Supplement Article

Hepatitis C Virus in Pregnancy and Early Childhood: Current Understanding and Knowledge Deficits

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Received March 19, 2014; accepted April 27, 2014.

Hepatitis C virus (HCV) is a well known cause of chronic liver disease in adults, but the burden of HCV in pregnant women and children is underappreciated. The leading route of HCV acquisition in children is vertical transmission. This review will discuss previous studies on the impact of HCV on pregnancy, risk factors for perinatal transmission, HCV transmission rates from mother to infant, what influence the virus has on the exposed or infected infant, and those areas where additional studies are required to advance our understanding of HCV pathogenesis during pregnancy. The rapid expansion of HCV treatment regimens free of interferon and ribavirin will expand future therapeutic opportunities for pregnant women and infected infants.

Key words. hepatitis C virus; pregnancy; vertical transmission.

HCV is an important cause of chronic liver disease in adults, with approx. 3.2 million persons in the US having chronic HCV infection and up to 17 000 new infections diagnosed annually [1]. The burden of HCV in pregnant women and children is underappreciated the public and medical providers and not accurately documented in the US population. Studies suggest HCV affects 0.6%–2.4% of all pregnancies with overall mother-to-infant transmission from 8% to 15%, which includes infants who resolve infection and who progress to chronicity [2, 3]. This review will describe what is known about the impact of HCV on pregnancy, previous attempts to identify risk factors for perinatal transmission, how frequently HCV is transmitted from mother to infant, what influence the virus has on the exposed or infected infant, and those areas where additional studies are required to advance our understanding of HCV pathogenesis during pregnancy.

Review of the Pathogen

HCV is an enveloped RNA virus that is a single-stranded, plus-sense RNA with a genome organized as 1 long open reading frame. The structural proteins of HCV (Core, E1 and E2) are at the 5 end of the genome and are followed by the nonstructural proteins [4]. The virus uses an internal ribosomal entry site for translation of the viral proteins [5]. HCV also co-opts a liver-specific microRNA called miR-122 as a replication or translation cofactor [6]. This use of a microRNA to promote gene expression is in marked contrast to conventional microRNA function in silencing gene expression. HCV exhibits genetic diversity with 6 main genotypes and also marked diversity within patients, with hypervariable regions within the E2 protein [7]. These regions generate "quasispecies" that allow the virus to evade the host immune response. Among the different genotypes of HCV, all can lead to long-term liver disease and all can be transmitted vertically. Genotypes 1a, 1b, and 4 are less susceptible to interferon-based therapies, whereas genotype 3 is more often associated with steatosis [8].

HCV primarily infects hepatocytes [9]. It may infect other cells within the liver, although definitive evidence is lacking. Because HCV is not cytopathic, infection does not necessarily result in symptoms. After infection, there is a period of viremia averaging 7 to 8 weeks but can vary from 2 to 26 weeks [10]. Symptoms with HCV infection often correspond to a robust cellular immune response mediated by HCV-specific CD4 and CD8 T cells, which often is accompanied by resolution of viremia [11]. Infected patients who progress to chronic infection develop an "exhausted" phenotype with ineffective CD8 T cells and little detectable CD4 T-cell responses [10, 12]. A humoral immune response is generated but does not neutralize the virus.

Journal of the Pediatric Infectious Diseases Society, Vol. 3, Suppl 1, pp. S13–S18, 2014. DOI:10.1093/jpids/piu045

Treatment of adults with chronic HCV has long relied on pegylated-interferon (peg-ifn) in combination with ribavirin for 24 to 48 weeks, with 40%–90% of patients achieving a sustained virologic response (SVR) that was highly dependent on viral genotype [13]. These agents were less than ideal for many reasons: eg, the need for weekly subcutaneous injections, extensive side effects for both agents, growth arrest in children, lack of FDA approval for use in pregnant women or young children. Two new HCV protease inhibitors, boceprevir and telaprevir, were approved in 2011 as the first direct-acting antiviral drugs (DAAs), and their addition to the prior regimens improved response rates into the 60%–80% range for genotype 1-infected patients [14, 15]. These agents were limited by side effects and modest potency, and they should be supplanted by the recently approved simeprevir, a new protease inhibitor with increased potency, few side effects, and broader genotype coverage [16]. Sofosbuvir, which is an HCV polymerase inhibitor, was approved by the US FDA for use in adults in December 2013 [17]. Sofosbuvir offers a multitude of benefits: for the first time, a treatment regimen that is all oral (in combination with ribavirin), only 12 weeks for most patients and improved SVR to almost 90% for almost all patients. Several other classes of drugs in late-stage clinical trials will magnify these benefits [13]. It is important to remember only peg-ifn and ribavirin are approved for use in children, and neither agent is recommended during pregnancy because of the known teratogenic effects of ribavirin. To date, there are no clinical trials of DAAs during pregnancy.

