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Use of Polymerase Chain Reaction and Antibody Tests in the Diagnosis of Vertically Transmitted Hepatitis C Virus Infection

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Data on patterns of polymerase chain reaction (PCR) and antibody test results in infants born to hepatitis C virus (HCV)-infected mothers were systematically reviewed to aid development of optimum testing schedules and diagnostic criteria for vertically exposed infants and to facilitate early identification of infected infants. Survival and cross-sectional analyses were used to estimate the timing of initial PCR positivity and subsequent PCR negativity in infected infants, and maternal antibody loss in uninfected infants was estimated as a weighted average of individual study findings. Of 74 eligible infants with strong evidence of HCV infection, an estimated 89% (90% confidence interval, 80–95%) were first PCR positive by 3 months of age, and less than 10% had subsequent PCR negativity attributable to intermittent viraemia or resolved infection in the first 18 months of life. The negative predictive value of PCR at 3 months of age was greater than 98% at an assumed rate of 5% vertical transmission, but as low as 88% at 25% transmission. The inclusion of 22 infants, each with a single PCR-positive result, increased the estimated frequency of resolved infections but made little difference to other estimates. A minority of PCR-positive infants had periods of antibody negativity by second- or third-generation assays, and among 297 uninfected infants, maternal antibody was not detected beyond 18 months. Thus, the majority of infected infants may be persistently PCR positive from 3 months of age, and the negative predictive value of PCR at 3 months is generally high. However, poor repeatability of PCR, inadequate infant follow-up, and inclusion of postnatally infected infants limits interpretation of the pooled data. Further studies using standardised PCR methodologies are needed.

Prospective studies of hepatitis C virus (HCV) vertical transmission mostly report low transmission rates among unselected maternal populations but increased rates among women coinfected with the human immunodeficiency virus (HIV) or with high levels of HCV viraemia (1–3). However, reported transmission rates vary widely (0–100%) (1, 4), and studies differ considerably in the definitions used for HCV infection and noninfection in vertically exposed infants and in the duration and frequency of infant follow-up (5).

Clarification of patterns of HCV RNA and antibody positivity in vertically exposed infants would assist in the development of standardised HCV diagnostic criteria for use in vertical transmission studies, improve comparability of study results, and allow early identification of infected infants. We therefore analysed data on the time at which HCV RNA is first detectable in vertically infected infants, the frequency of subsequent polymerase chain reaction (PCR)-negative results in these infants due to intermittent viraemia or resolved infection, and the patterns of antibody seropositivity in both infected and uninfected children.

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Materials and Methods

Data Identification. Published studies of HCV vertical transmission and the natural history of HCV infection in vertically infected infants were identified by computerised searches of Medline and Embase (January 1990 to July 1996), supplemented by hand searching selected journals and by scrutinising references from articles, reviews, and books. Conference abstracts were located by computerised literature searches of the Index of Scientific and Technical Proceedings and the British Library's Boston Spa Conferences Database and by hand searching printed proceedings from relevant conferences. Unpublished data were obtained via structured questionnaires on HCV vertical transmission sent to 63 researchers with published research or otherwise identified as carrying out work in this area. The questionnaire included questions on patterns of antibody positivity in uninfected infants.

Data Exclusion Criteria/Extraction. Excluded data comprised antibody test results from unspecified or first-generation assays (due to their poor sensitivity and specificity) (6), HCV RNA test results on cord blood or unspecified samples taken at birth, results from infants with no serological test results in the first year, and data duplicated in other published reports. Information was then extracted on testing schedules for infants, the assays used, and temporal patterns of infant HCV RNA and antibody test results.

Data Analyses. Eligible infants with at least one PCR-positive result were considered potentially HCV infected and were further categorised into two groups: group A (probably infected) comprised those with two or more PCR-positive results or with a single PCR-positive result and other evidence of infection (such as antibody seroconversion or a change in recombinant immunoblot assay (RIBA) reactivity); group B (possibly infected) comprised those with only a single PCR-positive result. Survival analysis for interval censored data was used to estimate when HCV RNA could first be detected in these infants, from the time of the last negative and first positive test result in each infant (using conception as the time of last negativity for infants who were positive on initial testing), and deriving likelihood ratio-based 90% confidence intervals (90% CI) (7, 8). Data for these analyses were restricted to studies in which the timing of the first PCR-positive result was reported for every infected infant to minimise bias due to selective reporting of unusual PCR findings.

