Hepatitis C virus (HCV) is considered the most common blood-borne chronic infection and is one of the leading causes for liver transplantation. Worldwide, the seroprevalence to HCV is about 0.8–1.5% in the adult population, with lower rates in children (0–0.1%). Although the clinical course of infection is variable, 10–20% of chronically infected patients will eventually develop cirrhosis or hepatocellular carcinoma, and in the US alone, an estimated 10 000 deaths per year are attributed to HCV-associated liver disease.

Hepatitis C virus is readily transmitted via blood exposure. The predominant mode of transmission in the developed world is needle sharing among intravenous drug users (IDU) which accounts for over 50% of HCV-infected patients. Moreover, in areas where HIV has spread primarily by injection drug use, the prevalence of HCV infection among HIV-infected people ranges from nearly 30% to over 90%. Unlike HIV, there is little evidence to support efficient spread of HCV via sexual contact. Perinatal exposure, however, has been implicated as an important mode for HCV transmission. Overall vertical transmission rate of HCV has been estimated at 5%, with higher

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rates from HCV-infected mothers co-infected with HIV. It has been proposed that this higher rate of vertical transmission results from higher HCV viral loads in mothers with immunosuppression secondary to HIV infection.

In observational studies published to date, methodological issues (small sample sizes, inadequate duration of infant follow-up, and lack of consensus concerning appropriate diagnostic criteria to define paediatric HCV infection) have limited accurate evaluation of HCV vertical transmission from mothers with concomitant HIV infection. As co-infection has been associated with elevated HCV viral load, and higher HCV viral load has been associated with enhanced vertical transmission, it seems probable that co-infected mothers will be more likely to transmit HCV to their infants. Therefore, a meta-analysis of current published and unpublished research was performed. In the following study, data from multiple studies were compiled, based on standardized diagnostic criteria, in an attempt to derive an estimate of the effect of maternal HIV co-infection on the risk of HCV vertical transmission.

Hepatitis C virus infection can be asymptomatic for more than 20 years prior to the development of liver disease. Most individuals, infected later in life, tend to die from unrelated conditions before HCV-induced liver disease becomes apparent. Given a longer duration of infection, however, the impact of chronic HCV infection on those infected as infants could pose a public health concern worthy of consideration. Therefore, a better understanding of risk factors contributing to enhanced rates of vertical transmission of HCV is needed.

Methods

Literature search and study identification

Articles published between the years 1992 (the first year that the HCV-enzyme immuno assay [EIA] 2.0 was available) and 2002 were identified by computerized literature searches of MEDLINE and AIDSLINE using the following keyword search strategy: HIV, hepatitis C, and perinatal or vertical. Identified articles were hand searched and appropriate references from all articles and reviews were checked. Relevant conference abstracts, including the Conference of Retroviruses and Opportunistic Infections, the International Conferences on AIDS, and the Infectious Disease Society of America annual meeting were also identified using AIDSLINE.

Data extraction

The following data were excluded from our analysis: case reports, data duplicated in updated reports, and articles in which diagnostic criteria to define maternal and/or infant HCV status were either unclear or failed to fit standardized criteria (discussed below). Studies with potential misclassification of some maternal/infant pairs were still utilized if enough information was available to recalculate transmission rates and odds ratios (OR) using only those maternal/infant pairs that met the inclusion criteria. Information extracted from each article included: the number of mother/infant pairs evaluated, maternal HIV serostatus, maternal characteristics (demographic information and selection criteria), the diagnostic criteria used to determine maternal and infant HCV infection (i.e. HCV-RNA and/or antibody test results), and duration and frequency of infant follow-up.

Data analysis

To reduce heterogeneity between studies, standardized diagnostic criteria were developed to define maternal and infant HCV infection status. In the initial analysis, maternal HCV infection was defined as a positive anti-HCV antibody assay during pregnancy (HCV-EIA 2.0 or higher and recombinant immunoblot assay [RIBA] HCV 2.0 or higher). A subanalysis of the same studies evaluated the risk of vertical transmission from only those mothers determined to be viraemic (HCV RNA+).

Infants were defined as HCV infected if they met one or more of the following criteria: polymerase chain reaction (PCR) positive for HCV-RNA on two or more separate occasions at least 3 months apart, anti-HCV antibody positive (as determined by HCV-EIA 2.0 or higher and RIBA HCV 2.0 or higher) at ≥18 months of age, and/or evidence of seroconversion (seroreversion followed by renewed seropositivity on enzyme-linked immunosorbent assay (ELISA) 2.0 or higher or a change in RIBA activity). Infants were considered HCV-uninfected if they: (1) were persistently PCR negative, or (2) seroreverted by 18 months. Seroconversion before 12 months of age required confirmation of antibody negativity at ≥18 months of age or a least one PCR-negative result from the age of 6 months.