Epidemiology

Although bloodborne transmission is most common in adults, vertical transmission is the leading route of HCV acquisition in children [2, 18]. The overall transmission of HCV from mother to infant is between 8% and 15%, but not every transmission event results in chronic infection [19–21]. High HCV viral load is consistently identified as a major risk factor, but it is not the only determining factor [19, 20]. Cesarean delivery has never been identified as having an impact on perinatal transmission and is not routinely recommended [20, 22]. One study examined amniotic fluid using polymerase chain reaction (PCR) and found no detectable HCV RNA [23]. Maternal human immunodeficiency virus (HIV) coinfection was initially identified as a major factor for transmission, but these findings came from the early studies in which maternal HIV infection was not controlled with anti-retroviral therapy [24, 25]. A recent study has shown that in the setting of highly active anti-retroviral therapy (HAART), the chance of perinatal transmission is no different with HIV coinfection than with HCV monoinfection [26]. Based on the identification of HCV in breast milk, breastfeeding was initially identified as a potential risk factor but has since been contested.

These clinical facts lead many to believe HCV vertical transmission is likely to occur in utero or intrapartum. There are several hypothesized mechanisms by which in utero transmission might happen: direct infection of trophoblasts that serve to transmit HCV, transcytosis of HCV in trophoblast cells without direct infection, and infection of placental and umbilical endothelial cells. There is circumstantial evidence to support infection of trophoblasts. Studies examining cell lines derived from first trimester trophoblasts demonstrate that they express the extracellular receptors present for HCV entry [27]. One study isolated primary trophoblasts and attempted infection using serum from patients infected with HCV [28]. Researchers reported very low levels of detectable viral RNA in some cells and electron micrographs showing what appeared to be viral particles. No direct staining of placental tissue has been performed that definitively demonstrates viral RNA within cells. Support for endothelial cell infection can only be found in studies reporting HCV infection of endothelial cells from other tissue such as brain [29]. Although intrapartum transmission likely results from direct exposure to maternal blood or bodily fluids during labor and delivery, the risk of transmission is likely directly dependent on maternal viremia [30].

Clinical Features HCV During Pregnancy

Women with chronic HCV infection often have uneventful pregnancies without worsening of liver disease or other maternal or infant adverse effects; some women may even have improvement. For example, in a series of 266 pregnant women infected with HCV, elevated serum alanine aminotransferase (ALT) levels were detected in 56% of women at the beginning of pregnancy but only 7% during the third trimester [31, 32]. However, 55% of women returned to elevated ALT levels by 6 months postpartum. Gervais et al [32] found the same results in a small group of 26 women infected with HCV. Such changes may be due to the significant changes in the maternal immune system during pregnancy.

Current data are conflicting as to whether the physiologic changes of pregnancy are associated with histologic progression of disease in women with chronic HCV. In a study comparing liver biopsies in 12 HCV-infected pregnant women (pre- and postpregnancy) and 12 matched HCV-infected nonpregnant women (no intervening pregnancy between 2 biopsy time points), pregnant women had a significant deterioration in necroinflammatory and fibrosis scores compared with nonpregnant controls [33].

However, another study found pregnancy had a beneficial effect on long-term progression of fibrosis [34].

Beyond liver-related complications, pregnant women infected with HCV may be at increased risk for pregnancy complications. In a population-based cohort study in Washington state that controlled for maternal substance use, Pergam et al [35] found infants born to women infected with HCV were more likely to be low birth weight, small for gestational age, and require neonatal intensive care and assisted ventilation. In the same cohort, women infected with HCV had an increased risk for gestational diabetes but only when combined with excessive gestational weight gain. There was also a nonsignificant increase in preterm birth associated with maternal HCV infection. In an analysis of the US Nationwide Inpatient Sample, Reddick et al [3] found similar results with an increased risk of gestational diabetes among HCV-infected women and nonsignificant increases in preterm birth and cesarean delivery. They did not find an association between maternal HCV and small for gestational age.

