 (H102) seroconverted from anti-HCV positive to RIBA indeterminate between ages 48 and 60 months. Both of these seroconversions occurred after clearance of HCV RNA. In 5 infants, loss of anti-HCV or seroconversion to anti-HCV positive was preceded by EIA-positive but RIBA-indeterminate or -negative results.

None of the infected infants had any clinical signs or symptoms of hepatitis during 5 years of follow-up, although abnormal ALT activity was detected at least once in all infants (figure 2). Three (33%) of the infected infants (H071, H102, and K048) appeared to resolve their infection and were persistently HCV RNA negative with normal ALT levels beginning at age 12–18 months (figure 2).

Duration of anti-HCV and ALT results among uninfected infants. Among the 235 uninfected infants with ≥ 12 months of follow-up, maternal anti-HCV was detectable by EIA in 96.8% (215/222) at birth, 15.3% (30/196) at age 12 months, 4.8% (9/186) at age 15 months, 1.6% (3/190) at age 18 months, and 1.0% (2/196) at age 24 months (figure 3). All were found to be anti-HCV negative by EIA at age 30 months. Of uninfected infants with EIA-positive results, the proportion that were RIBA positive or RIBA indeterminate declined from 100% (215/215) at birth to 73% (22/30) at age 12 months. At age 15 months, 0 of 9 uninfected infants who still tested EIA positive were RIBA positive, although 1 infant was RIBA indeterminate. At age 18 months, all 3 infants with EIA-positive results were RIBA negative.

At least 1 abnormal ALT level was detected in 46 (19.3%)

Table 2. Risk of hepatitis C virus (HCV) infection among infants born to HCV RNA–positive, HIV-negative mothers, by maternal characteristics (univariate analysis).

Maternal characteristic	Infants, no. (%)		RR (95% CI)	P
	Total	Infected		
HCV RNA level, genome copies/mL				
≤10 ⁶	61 (33.5)	1 (1.6)03 ^a
>10 ⁶ , <10 ⁷	87 (47.8)	2 (2.3)	...	
≥10 ⁷	34 (18.7)	4 (11.8)	...	
Age at delivery, years				
≥30	100 (55.3)	5 (5.0)	2.0 (0.4–10.2)	.46
<30	81 (44.8)	2 (2.5)	...	
Prior pregnancies, no.				
>4	73 (40.1)	2 (2.7)	0.6 (0.1–3.0)	.70
≤4	109 (59.9)	5 (4.6)	...	
ALT level at delivery, U/L				
>35	45 (24.7)	3 (6.7)	2.3 (0.5–9.8)	.37
≤35	137 (75.3)	4 (2.9)	...	
Mode of delivery				
Vaginal	151 (83.4)	6 (4.0)	1.0 (reference)	1.0
Elective cesarean	12 (6.6)	0 (0.0)	Undefined	
Emergency cesarean	18 (9.9)	1 (5.5)	1.4 (0.2–11.1)	
Fetal monitoring				
Internal	16 (8.8)	3 (18.8)	7.7 (1.9–31.6)	.02
External	165 (91.2)	4 (2.4)	...	
Rupture of membranes before onset of labor				
Yes	45 (24.7)	4 (8.9)	4.1 (0.9–17.5)	.06
No	137 (75.3)	3 (2.2)	...	
Duration of membrane rupture, h				
<1	53 (29.1)	0 (0.0)02 ^a
1–5	59 (32.4)	1 (1.7)	...	
6–12	40 (22.0)	4 (10.0)	...	
≥13	30 (16.5)	2 (6.7)	...	
Duration of labor, h				
≤6	84 (47.7)	2 (2.4)78 ^a
7–12	48 (27.3)	4 (8.3)	...	
≥13	44 (25.0)	1 (2.3)	...	
Amniotic fluid				
Clear	129 (72.1)	2 (1.6)	1.0 (reference)	.03
Meconium	40 (22.4)	4 (10.0)	6.5 (1.2–33.9)	
Bloody	10 (5.6)	1 (10.0)	6.5 (0.6–65.2)	
Cigarette smoking during pregnancy				
Yes	99 (54.4)	1 (1.01)	0.14 (0.02–1.1)	.05
No	83 (45.6)	6 (7.23)	...	
Alcohol intake during pregnancy				
Yes	42 (23.1)	1 (2.4)	0.6 (0.1–4.5)	1.0
No	140 (76.9)	6 (4.3)	...	
History of injection drug use				
Yes	94 (51.7)	1 (1.1)	0.2 (0.02–1.27)	.06
No	88 (48.4)	6 (6.8)	...	