Duration of infant follow-up was an important aspect of the standardized diagnostic criteria developed for our analysis. Passively acquired maternal anti-HCV antibodies usually become undetectable by 6–12 months of age. However, studies have documented persistence of maternal antibody longer in infants born to mothers co-infected with HIV. To prevent overestimates of transmission resulting from misclassification, infants followed for less than 18 months were eliminated from the analysis.

Statistical methods

Odds ratios and exact 95% CI for individual studies were calculated with maternal HIV serostatus as the predictor variable and perinatal transmission of HCV as the outcome variable. To ascertain whether the results of the individual studies were similar within each comparison, homogeneity was tested using the Mantel-Haenszel method. Under the null hypothesis of the test of heterogeneity, there is no difference in treatment effect between groups (this follows a distribution with d.f., where k is the number of studies contributing to the meta-analysis). Study results were considered heterogeneous if the resultant P-value was <0.1.

As observed differences between the results of individual studies were not statistically significant (P = 0.28 for the analysis of anti-HCV+ mothers and P = 0.13 for the analysis of HCV viraemic mothers), a fixed effects model (which ignores between study variation) or random effects model could have been used to summarize the effect across studies. The random effects model provides a more conservative estimate of significance. This model operates under the assumption that included studies are only a random sample of all studies that will be conducted so that heterogeneity between individual studies will result in a wider CI of the summary estimate. Therefore, using the DerSimonian and Laird random effects model, the reported summary estimate was calculated as an average of the individual study results weighted by the inverse of their variance.
Quality assessment
The sensitivity of the results of the analysis to changes in assumptions that were made in the process of conducting the study was evaluated. First, the data were re-analysed following inclusion of the excluded studies (using less stringent methodological cutpoints or inputting reasonable values for missing data). In addition, the data were re-analysed using the Mantel-Haenszel fixed effects model for comparison with the summary estimate obtained by the DerSimonian and Laird random effects approach. Finally, to ascertain the likelihood of publication bias, a funnel plot was generated by plotting the log OR versus sample size for individual studies.

Results
In total, 15 studies were identified that evaluated HCV-infected pregnant woman with and without HIV infection (14 published articles and 1 abstract). Table 1 summarizes the 10 articles that fit the inclusion criteria for the meta-analysis.5,7,8,10,16,17,20,21,23,25 Two studies were excluded from analysis due to short duration (≤12 months) of infant follow-up.26,27 A study by Gibb et al. was excluded as the authors could not definitively diagnose or exclude HCV infection in most children because of the timing of the antibody tests and uncertainty about the sensitivity and specificity of the HCV RNA PCR.28 A study by Fischler et al. could not be used in the analysis as only 4% (2/55) of the HCV-infected mothers were co-infected with HIV.29 Finally, a report by Conte et al. was excluded due to the limited number of HIV co-infected mothers (only 4%), inadequate duration of infant follow-up, and missing information.30

Hepatitis C virus antibody positive mothers
A total of 2382 mother/infant pairs from 10 studies were included in the initial analysis of infants born to HCV antibody positive mothers (anti-HCV+). When these studies were combined, the overall risk estimate (OR) of HCV vertical transmission was 2.82 (95% CI: 1.78–4.45; P = 0.00001) for anti-HCV+/HIV+ co-infected mothers when compared with anti-HCV+/HIV– mothers (Figure 1).

Viraemic mothers
Previous reports have indicated that maternal HCV viraemia increases the likelihood of vertical transmission of the virus.1,8,12,13 To control for maternal HCV viraemia, a sub-analysis was performed. Data from seven studies, including 1327 infants born to HCV viraemic (i.e. HCV RNA+) mothers, determined the overall risk estimate of HCV vertical transmission to be 1.97 (95% CI: 1.04–3.74; P = 0.04) for HCV viraemic/HIV+ co-infected mothers when compared with HCV viraemic/HIV– mothers (Figure 2).

Sensitivity analyses
Sensitivity analyses were performed to determine whether the results of our meta-analysis would change under different assumptions. Re-analysis following inclusion of the excluded studies provided a nearly identical risk estimate for vertical transmission (OR = 2.71; 95% CI: 1.85–3.98). Re-analysing the data using a Mantel-Haenszel fixed effects model also resulted in comparable summary estimates for vertical transmission from anti-HCV+/HIV+ co-infected mothers (OR = 2.64; 95% CI: 1.85–3.76) and HCV viraemic/HIV+ co-infected mothers (OR = 1.77; 95% CI: 1.20–2.61). Failure of sensitivity analyses to significantly alter the results of this study should strengthen the confidence that can be placed in the findings.