The survival analysis approach assumes that once initial PCR positivity occurs, infected infants will remain PCR positive. This may not be the case in HCV infection, as intermittent viraemia or resolution of infection has been reported (9, 10). To quantify subsequent PCR negativity among infected infants in the first 18 months, cross-sectional analyses of PCR results at various times after birth were carried out. Infected infants with one or more subsequent negative result were categorised as having intermittent viraemia if they remained antibody positive (or lost antibody only on first-generation tests) and any PCR-negative result was followed by renewed PCR positivity; as having probable resolved infection if there was persistent loss of viraemia and antibody seroreversion; or being in either category if the last PCR result was negative without evidence of antibody seroreversion.

The negative predictive value of a PCR test at age 3 months was calculated using the standard formula (11), deriving test sensitivity from the proportion of infants ultimately categorised as infected who were PCR negative at 3 months, and assuming a range of vertical transmission rates and levels of PCR false positivity. Upper and lower estimates of negative predictive value were similarly calculated using the upper and lower values of the exact 90% CI for the proportion of infected infants who were PCR negative at 3 months, after deriving this interval from the binomial distribution. In uninfected infants, age at loss of maternal antibody was estimated by calculating the weighted average of findings from studies with complete ascertainment of such loss.

Results

Published and unpublished data fulfilling inclusion criteria were identified from 48 studies, six of which (12, 13, F. Bartolotti et al., MG Ercilla et al.,

Table 1: Pooled polymerase chain reaction (PCR) test results for 74 hepatitis C virus (HCV)-infected infants at various times in the first 18 months of life, with categorisation of PCR-negative infants at each age into those negative on initial testing (column 4), those previously tested with at least one previous PCR-positive result (column 5), and those previously tested but never previously PCR positive (column 6).

Infant age	No. tested by PCR	Total no. (%) PCR negative	Infants found to be PCR negative		
			No. not tested previously (age when first PCR positive)[a]	No. tested previously	
				Previously PCR-positive (ref.)[b]	Never previously PCR positive
Birth	3	0 (0)	–	–	–
1–29 days	11	2 (18)	2 (4,4)	0	0
1 month	14	3 (21)	3 (2, 3, 3)	0	0
2 months	8	0 (0)	–	–	–
3 months	39	5 (13)	5 (5, 6, 6, 8, 17)	0	0
4–6 months	48	1 (2)	0	1 (6)	0
7–9 months	20	1 (5)	0	1 (5)	0
10–12 months	39	4 (10)	0	4 (1, 2, 5, 6)	0
13–15 months	23	2 (9)	0	2 (4, 6)	0
16–18 months	18	4 (22)	0	4 (1, 3, 4, 5)	0

[a] Age at first subsequent PCR-positive result (months).
[b] References to individual infants (further detailed in Figure 2).

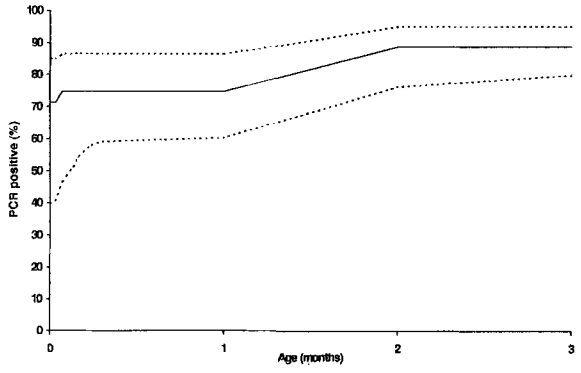

Figure 1: Estimated timing of first polymerase chain reaction (PCR) positivity in 74 infants with more than one PCR-positive test or with other evidence of hepatitis C virus infection. Solid line, percent PCR positive; dotted line, 90% confidence intervals.

GV Zuccotti et al., and R. Giacchino et al., IX Triennial International Symposium on Viral Hepatitis and Liver Disease, 1996, Abstract no. C35, Abstract no. B240, Abstract no. B182, and Abstract no. B169, respectively) contained data reported in six other publications (2, 14–18).