NOTE. ALT, alanine aminotransferase; CI, confidence interval; RR, relative risk.

^a Cochran-Armitage test for trend.

of 238 uninfected infants during follow-up; 32 (13.4%) had 1, 10 (4.2%) had 2, 3 (1.3%) had 3, and 1 (0.4%) had 4 abnormal ALT levels. Abnormal levels ranged from 1.02 to 4.93 (mean, 1.86) times the upper limit of normal.

DISCUSSION

The rates of HCV transmission to infants from HCV-infected, HIV-negative mothers (3.8%) and from HCV/HIV-coinfected

Table 3. Risk of hepatitis C virus (HCV) infection among infants born to HCV RNA-positive, HIV-negative mothers, by infant characteristic (univariate analysis).

Infant characteristic	Infants, no (%)		RR (95% CI)	P
	Total	Infected		
Sex				
M	85 (47.0)	2 (2.3)	0.45 (0.09–2.27)	.45
F	96 (53.0)	5 (5.2)		
Gestational age, weeks				
<37	27 (14.8)	0 (0.0)	Undefined	.6
≥37	155 (85.2)	7 (4.5)		
Birth weight, g				
<2500	22 (12.1)	1 (4.6)	1.2 (0.2–9.6)	1.0
≥2500	160 (87.9)	6 (3.8)		
Apgar score ^a at 5 min				
≤8	21 (11.5)	0 (0.0)	Undefined	1.0
>8	161 (88.5)	7 (4.4)		
Breast-fed				
Yes	62 (34.1)	2 (3.2)	0.8 (0.2–3.9)	1.0
No	120 (65.9)	5 (4.2)		

NOTE. CI, confidence interval; RR, relative risk.

^a The Apgar score measures an infant's heart rate, breathing muscle tone, reflex response, and color at birth.

mothers (25%) in the present study are consistent with finding from other studies [2]. Our results provide information that can be used for counseling regarding the risk of transmission through breast-feeding, the timing of follow-up to distinguish infected from uninfected infants, and the course of infection during the first 5 years of life. The study also provides insight into risk factors that might facilitate and interventions that might prevent perinatal HCV transmission.

Transmission occurred only from mothers who were HCV RNA positive, which is consistent with finding from other studies. The only 2 documented episodes of transmission from HCV RNA-negative mothers [11, 12] could be explained as resulting from the use of testing methods that were not sensitive enough to detect low levels of HCV RNA or from intermittent HCV RNA detection in an HCV-infected pregnant woman [13]. Although we detected HCV RNA intermittently among some women, >96% had consistent results when tested both prenatally and at delivery, indicating that a single HCV RNA test during pregnancy should be sufficient for diagnosis and counseling. Maternal HCV RNA testing should be required in studies of perinatal HCV transmission, and analyses should be restricted to HCV RNA-positive mothers or stratified by HCV RNA status.

Although higher maternal viral levels increase the risk of perinatal transmission of HIV and hepatitis B virus (HBV) [14–16], data on associations between maternal HCV RNA level and perinatal transmission have been inconsistent. In univariate analysis of HCV-positive, HIV-negative mothers, the risk of

transmission has been found to increase with increasing maternal HCV RNA level, and mean levels were found to be higher in transmitting than in nontransmitting mothers. However, maternal HCV RNA level was not independently associated with transmission in multivariate analysis among HIV-negative women. A direct relationship between maternal viral level and increased transmission rates has been reported by some investigators [4, 5, 8, 17–20] but not by others [10, 21, 22]. Furthermore, among the studies reporting an association, the threshold viral level associated with transmission has differed. Reasons for the inconsistencies could include the use of different methods to quantify HCV RNA levels, combining HIV-negative and HCV/HIV-coinfected women in analyses, and unrecognized statistical interactions between viral level and other factors. Given the inconsistency of results and the evidence for transmission from women with HCV RNA levels <10⁶ genome copies/mL, we believe that HCV RNA level cannot be used to counsel HCV RNA-positive women about their risk for perinatal HCV transmission.