Risk Factors for Perinatal Transmission

Maternal-to-infant HCV transmission is generally limited to women with detectable HCV RNA during pregnancy [36, 37]. Although cases have been reported in women without detectable HCV RNA, they are likely due to fluctuating viral load so an initial negative HCV RNA test could become positive later in gestation [21]. Furthermore, numerous studies have shown a correlation between HCV viral load and risk of perinatal transmission. Thus, identifiable and potentially modifiable risk factors for intrapartum vertical transmission are those resulting in increased exposure to maternal virus. In a cohort study of 244 infants born to mothers infected with HCV, multivariate analysis demonstrated an increased risk of transmission associated with prolonged rupture of amniotic membranes (>6 hours) and invasive fetal monitoring [20]. However, this finding was not duplicated in another study [38]. Furthermore, there is evidence of discordant transmission in 4 twin pregnancies, with the second twin more likely to be infected with HCV [39–42]. Although not clearly understood, possible etiologies of discordant vertical HCV transmission include placental separation at the time of delivery of the 1st twin resulting in increased exposure to the 2nd twin and differences in genetic susceptibility. Cesarean delivery has been proposed as a means of preventing peripartum transmission by reducing the chance of infant exposure to maternal blood [43]. However, cesarean delivery does not reduce the risk of transmission, and labor should be actively managed with efforts made to avoid early amniotomy and thus shorten the active labor phase [38, 44].

Peripheral blood mononuclear cell (PBMC) infection appears to be a clear risk factor for mother-to-infant HCV transmission, reaching rates of 100% vertical transmission [45, 46]. PBMCs may serve as the vector for HCV, or there may be a variant HCV virus with specific capacity for both infecting PBMCs and overcoming placental immune cells [45]. Past or ongoing maternal intravenous (IV) drug use has also been associated with an increased risk of perinatal HCV, although this result is likely due to a higher incidence of PBMC infection among women with a history of IV drug use [45]. PBMC HCV infection appears to be more common among IV drug users due to the high viral heterogeneity related to repeated superinfections with different HCV strains and quasispecies from blood-contaminated needles.

HIV coinfection has been associated with an increased risk of perinatal HCV infection. A systematic review of 8 studies estimated the risk of perinatal transmission of HCV was 4-fold higher than in HIV-uninfected women (19% vs 4%) [47]. This increased likelihood of transmission is most likely explained by HIV-mediated immunosuppression, which leads to elevations in HCV viral load and thus a higher chance of perinatal transmission. Many studies of HIV-HCV coinfection were conducted before the routine use of HAART. In a study of HIV-infected women receiving HAART, no HCV-coinfected women transmitted HCV to their infants and their mean HCV viral load was similar to that in HCV monoinfected mothers [26]. Therefore, the higher risk associated with HIV coinfection is likely attenuated through the use of HAART.

HCV is detectable in breast milk, although at levels 100–1000 times lower than in plasma [44, 48, 49]. Studies attributing transmission to breastfeeding did not exclude in utero or peripartum transmission. Most other studies have not shown increased transmission risk with breastfeeding, and clinical guidelines do not prohibit breastfeeding in women infected with HCV [50]. One potential explanation is the presence of lipases in human milk that have antiviral activity against enveloped viruses, particularly against HCV [51].

One interesting observation of HCV infection during pregnancy is there is a small but consistent percentage of women who clear their viremia in the postpartum period [52]. Most women experience an increase in viral RNA during the course of pregnancy, which is followed by a decline after delivery. Approx. 10% of infected women would fall to undetectable levels and never subsequently have detectable RNA. This pattern has been attributed to physiologic impairment of cellular immunity during the

immunotolerant phase of pregnancy, which is followed by a surge of HCV-specific T lymphocytes that re-exert immune pressure on the virus after delivery. A recent study describes the changes in maternal immunity in detail by also observing the viral species during the course of successive pregnancies and demonstrating that wild-type HCV emerges during pregnancy, replacing an HCV-specific cytotoxic T lymphocyte escape mutant, which then re-emerged after delivery once immune pressure was restored [53].