Timing of Initial Polymerase Chain Reaction Positivity. Ninety-six eligible PCR-positive infants were identified from the 26 studies containing sufficient information on PCR positivity for every infected infant (2–4, 14, 15, 17–37). Seventy-four of the 96 infants were categorised as group A (probably infected). These 74 infants were first tested at ages ranging from 0 to 12 months, with 26 (35%) tested at least once by age 1 month and 50 (70%) by age 3 months. Table 1 summarises cross-sectional analyses of all PCR-negative results among the 74 infants in the first 18 months of life. Ten (14%) infants were PCR negative on initial testing (column 4), with the first PCR-positive result documented at age 2 to 17 months. Figure 1 illustrates the estimated proportion of the 74 infants who became PCR positive in the first three months of life, taking into account the censored nature of the data. An estimated 74.8% of infants (90% CI, 60.4–86.5%) were first PCR-positive by 1 month of age, and 88.8% (90% CI, 79.8–95.1%) by 3 months.

Twenty-two of the 96 PCR-positive infants had only a single eligible PCR-positive result and were

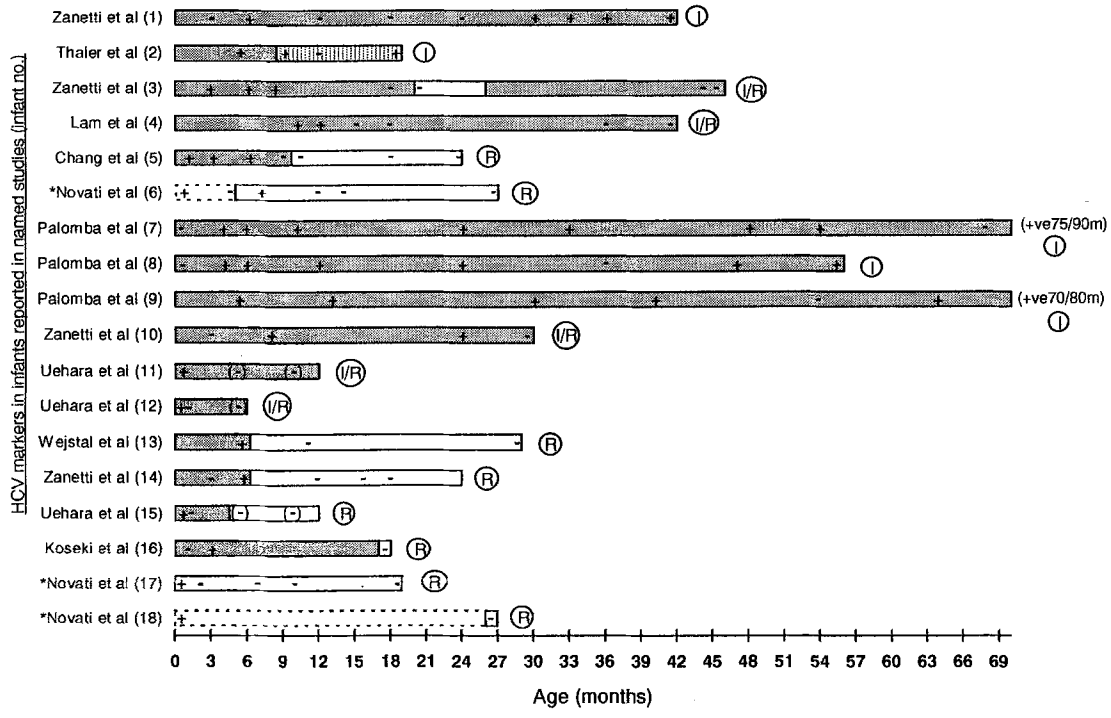

Figure 2: Temporal patterns of subsequent hepatitis C virus (HCV) polymerase chain reaction (PCR) negativity in 18 infants following one or more PCR-positive result (adapted from data reported in references 2, 4, 19, 20, 27, 29-31, 37). Infant numbers correspond to those discussed in the text and in Table 1. Solid shading indicates periods of antibody seropositivity; no shading indicates periods of antibody seronegativity by second- or third-generation assays; striped shading indicates periods of antibody seronegativity by first-generation assays; dotted lines indicate antibody serostatus was not determined. *, RIBA-2 testing only; +, positive PCR result; −, negative PCR result; (−), negative PCR result reported within broad time bands only. I, possible intermittent viraemia; R, possible resolved infection; I/R, either intermittent viraemia or resolved infection.

categorised as group B (possibly infected). These infants had a mean of 2.3 (range, 1–5) PCR tests in the first 18 months. The timing of the single PCR-positive result ranged between 0 and 15 months, with 50% of the infants positive by age 1 month. Eight infants had one or more initial PCR-negative result. Adding these infants to survival analyses slightly lowered estimates of the timing of initial PCR positivity, with an estimated 83.8% (90% CI, 71.3–90.3%) of infants positive by age 3 months.