Perinatal HCV transmission could occur in utero, during the intrapartum period, or postnatally. We found no evidence for postnatal transmission, because all infected infants were HCV RNA positive by age 2 months, which is consistent with HCV exposure occurring at or before the time of delivery. This finding contrasts with the pattern of mother-to-child HBV transmission, in which as many as 40% of infants born to HBV-infected mothers who are not infected during the intrapartum period become infected during the first 18 months of life [23].

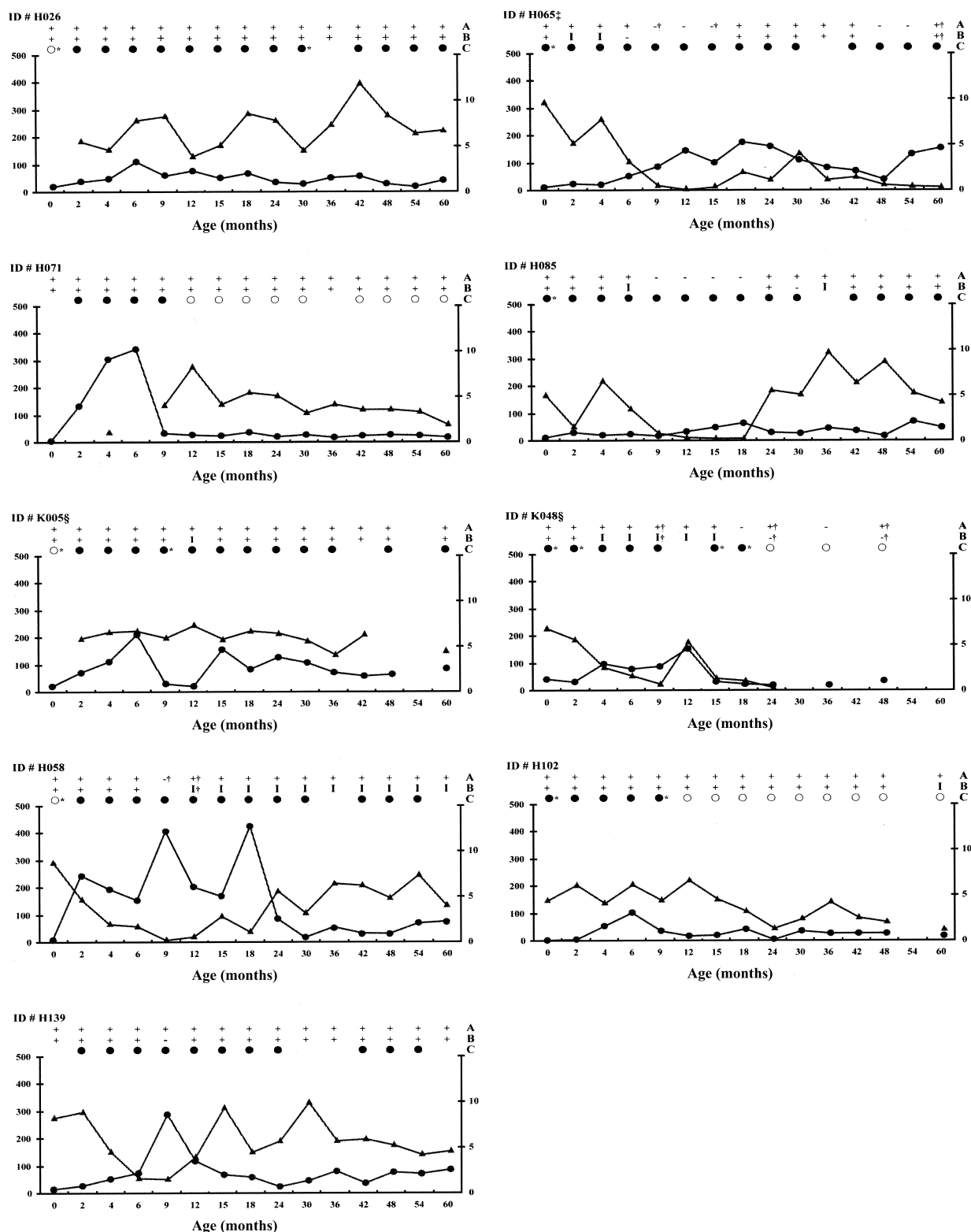


Figure 2. Levels of alanine aminotransferase in units per liter (circles, left y axis) and EIA signal-to-cutoff ratio (triangles, right y axis) for the 9 infants with perinatal hepatitis C virus (HCV) infection. Results of EIA to detect antibody to HCV (anti-HCV) are shown in line A (+/-). Supplemental anti-HCV test results are shown in line B (+/-/I [indeterminate]). HCV RNA results are shown in line C (● [positive]/○ [negative]). Asterisks (*) indicate nested polymerase chain reaction test results of samples testing negative by AMPLICOR, daggers (†) indicate EIA/RIBA 3.0 test results of samples testing negative by EIA 2.0, double daggers (§) indicate HIV infection in the infant, and section symbols (§) indicate that the infant was breast-fed.

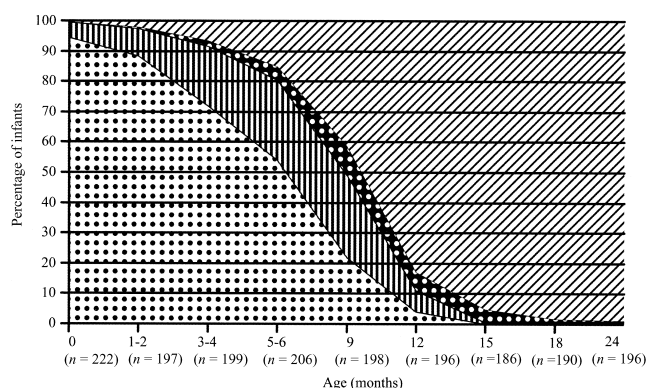


Figure 3. Persistence of maternal antibody to hepatitis C virus (anti-HCV) among uninfected infants. Shaded areas, from left to right, are as follows: (1) the percentage of infants with EIA-positive/RIBA-positive anti-HCV results, (2) the percentage of infants with EIA-positive/RIBA-indeterminate anti-HCV results, (3) the percentage of infants with EIA-positive/RIBA-negative anti-HCV results, and (4) the percentage of infants with EIA-negative results.