HCV During Infancy and Early Childhood

Reliable and early detection of infected infants has been an ongoing challenge. Maternal antibody that persists in the first 12–18 months of life prevents meaningful use of the usual anti-HCV antibody diagnostic tests during that time [19]. The American Academy of Pediatrics recommends "The duration of presence of passive maternal antibody in infants can be as long as 18 months. Therefore, testing for anti-HCV should not be performed until after 18 months of age. If earlier diagnosis is desired, an NAAT to detect HCV RNA may be performed at or after the infant's first well-child visit at 1 to 2 months of age" [54]. Waiting until 18 months of age for definitive testing is not feasible for many anxious parents who want to know if their child has HCV infection. The recommendation for early testing using HCV RNA is based on studies done using now outdated PCR methods. These early studies demonstrated that infants had intermittent episodes of viremia during the first year, which could be due to resolving infection or an abortive infection [19,21,55]. Most laboratories have converted to newer, real-time PCR-based assays with improved sensitivity, but there have been no studies in HCV-exposed infants to determine the test performance characteristics in this population. An alternate but unstudied approach would be to test using the HCV PCR test within the first few months, and then repeat the test several months later. If both tests are negative, it is unlikely the infant is infected. Any other scenario requires further evaluation and possible consultation with an HCV-experienced specialist.

HCV infection in infancy is almost always asymptomatic. It has not been shown to have any negative effect on growth or development in the early years. Several case reports have outlined that infants known to have been exposed and infected after birth can develop significant liver enzyme elevations in the first year of life along with high levels of viremia [56-58]. For reasons not understood, up to 50% of these infants will spontaneously resolve this viremia and hepatitis [19, 21, 59]. One study attempted to examine the long-term outcomes of infants infected after congenital heart surgery from infected blood products [60]. The authors found the same level of resolution in this group, suggesting that age and developmental stage of the immune system or the liver are primary factors in resolution and not mode of transmission. Long-term complications of chronic HCV can present in childhood but are infrequent [61, 62]. There is approx. 1%–2% risk of cirrhosis in childhood, and there are case reports of teenagers with HCV-induced hepatocellular carcinoma [63].

There are no clear indications for treatment of HCV in a young child. This observation is partly due to the lack of symptoms, slow rate of fibrosis development, and significant side effects and poor efficacy with the current standard of care. With increased experience using DAAs that include studies in children, the promise of near universal response will mean treatment indications will expand considerably.

Current and Future Research

With the coming of new DAAs against multiple HCV targets, there are many who believe HCV will no longer be a problem. The extreme costs of these agents coupled with potential risks and limited access mean many patients will still not be treated. Pregnant women and children will wait the longest to reap the benefits brought by new drugs. There are several areas related to HCV in pregnancy and vertical transmission where more research is needed. These topics are summarized in Table 1 and discussed briefly below.

More research is needed on the influence of HCV on adverse outcomes like preterm labor and gestational diabetes. A more in-depth understanding of the dynamic changes in the maternal immune response to HCV during pregnancy will enable us to better predict who will resolve their viremia or who should be targeted with DAAs. A better understanding of which infants are likely to acquire HCV through vertical transmission and what factors govern resolution early in life will enable more precise use of DAAs for those children who will progress to chronic infection. Finally, testing methods need more validation in infants infected with HCV so the recommendations for screening reflect the current standard of care.

In an effort to achieve some of these ends, the National Institute of Child Health and Human Development is conducting an observational study of HCV in pregnancy within the Maternal Fetal Medicine Units (MFMU) Network. The objectives are to: identify risk factors for mother-to-child transmission of HCV; determine risk factors for HCV seropositivity in pregnant women; determine the seroprevalence of HCV infection in the MFMU Network population; determine a threshold of viremia below which