Intermittent Viraemia and Resolution of Infection. Eighteen of the 74 group A infants were reported as repeatedly PCR positive without further details (14, 25). The remaining 56 infants were tested subsequently by PCR up to ten times during two to 90 months of follow-up, with a mean of 2.6 (range, 0–6) tests performed in the first 18 months. Six of these infants had at least one subsequent PCR-negative result within 18 months of birth (a total of 12 negative results, Table 1, column 5). Test results in these six infants are summarised in Figure 2 (infants 1–6). In two cases (infants 1 and 2) the results suggested intermittent viraemia, and in two other cases (infants 3 and 4) markers were consistent with either intermittent viraemia or resolved infection. Depending on how infants 3 and 4 are classified, the minimum frequency of intermittent viraemia in the first 18 months is therefore either 2.7% (2/74; 90% CI, 0.5–8.3), or 5.4% (4/74; 90% CI, 1.9–11.9). In the two remaining cases (infants 5 and 6; Figure 2), viraemia disappeared within 18 months and antibody tests became negative, suggesting resolved infection. The minimum frequency of resolved infection is therefore either 2.7% (2/74) or 5.4% (4/74) (90% CI, 0.5–11.9). Four other infants had PCR-negative results occurring later in childhood, between 30 and 68 months of age (infants 7–10; Figure 2). All four had patterns consistent with intermittent viraemia, and one (infant 10) had a pattern that was also consistent with resolved infection.

Eight of the 22 group B infants were tested on one or more occasions after the single PCR-positive test result. Seven infants became PCR negative in the first 18 months, five of whom could be categorised as having resolved infection (infants 13–18; Figure 2) and two as having either intermittent viraemia or resolved infection (infants 11 and 12). If these infants are included in analyses, the frequency of intermittent viraemia in the first 18 months may be as high as 6.2% (6/96; 90% CI, 2.8–12.0), and that of resolved infection as high as 11.5% (11/96; 90% CI, 6.6–18.2).

Table 2: Negative predictive value (NPV) of a polymerase chain reaction (PCR) test at age 3 months in vertically exposed infants at varying PCR specificities and vertical transmission rates.

	NPV of PCR at 3 months (90% CI)[a]		
PCR specificity	5% VTR	10% VTR	25% VTR
60%	98.9% (97.8–99.5)	97.7% (95.6–99.0)	93.4% (87.8–97.2)
80%	99.2% (98.4–99.7)	98.3% (96.6–99.3)	94.9% (90.5–97.9)
95%	99.3% (98.6–99.7)	98.5% (97.1–99.4)	95.7% (91.9–98.2)

[a]Sensitivity = 87.2% (90% CI, 74.9–94.8), from cross-sectional analyses of PCR-negative results at age 3 months in 74 group A infants with stronger evidence of HCV infection (see Table 1). VTR, vertical transmission rate.

Negative Predictive Value of Polymerase Chain Reaction. Among the 39 group A infants tested at 3 months of age, 87.2% (90% CI, 74.9–94.8%) were PCR positive (Table 1). Negative predictive values of PCR at 3 months at varying vertical transmission rates and levels of PCR false positivity are summarised in Table 2. Overall, the negative predictive value was high (> 98%) at the lowest transmission rates, even at high levels of PCR false positivity. However, at a 25% transmission rate, negative predictive value point estimates fell below 95%, with the lower bound of the 90% CI as low as 88%. Estimates of negative predictive value were only slightly lower if data from group B infants were included, with the corresponding lowest estimate at 91.7% (90% CI, 86.4–95.8%) (data not shown).