In addition, breast-feeding was not associated with transmission, which is consistent with finding of other studies [3, 12, 19, 22, 24–27] and provides further support for recommendations that HCV-positive, HIV-negative women can safely breast-feed [28, 29]. Differentiation of in utero from intrapartum transmission is primarily based on detection of HCV RNA at the time of birth. Although HCV RNA was detected in peripheral venous serum samples from some infected infants at birth by use of nested PCR in the present study, HCV RNA was also detected in uninfected infants, suggesting that HCV RNA positivity at birth may reflect passive transferral of maternal virus rather than intrauterine transmission.

Recommendations for screening and follow-up of infants born to HCV-infected mothers include anti-HCV testing at age >15 months or nucleic acid testing on 2 occasions between ages 2 and 6 months [30]. Our finding suggest that it may be prudent to delay anti-HCV testing until age >18 months, because 2 infected infants who were anti-HCV negative at 15 months subsequently seroconverted to anti-HCV positivity, one between age 15 and 18 months and the other between age 18 and 24 months. Testing at age >18 months also eliminated all false-positive results in infants with passively acquired anti-HCV, if RIBA testing was used to verify anti-HCV EIA screening test–positive results [31]. Our finding support the recommendation for nucleic acid testing, because all infected infants were HCV RNA positive at ages 2, 4, and 6 months. Testing before age 2 months cannot be recommended, because detection of HCV RNA in both cord and peripheral venous serum samples collected at birth is likely to indicate contamination with maternal blood or passive transfer of maternal HCV RNA, rather than infant infection.

Although none of the infected infants in this study had clin-

ical evidence of hepatitis during 5 years of follow-up, all had abnormal ALT levels at some point. These findings indicate that liver disease among HCV-infected infants is generally mild [32, 33]. The higher proportion of HCV-infected infants in the present study who resolved their infections, compared with those in follow-up studies of persons infected at older ages [5, 32–35], demonstrates the importance of follow-up testing of HCV-infected infants to determine the clinical progression of infection.