Evaluation of publication bias
The possibility that results of unpublished data differ substantially from published data is always a potential source of bias when performing a meta-analysis. In an attempt to control for publication bias in this report, relevant conference abstracts were reviewed as well. To ascertain the likelihood for publication bias in our analysis, a funnel plot was generated by plotting the log OR versus sample size for individual studies (Figure 3). Publication bias was not evident from this plot, which reflects a fairly symmetric pattern.

Discussion
Meta-analytical methods have often been applied to randomized controlled trials (RCT) to arrive at conclusions regarding a particular intervention. Quite often, however, RCT designs are not feasible and only observational data are available (e.g. studies of risk factors cannot be randomized because they either deal with inherent human characteristics or would involve unethical exposure of subjects to harmful risk factors). As observational designs lack the experimental element of random allocation to an exposure, they are prone to inherent biases that can make the calculation of a single summary estimate of effect of exposure misleading. Therefore, accounting for differences among individual studies is an important consideration when combining multiple observational studies into a single meta-analysis. Variation in results obtained from individual studies included in this meta-analysis may reflect differences in: (1) participant selection and maintenance, (2) laboratory measurement, (3) misclassification of maternal or infant infection status, and/or (4) inability to account for potential confounding factors or co-variates.

With regard to participant selection, nearly all mothers were identified from prospective longitudinal cohorts of ‘unselected’ or high-risk (i.e. IDU) pregnant women. In prospective studies, one of the most significant biases results from differential loss to follow-up. This would represent an important source of bias if loss to follow-up was associated with maternal HIV status (i.e. if HIV-infected mothers are more or less likely to return). In addition, while most authors reported the number of pregnant women who consented to participate in their respective studies, failure to report the number and/or socio-demographic characteristics of those who declined could also represent a source of selection bias in these studies. In the only study included in this meta-analysis to address the issue of selection bias, the investigators found no difference in historical or clinical data between those who dropped out (n = 121) and those who completed the study (n = 1372).20

Differences in laboratory measurement resulting from varying sensitivities of PCR assays and/or a lack of standardization of HCV antibody or PCR-based assays between laboratories can also account for variation between studies.31 The ‘protective’ effect of maternal HIV co-infection on vertical transmission of HCV reported by Lam et al. appears discrepant when compared with the other studies included in this analysis and may have resulted from retrospective evaluation of the
Table 1 Summary of studies included in the meta-analysis relating the impact of maternal human immunodeficiency virus (HIV) co-infection on vertical transmission of hepatitis C virus (HCV)