Antibody Positivity in Uninfected Infants. Table 3 summarises age at loss of maternal antibody in eight studies in which all uninfected infants were monitored until loss of maternal antibody was documented or in which analyses were restricted to infants monitored for at least 12 months in ongoing studies. A minority of infants were still seropositive at 12 months, but persistence of antibody was not documented beyond 18 months. Similar results were reported in 19 studies with less complete ascertainment of loss of antibody among a total of 666 putatively uninfected infants (data not shown) (1, 12, 19, 25, 26, 29, 30, 32, 38–41; A. Zanetti, T. Guerra et al., T. Fujisawa, A. Miyamura, A. Ruiz-Extremera, J. Suarez, J. Goedert, unpublished data): enzyme immunoassay (EIA) positivity beyond 18 months was reported in only three cases (19; J. Goedert, unpublished data). Few studies reported maternal determinants of infant antibody loss. Infant antibody clearance was associated with maternal antibody titre in two studies

Table 3: Persistence of maternal antibody in 297 hepatitis C virus uninfected infants.

Study (reference)	No. of uninfected infants	Antibody assay	Antibody positive results amongst uninfected infants (%)				
			By 9 m	By 12 m	By 15 m	By 18 m	> 18 m
Zanetti et al. (2)	108	E2/E3 + R2/R3	NR	0	–	–	–
Giacchino et al. (18)	31	E2 + R2	NR	0	–	–	–
Zuccotti et al. (17)	29	E3 + R3	NR	0	–	–	–
Chen (unpublished)	28	E2	3.6	0	–	–	–
Suarez (unpublished)	10	E2 + R2	20	0	–	–	–
Manzini et al. (24)	44	E2	NR	6.8	0	–	–
Scaravelli et al. (unpublished)	30	E2/E3 + R2/R3	NR	25	NR	0	–
Resti et al. (15)	17	E2 + R2	NR	5.6	NR	NR	NR
Weighted average			7.9	3.9	0	0	–

E2, EIA-2; E3, EIA-3; R2, RIBA-2; R3, RIBA-3.
NR not reported.

(Di Domenico et al, IX Triennial International Symposium on Viral Hepatitis and Liver Disease, 1996, Abstract no. B235; T. Fujisawa, unpublished data) and with the number of RIBA band reactivities on maternal sera in one study (24).

Antibody Negativity in Infected Infants. Most infected infants remained antibody positive throughout the follow-up period. Several reports of changes in infant RIBA reactivity patterns between 4 and 24 months of age suggested endogenous antibody production (16, 18, 30, 33), as did two reports of a fall and then subsequent rise in antibody titres after two to ten months (26; T. Fujisawa, unpublished data). A minority of PCR-positive infants demonstrated periods of antibody negativity (using second- or third-generation assays) without evidence of resolving infection. Figure 3 summarises the findings for ten such infants for whom the timing of both PCR and antibody negativity were reported. In three cases (infants 3, 20, and 26) transient seronegativity of three to six months duration was reported between the ages of 6 and 24 months. In five cases (infants 19,