Among infants born to HCV-infected, HIV-negative mothers, longer duration of membrane rupture and invasive fetal monitoring were associated with transmission. Of 2 studies that evaluated duration of membrane rupture [5, 36], 1 found increased transmission rates associated with longer duration [5]. One study found increased transmission rates associated with fetal scalp monitoring [37], and another found increased transmission rates associated with intrapartum exposure to maternal blood [4]. One hypothesis to explain these findings is that perinatal transmission generally occurs during the intrapartum period and is related to infant exposure to maternal genital tract secretions or blood. Although one study found no evidence of virus in genital tract secretions [38], another detected virus in the cellular fraction of cervicovaginal secretions of 27% of infected women [39]. Both longer duration of exposure of infant mucous membranes and percutaneous inoculation of the infant could enhance transmission.

Our findings suggest that avoiding internal fetal monitoring and/or performing cesarean section before or soon after membrane rupture could decrease the risk of perinatal transmission from HCV-infected mothers who were identified prenatally. However, results from studies comparing risk for HCV transmission among infants delivered vaginally and infants delivered by cesarean section are conflicting [2, 3, 11, 12, 18, 26]. In addition, in virtually all of these studies, most study participants were HCV/HIV coinfecting, coinfecting and HIV-negative women were combined for analysis, and, in some instances, other potential risk factors were not accounted for. These features make it difficult to determine whether the results apply to infants born to women infected only with HCV. In addition, only 1 study differentiated elective cesarean sections performed before membrane rupture from emergency cesarean sections; that study found a lower transmission rate from mothers who delivered by elective cesarean section than from mothers who delivered by emergency cesarean section, although the analysis also included HCV/HIV-coinfecting women [3]. Among HIV-infected women, elective cesarean section performed before onset of labor and rupture of membranes not only was associated with lower HIV transmission rates but also was shown, in a randomized trial, to be an effective intervention [40, 41]. In our study, no transmission occurred from mothers who delivered by elective cesarean section, but the number of such deliveries was low.

Our finding support existing recommendations to avoid internal fetal monitoring and prolonged labor after rupture of membranes in HCV-infected pregnant women [30]. Current recommendations regarding the need for cesarean section versus vaginal delivery are not based on HCV infection status. Any changes in cesarean section practices for HCV-infected pregnant women should be considered cautiously and should be based on separate studies of HCV/HIV-coinfected women and women infected only with HCV. Ideally, prospective studies should be conducted to determine whether elective cesarean section delivery reduces the risk for perinatal HCV transmission.

Acknowledgments

We thank the patients who volunteered to participate in this study and gratefully acknowledge assistance from the following members of the study team: from the University of Texas Health Science Center, Houston: Cathy Troisi and Margaret Dybala (study coordination); Irene Collier and Carolyn Grimes (laboratory testing); Carolyn Kelley and Madelyn Randle (patient enrollment and follow-up); Fen-Yuan Xia, Lara Bull, and Jennifer Karama (data collection and data management); Nancy Ericson; and Marilyn Doyle (patient enrollment and follow-up); from the Houston Department of Health and Human Services: Sulabha Hardikar and Vern Juchau (laboratory testing); from the Texas Medical Center, Baylor Medical College: Hunter Hammill, Larry Taber, and Garcia Pratt (patient enrollment and follow-up); from the Kapiolani Medical Center and University of Hawaii School of Medicine, Honolulu: Eunice Irinaka, Patty Iwamoto, and Arwind Diwan (study coordination); from the Centers for Disease Control and Prevention, Atlanta: Christine Arcari, Mack Kelly, and Christy Ray (data management); David Culver and Greg Armstrong (statistical analysis); Steve Lambert, Margaret Gallagher, Wendi Kuhnert, Mar Than, Feng-Xiang Gao, and Feng Chai (laboratory testing); and Allison Greenspan (manuscript preparation).