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Geographical location</th>
<th>Study design</th>
<th>Maternal selection</th>
<th>Total no. of infants</th>
<th>Follow-up</th>
<th>Diagnostic assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lam</td>
<td>1993</td>
<td>UK: Edinburgh</td>
<td>Retrospective cohort</td>
<td>Anti-HCV+ child-bearing women at high risk for HCV and HIV (IDU)</td>
<td>66</td>
<td>12–72 months</td>
<td>EIA, 2.0, RIBA, RNA</td>
</tr>
<tr>
<td>Manzini</td>
<td>1995</td>
<td>Italy: Turin</td>
<td>Prospective cohort</td>
<td>Consecutive, unselected anti-HCV+ mothers</td>
<td>45</td>
<td>birth, every 3 months (12–27 months)</td>
<td>EIA 2.0, RIBA II, RNA, ALT</td>
</tr>
<tr>
<td>Zuccotti</td>
<td>1995</td>
<td>Italy: Milan</td>
<td>Prospective cohort</td>
<td>Anti-HCV+ pregnant women (mainly IDU)</td>
<td>21</td>
<td>birth, 1 month, every 3 months for 18 months</td>
<td>EIA 2.0, RIBA, RNA, ALT</td>
</tr>
<tr>
<td>Paccagnini</td>
<td>1995</td>
<td>Italy: Milan</td>
<td>Prospective cohort</td>
<td>Anti-HCV+ pregnant women (mainly IDU)</td>
<td>70</td>
<td>birth, 3, 6, 9, 12, 15, 18 months</td>
<td>EIA 2.0, RIBA, RNA, ALT</td>
</tr>
<tr>
<td>Bossi</td>
<td>1996</td>
<td>Italy: Pavia</td>
<td>Prospective cohort</td>
<td>Anti-HCV+ pregnant women (mainly IDU)</td>
<td>64</td>
<td>bimonthly from birth for &gt; 2 years</td>
<td>EIA 2.0, RNA</td>
</tr>
<tr>
<td>Tovo</td>
<td>1997</td>
<td>Italy: Turin, Milan, Bologna, Padua, Pavia</td>
<td>Prospective cohort</td>
<td>Anti-HCV+ pregnant women (mainly IDU) from 12 participating centres</td>
<td>245</td>
<td>birth, every 3–5 months for 18 months (19–42 months)</td>
<td>EIA 2.0, RIBA II/III, RNA</td>
</tr>
<tr>
<td>Zanetti</td>
<td>1998</td>
<td>Italy: Lombardy</td>
<td>Prospective cohort</td>
<td>Anti-HCV+ pregnant women identified from cross-sectional antenatal screening</td>
<td>291</td>
<td>birth, every 3 months for 1 year then every 6 months (12–46 months)</td>
<td>EIA 2.0, RIBA, RNA</td>
</tr>
<tr>
<td>Mazza</td>
<td>1998</td>
<td>Italy: Brescia</td>
<td>Prospective cohort</td>
<td>Anti-HCV+ women during pregnancy or at delivery (mainly IDU)</td>
<td>70</td>
<td>6–32 months (mean 15 months)</td>
<td>EIA, RIBA, RNA</td>
</tr>
<tr>
<td>Granovsky</td>
<td>1998</td>
<td>US: New York (5 clinics in Brooklyn and the Bronx)</td>
<td>Prospective cohort</td>
<td>Anti-HCV+ women from cohort of HIV+/HIV– pregnant women (mainly IDU)</td>
<td>122</td>
<td>monthly for 6 months, every 3 months through 18 months, then every 6 months through 4 years</td>
<td>EIA 2.0/3.0, RIBA II/III, RNA</td>
</tr>
<tr>
<td>Resti</td>
<td>2002</td>
<td>24 medical departments distributed throughout Italy</td>
<td>Prospective cohort</td>
<td>Consecutive, unselected anti-HCV+ mothers from multicentre study</td>
<td>1372</td>
<td>birth, &gt;3 times in subsequent 2 years</td>
<td>EIA 2.0, Western Blot, RNA</td>
</tr>
</tbody>
</table>

a Enzyme immuno assay.
b Recombinant immunoblot assay.
c Alanine aminotransferase.
The authors note that serum samples from HCV-infected mothers were several years old and had been stored at 4°C for 1–2 weeks prior to long-term storage which may have led to degradation of viral RNA and thus an underestimate of the frequency of HCV viraemia. Misclassification of infant infection status can result from ‘immunosilent’ HCV infection (i.e. viraemic seronegative infection) in some infants. Results from three separate studies included in our analysis revealed prolonged seronegative results (up to 36 months), despite persistent HCV viraemia in six HCV-infected infants co-infected with HIV. If HCV seronegative infections are, in fact, more common in HIV-infected infants, this finding would likely result in misclassification that would bias results toward the null for studies that used antibody assays for diagnosis of paediatric infection. Therefore, in our analysis, seronegative HCV infections may have resulted in an underestimate of the risk of HCV vertical transmission for infants born to HCV+/HIV+ co-infected mothers. Misclassification of HCV-infected mothers is also a possibility in this study. Results from several laboratories have shown that at least 15% of individuals acutely infected with HCV are...
able to clear the virus. Therefore, in our analysis, it is possible that at least 15% of mothers with anti-HCV antibody in the absence of detectable levels of HCV RNA may have actually cleared the virus. If, due to immunosuppression, HCV co-infected women were less likely to eradicate HCV infection, this potential misclassification would result in an apparently higher risk of vertical HCV transmission for infants born to anti-HCV+/HIV+ co-infected mothers. A recent report, however, revealed an HCV clearance rate in HIV-infected patients (15%) that was identical to clearance rates reported in HIV-uninfected patients. Therefore, this misclassification would appear to be non-differential across HIV exposure groups and should, therefore, bias the results toward the null.

Nearly all of the studies included in our analysis lacked the information and/or sample size necessary to evaluate the contribution of other potential confounding factors or co-variates that may impact the estimate of risk of vertical transmission of HCV (e.g. breastfeeding, delivery mode, HCV genotype, severity of HCV-induced liver disease, stage of HIV infection, antiretroviral experience, and current IDU status). Therefore, this misclassification would appear to be non-differential across HIV exposure groups and should, therefore, bias the results toward the null.