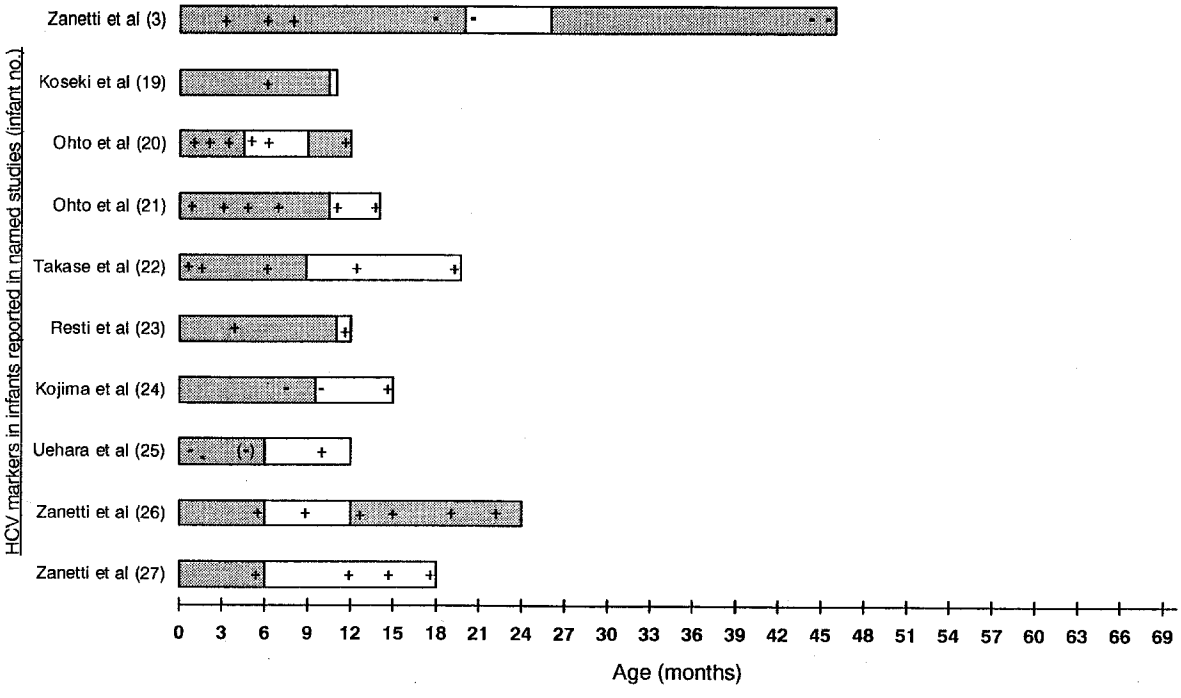

Figure 3: Patterns of antibody seronegativity in ten hepatitis C virus viraemic infants (adapted from data reported in references 2, 3, 15, 21, 28, 29, 31). Infant numbers correspond to those discussed in the text and in Table 1. Solid shading indicates periods of antibody seropositivity; no shading indicates periods of antibody seronegativity; +, positive PCR result; −, negative PCR result.

21, 23, 24 and 25) antibody negativity of six months or less occurred at the end of the follow-up period, and these infants may have become antibody positive again on further follow-up. Three of these infants (infants 19, 24, and 25) were PCR positive on a single occasion only and may not have been truly infected. Similar transient antibody negativity (with fewer details of timing) was reported for 11 infants in three other studies (data not shown) (12, 13, 16).

However, in two cases (infants 22 and 27) antibody negativity lasted nine to 12 months, with two to four PCR-positive results documented after loss of antibody. Five other studies have reported in less detail antibody negativity in PCR-positive infants, in seven of nine (V. Papaevangelou, unpublished data), four of 17 (14), seven of 11 (M.K. Joung et al, Third International Symposium on HCV, 1991, Abstract no. E56), five of 19 (A. Ruiz-Extremera, unpublished data), and one of 19 (M.G. Ercilla et al, IX Triennial International Symposium on Viral Hepatitis and Liver Disease, 1996, Abstract no. B240) infants. Thirteen of these 24 additional infants were reported to be repeatedly PCR positive, and duration of antibody negativity was up to five years.

Discussion

Approximately 90% of vertically infected infants were found to be PCR positive by 3 months of age, and subsequent PCR negativity due to intermittent viraemia or resolving infection was uncommon. The negative predictive value of a single PCR-negative test at age 3 months was high if vertical transmission was 10% or less, but misclassification of infected infants may be a problem at higher transmission rates.

Quality control studies have shown that the repeatability of PCR can be poor. In the first Eurohep HCV RNA quality control study, only 16% of 31 laboratories performed faultlessly, 23% missed a weakly positive sample, and 61% reported false-positive and/or false-negative results (42). Similar results were obtained in the second study, with 16% of 86 laboratories performing faultlessly, 29% missing the weakly positive sample, and 55% reporting false-positive and/or false-negative results (43). In both studies false positivity was more frequent than false negativity. Differences between studies in patterns of PCR positivity and in rates of intermittent viraemia or resolution of infection may therefore be partly attributable to differences in PCR methodologies. Many of the 22 group B infants with single PCR-positive results may not have been truly infected, but inclusion of these infants in analyses made little difference to estimates of the timing of initial PCR positivity or the negative predictive value of PCR at age 3 months. However, exclusion of these infants may have resulted in underestimation of the frequency of resolved infection.