References

- Alter MJ, Kruszon-Moran D, Nainan OV, et al. The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. *N Engl J Med* **1999**; 341:556–62.
- Yeung LT, King SM, Roberts EA. Mother-to-infant transmission of hepatitis C virus. *Hepatology* **2001**; 34:223–9.
- Gibb DM, Goodall RL, Dunn DT, et al. Mother-to-child transmission of hepatitis C virus: evidence for preventable peripartum transmission. *Lancet* **2000**; 356:904–7.
- Steininger C, Kundi M, Jatzko G, Kiss H, Lischka A, Holzmann H. Increased risk of mother-to-infant transmission of hepatitis C virus by intrapartum infantile exposure to maternal blood. *J Infect Dis* **2003**; 187:345–51.
- Spencer JD, Latt N, Beeby PJ, et al. Transmission of hepatitis C virus to infants of human immunodeficiency virus-negative intravenous drug-using mothers: rate of infection and assessment of risk factors for transmission. *J Viral Hepat* **1997**; 4:395–409.
- Dunn DT, Gibb DM, Healy M, et al. Timing and interpretation of tests for diagnosing perinatally acquired hepatitis C virus infection. *Pediatr Infect Dis J* **2001**; 20:715–6.
- Palomba E, Manzini P, Fiammengio P, Maderni P, Saracco G, Tovo PA. Natural history of perinatal hepatitis C virus infection. *Clin Infect Dis* **1996**; 23:47–50.
- Ohto H, Terazawa S, Sasaki N, et al. Transmission of hepatitis C virus from mothers to infants. The Vertical Transmission of Hepatitis C Virus Collaborative Study Group. *N Engl J Med* **1994**; 330:744–50.
- European Paediatric Hepatitis C Virus Infection Network, Tovo PA, Pembrey LJ, Newell ML. Persistence rate and progression of vertically acquired hepatitis C infection. *J Infect Dis* **2000**; 181:419–24.
- Resti M, Azzari C, Mannelli F, et al. Mother to child transmission of hepatitis C virus: prospective study of risk factors and timing of infection in children born to women seronegative for HIV-1. Tuscany Study Group on Hepatitis C Virus Infection. *BMJ* **1998**; 317:437–41.
- Granovsky MO, Minkoff HL, Tess BH, et al. Hepatitis C virus infection in the Mothers and Infants Cohort Study. *Pediatrics* **1998**; 102:355–9.
- Resti M, Azzari C, Galli L, et al. Maternal drug use is a preeminent risk factor for mother-to-child hepatitis C virus transmission: results from a multicenter study of 1372 mother-infant pairs. *J Infect Dis* **2002**; 185:567–72.
- Dore GJ, Kaldor JM, McCaughan GW. Systematic review of role of polymerase chain reaction in defining infectiousness among people infected with hepatitis C virus. *BMJ* **1997**; 315:333–7.
- Garcia PM, Kalish LA, Pitt J, et al. Maternal levels of plasma human immunodeficiency virus type 1 RNA and the risk of perinatal transmission. Women and Infants Transmission Study Group. *N Engl J Med* **1999**; 341:394–402.
- Mofenson LM, Lambert JS, Stiehm ER, et al. Risk factors for perinatal transmission of human immunodeficiency virus type 1 in women treated with zidovudine. Pediatric AIDS Clinical Trials Group Study 185 Team. *N Engl J Med* **1999**; 341:385–93.
- Beasley RP, Trepo C, Stevens CE, Szmuness W. The e antigen and vertical transmission of hepatitis B surface antigen. *Am J Epidemiol* **1977**; 105:94–8.
- Lin HH, Kao JH, Hsu HY, et al. Possible role of high-titer maternal viremia in perinatal transmission of hepatitis C virus. *J Infect Dis* **1994**; 169:638–41.
- Okamoto M, Nagata I, Murakami J, et al. Prospective reevaluation of risk factors in mother-to-child transmission of hepatitis C virus: high virus load, vaginal delivery, and negative anti-NS4 antibody. *J Infect Dis* **2000**; 182:1511–4.
- Moriya T, Sasaki F, Mizui M, et al. Transmission of hepatitis C virus from mothers to infants: its frequency and risk factors revisited. *Biomed Pharmacother* **1995**; 49:59–64.
- Thomas DL, Villano SA, Riester KA, et al. Perinatal transmission of hepatitis C virus from human immunodeficiency virus type 1-infected mothers. Women and Infants Transmission Study. *J Infect Dis* **1998**; 177:1480–8.
- La Torre A, Biadaoli R, Capobianco T, et al. Vertical transmission of HCV. *Acta Obstet Gynecol Scand* **1998**; 77:889–92.
- Conte D, Fraquelli M, Prati D, Colucci A, Minola E. Prevalence and clinical course of chronic hepatitis C virus (HCV) infection and rate of HCV vertical transmission in a cohort of 15,250 pregnant women. *Hepatology* **2000**; 31:751–5.
- Beasley RP, Hwang LY. Postnatal infectivity of hepatitis B surface antigen-carrier mothers. *J Infect Dis* **1983**; 147:185–90.
- Dal Molin G, D'Agaro P, Ansaldi F, et al. Mother-to-infant transmission of hepatitis C virus: rate of infection and assessment of viral load and IgM anti-HCV as risk factors. *J Med Virol* **2002**; 67:137–42.
- Tovo PA, Palomba E, Ferraris G, et al. Increased risk of maternal-infant hepatitis C virus transmission for women coinfected with human immunodeficiency virus type 1. Italian Study Group for HCV Infection in Children. *Clin Infect Dis* **1997**; 25:1121–4.
- Paccagnini S, Principi N, Massironi E, et al. Perinatal transmission and manifestation of hepatitis C virus infection in a high risk population. *Pediatr Infect Dis J* **1995**; 14:195–9.
- Mazza C, Ravaggi A, Rodella A, et al. Prospective study of mother-to-infant transmission of hepatitis C virus (HCV) infection. Study Group for Vertical Transmission. *J Med Virol* **1998**; 54:12–9.
- Roberts EA, Yeung L. Maternal-infant transmission of hepatitis C virus infection. *Hepatology* **2002**; 36(Suppl):S106–13.
- Centers for Disease Control and Prevention. Recommendations for prevention and control of hepatitis C virus (HCV) infection and HCV-related chronic disease. *MMWR Recomm Rep* **1998**; 47(RR-19):1–39.