Figure 3 Funnel plot to assess publication bias among studies relating the risk of hepatitis C virus (HCV) vertical transmission from HCV-infected mothers with and without concomitant human immunodeficiency virus (HIV) infection

The potential association of maternal HCV-induced liver disease on vertical transmission was evaluated by Zuccotti et al. This group noted that among the six transmitting mothers, three (1/4 co-infected mothers and 2/2 mothers infected with HCV alone), had chronic hepatitis as demonstrated by elevated alanine aminotransferase (ALT) levels. However, results from a larger study of 291 mothers conducted by Zanetti et al. indicated that the rate of HCV transmission was not associated with elevated ALT or chronic liver disease in HCV+ mothers. In this study, the percentage of individuals with elevated ALT and liver disease were similar between groups of HIV− and HIV+ mothers. Paccagnini et al. also reported that the rate of HCV infection was not significantly higher for infants born to mothers with elevated ALT (50–445 IU/l) when compared with infants born to mothers with normal ALT (<45 IU/l).

Although most studies included in our analysis failed to document the stage of maternal HIV infection, two groups determined that the presence of HIV-related symptoms in the mother was not predictive of HCV infection in the infant. In addition, Granovsky et al. found no association between CD4+ T cell count (<20% versus ≥20%) and vertical transmission. In a study of 70 HIV-infected women conducted by Paccagnini et al., transmission was not associated with HIV stage (29% Class IV and 20% Class II or III; P > 0.05) or maternal CD4 count at delivery (23% with CD4 >400 versus 19% with CD4 <400; P > 0.05). Of particular interest, only 1 of the 15 studies originally identified for this analysis reported the number of HIV-infected pregnant women on antiretroviral therapy. These investigators failed to document vertical transmission of HCV from any of the 15 HIV co-infected mothers; all of who were on antiretroviral therapy at the time of pregnancy. However, this study was not included in our analysis due to inadequate infant follow-up, missing data, and the limited number of HCV+/HIV+ co-infected mothers evaluated (only 4% [15/370]).

Hepatitis C virus/HIV co-infection is common due to shared risk factors for transmission, particularly injection drug use. As HIV-1 positivity and injection drug use are highly correlated and both are associated with immunosuppression, it has been difficult to evaluate the individual risk from each variable. Using multi-variate analysis, Resti et al. found that maternal injection drug use but not HIV-1 co-infection was significantly associated with vertical HCV transmission. It should be noted, however, that this group failed to: quantify maternal HCV viral load, discriminate the stage of maternal HIV infection, and report maternal antiretroviral experience. Several studies have shown that highly active antiretroviral therapy (HAART) may result in transient increases in HCV viral load and liver enzymes (including ALT). Moreover, some antiretrovirals are hepatotoxic which may have an adverse affect on HCV co-infected mothers thereby facilitating transmission. Therefore, without quantified HCV viral loads and stratification based on stage of HIV disease and/or antiretroviral experience, it is difficult to fully evaluate the association of injection drug use on vertical transmission of HCV.

Conclusions

Overall, results from this meta-analysis indicate a higher risk of HCV vertical transmission for infants born to anti-HCV+/HIV+ co-infected women. Results from a sub-analysis that controlled for maternal HCV viraemia indicated that the overall risk of
HCV vertical transmission, though less pronounced, was still higher for co-infected mothers than for mothers infected solely with HCV. Clearly, detectable maternal HCV RNA plays an important role in the risk of HCV vertical transmission. These findings are consistent with evidence in the literature that supports the biological plausibility that HIV-induced immunosuppression results in enhanced HCV replication that facilitates vertical transmission of the virus.8,13

There is currently no effective means to prevent vertical transmission of HCV, and treatment options for infected individuals are limited by relatively ineffective and toxic therapies. Moreover, chronic HCV infection has also been associated with elevated rates of HIV vertical transmission.13,40 Therefore, a better understanding of HIV co-infection and other potential co-variates contributing to enhanced vertical transmission of HCV is needed. Delineating these risk factors may facilitate policy development targeting prevention of vertical HCV transmission through antenatal HCV screening and/or counselling, particularly in high-risk women such as previous or current injection drug users.

KEY MESSAGES

- Methodological issues have limited accurate evaluation of the contribution of maternal co-infection with human immunodeficiency virus (HIV) on the risk of vertical transmission of hepatitis C virus (HCV).
- Results from this meta-analysis of observational studies suggest that the risk of HCV vertical transmission is higher in infants born to HIV co-infected mothers.

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