Studies differed in the frequency and timing of infant PCR tests and in the level of detail of reporting of results. The frequency of intermittent viraemia is a minimum estimate, as many studies tested infants infrequently or followed them up for less than 18 months. Some studies with a higher proportion of infants with intermittent viraemia were excluded from these analyses because of lack of temporal details (12, F. Bortolotti et al., IX Triennial International Symposium on Viral Hepatitis and Liver Disease, 1996, Abstract no. C35). Other studies, however, in which frequent PCR testing was performed on all (17) or most (3, 26, 27) infected infants, have documented persistent viraemia in the first nine to 18 months of life. Infrequent testing of intermittently viraemic infants may have led to biased estimates of the timing of initial PCR positivity by missing early positivity and finding infants to be PCR negative when first tested. Conversely, studies with brief follow-up periods may have misclassified infected infants who were initially PCR negative as uninfected, biasing results towards earlier initial positivity.

Differences between studies in initial PCR positivity may also have been due to differences in the proportion of infants who were infected prenatally, perinatally, or postnatally (via contaminated transfusions or possibly via household contact or breastfeeding). Inclusion of PCR results from postnatally infected infants in survival analyses may result in underestimation of early viraemia in vertically infected infants. It may be difficult to distinguish vertical from postnatal infection, as a lack of PCR positivity in the first few months of life could also result from inefficient viral replication in the newborn liver, and some vertically exposed infants fostered with HCV-negative families and with no apparent postnatal exposure have demonstrated late onset of PCR positivity (6–14 months) (16, 24). One option is to consider vertically exposed infants to be born into a wider environment of HCV infection risk and follow them up accordingly. However, it is important to quantify the risks of transmission from each route in

order to enable development of effective HCV prevention strategies.

Among uninfected infants, persistence of maternal antibody beyond 18 months was rare. The timing of seroreversion depends on which generation of antibody assay is used (including whether supplementary assays are employed) and on the presence of determinants of antibody persistence. Maternal HCV antibody titre may depend on a number of factors, such as viral load or viral genotype (44). Among infected infants, most remained antibody positive throughout the follow-up period, but a minority were transiently antibody negative between the ages of 2 and 24 months. The frequency of such negativity may have been underestimated, due to infrequent testing schedules or to possible misclassification of infected infants who were no longer followed up after seroreversion. It may therefore be important to continue to monitor infants who serorevert early, particularly if PCR testing is infrequent, to ensure that transmission has not occurred.

Reports of more prolonged antibody negativity in infected infants can be attributed in some cases to the use of insensitive first-generation antibody assays (4, 45), but other cases are more difficult to explain (2, 14; V. Papaevangelou, unpublished data). Seven infected infants with persistent antibody negativity were known to be coinfected with HIV, and one of these infants was hypogammaglobulinaemic. Other HCV-infected children on immunosuppressive therapy have demonstrated delayed or absent antibody seropositivity (46, 47). However, it seems unlikely that infants with early HIV infection would fail to mount an immune response to HCV. One possibility is that infection was acquired early in utero and led to immune tolerance. Alternatively, there may be some methodological explanation for the higher proportion of seronegative infections reported by a handful of studies, although many of these infants were repeatedly PCR positive, suggesting that they were truly infected.

Other markers of HCV infection in vertically exposed infants appear to be less reliable. Limited existing data from vertically infected infants suggests that detection of HCV immunoglobulin M (IgM) antibodies using existing assays may be of limited diagnostic use (27, 37). Studies of adults have suggested that an IgM response may be late or absent in acute infection (48) and that IgM core antibodies may be present in chronic infection (49). Similarly, the sensitivity and specificity of serum alanine aminotransferase (ALT) levels for HCV vertical infection may be limited. Raised ALT levels may be transient in HCV vertical transmission and easily missed if sampling is infrequent, or may be attributable to other hepatotropic viruses (27, 38).

Further research is needed on the patterns of serum markers of HCV infection in vertically exposed infants. Infected children with long-term loss of viraemia may have resolved infection or quiescent ongoing infection (M.G. Ercilla et al. and R. Giacchino et al., IX Triennial International Symposium on Viral Hepatitis and Liver Disease, 1996, Abstract no. B240 and Abstract no. B169, respectively; T. Moriya, unpublished data), and information is needed on the possible duration of PCR negativity in infants with intermittent viraemia and on the duration of antibody positivity after resolution of infection. There is a lack of information on viral or host determinants of resolution of infection and on the frequency and determinants of antibody-negative infection. Polymerase chain reaction methodologies need standardisation, so that data from individual studies can be better combined. Clarification of these issues will aid future decisions about testing schedules and diagnostic criteria to be used in HCV vertical transmission studies.

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