30. National Institutes of Health Consensus Development Conference Statement. Management of hepatitis C: 2002. June 10–12, 2002. Available at: <http://consensus.nih.gov/2002/2002HepatitisC2002116html.htm>. Accessed 17 October 2005.
31. Alter MJ, Kuhnert WL, Finelli L. Guidelines for laboratory testing and result reporting of antibody to hepatitis C virus. Centers for Disease Control and Prevention. MMWR Recomm Rep **2003**; 52(RR-3):1–13, 15.
32. Resti M, Jara P, Hierro L, et al. Clinical features and progression of perinatally acquired hepatitis C virus infection. J Med Virol **2003**; 70: 373–7.
33. Bortolotti F, Resti M, Giacchino R, et al. Hepatitis C virus infection and related liver disease in children of mothers with antibodies to the virus. J Pediatr **1997**; 130:990–3.
34. Ni YH, Lin HH, Chen PJ, Hsu HY, Chen DS, Chang MH. Temporal profile of hepatitis C virus antibody and genome in infants born to mothers infected with hepatitis C virus but without human immunodeficiency virus coinfection. J Hepatol **1994**; 20:641–5.
35. Xiong SK, Okajima Y, Ishikawa K, Watanabe H, Inaba N. Vertical transmission of hepatitis C virus: risk factors and infantile prognosis. J Obstet Gynaecol Res **1998**; 24:57–61.
36. Healy CM, Cafferkey MT, Conroy A, et al. Outcome of infants born to hepatitis C infected women. Ir J Med Sci **2001**; 170:103–6.
37. European Paediatric Hepatitis C Virus Network. Effects of mode of delivery and infant feeding on the risk of mother-to-child transmission of hepatitis C virus. BJOG **2001**; 108:371–7.
38. Hsu HH, Wright TL, Luba D, et al. Failure to detect hepatitis C virus genome in human secretions with the polymerase chain reaction. Hepatology **1991**; 14:763–7.
39. Belec L, Legoff J, Si-Mohamed A, et al. Cell-associated, non-replicating strand⁽⁺⁾ hepatitis C virus-RNA shedding in cervicovaginal secretions from chronically HCV-infected women. J Clin Virol **2003**; 27:247–51.
40. The European Mode of Delivery Collaboration. Elective caesarean-section versus vaginal delivery in prevention of vertical HIV-1 transmission: a randomised clinical trial. Lancet **1999**; 353:1035–9.
41. The International Perinatal HIV Group. The mode of delivery and the risk of vertical transmission of human immunodeficiency virus type 1—a meta-analysis of 15 prospective cohort studies. N Engl J Med **1999**; 340:977–87.