Table 1. Research Gaps Related to HCV in Pregnancy and Vertical HCV Transmission

HCV in Pregnancy
1) When and how does HCV influence specific adverse outcomes in pregnancy such as gestational diabetes, preterm birth, and fetal growth restriction?
2) What changes occur in the HCV-specific immune response during pregnancy: what triggers the surge in HCV-specific T cells?
3) Can we predict the minority of women who will resolve their viremia postpartum?
4) Can we use an abbreviated treatment protocol with new DAAs in this window to achieve high rates of viral clearance?
5) What, if any, adverse effects do DAAs have on pregnancy and postpregnancy outcomes such as breastfeeding?
6) Is there a role now for universal screening for HCV during pregnancy, both to increase awareness of infection and to increase those women that could be treated?
HCV Vertical Transmission
1) What key host and viral factors allow HCV-infected women to transmit virus to their infants?
2) When during pregnancy does transmission occur?
3) Can single or multiple DAAs interrupt transmission?
HCV in Infancy
1) When and how is the best way to reliably identify infected and uninfected infants in the first few months of life?
2) Which infants will spontaneously resolve their viremia?
3) Can we use DAAs in early infancy to prevent chronic infection from being established?

Abbreviations: DAA, direct-acting antiviral drugs; HCV, hepatitis C virus.

mother-to-child transmission does not occur; determine the risk of adverse pregnancy outcomes among women seropositive for HCV; describe the natural history of HCV in children infected via mother-to-child transmission; and evaluate if cesarean delivery is protective against mother-to-child transmission.

Summary

The paradigm for treating HCV will change considerably over the next several years, and many adults infected with HCV will be cured of any detectable virus. It is imperative that the medical community work to ensure the patients with HCV that will not immediately benefit, pregnant women and children chief among them, continue to receive the benefit that further research can bring. Each population offers the potential to reduce the overall burden of HCV while at the same time improving individual outcomes.

Supplementary Data

Supplementary materials are available at the *Journal of The Pediatric Infectious Diseases Society* online (http://jpids.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Acknowledgments

Financial support. This work was supported by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development—National Institutes of Health.

Potential conflicts of interest. Both authors: No reported conflicts.

Both authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

References*

1. CDC, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention. Hepatitis C FAQs for health professionals. Available at: http://www.cdc.gov/hepatitis/HCV/HCVfaq.htm#section1. Accessed 20 November 2013.
2. Arshad M, El-Kamary SS, Jhaveri R. Hepatitis C virus infection during pregnancy and the newborn period–are they opportunities for treatment? J Viral Hepat **2011**; 18:229–36.
3. Reddick KL, Jhaveri R, Gandhi M, et al. Pregnancy outcomes associated with viral hepatitis. J Viral Hepat **2011**; 18:e394–8.
4. Steinmann E, Pietschmann T. Cell culture systems for hepatitis C virus. Curr Top Microbiol Immunol **2013**; 369:17–48.
5. Niepmann M. Hepatitis C virus RNA translation. Curr Top Microbiol Immunol **2013**; 369:143–66.
6. Jopling CL, Yi M, Lancaster AM, et al. Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. Science **2005**; 309:1577–81.
7. Simmonds P. The origin of hepatitis C virus. Curr Top Microbiol Immunol **2013**; 369:1–15.
8. Forns X, Bukh J. The molecular biology of hepatitis C virus. Genotypes and quasispecies. Clin Liver Dis **1999**; 3:693–716, vii.
9. Yamane D, McGivern DR, Masaki T, Lemon SM. Liver injury and disease pathogenesis in chronic hepatitis C. Curr Top Microbiol Immunol **2013**; 369:263–88.
10. Rehermann B. Hepatitis C virus versus innate and adaptive immune responses: a tale of coevolution and coexistence. J Clin Invest **2009**; 119:1745–54.
11. Neumann-Haefelin C, Thimme R. Adaptive immune responses in hepatitis C virus infection. Curr Top Microbiol Immunol **2013**; 369:243–62.
12. Prasad MR, Honegger JR. Hepatitis C virus in pregnancy. Am J Perinatol **2013**; 30:149–59.
13. Pawlotsky JM. Treatment of chronic hepatitis C: current and future. Curr Top Microbiol Immunol **2013**; 369:321–42.
14. McHutchison JG, Everson GT, Gordon SC, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J Med **2009**; 360:1827–38.
15. Poordad F, McCone J Jr, Bacon BR, et al. Boceprevir for untreated chronic HCV genotype 1 infection. N Engl J Med **2011**; 364:1195–206.

16. Fried MW, Buti M, Dore GJ, et al. Once-daily simeprevir (TMC435) with pegylated interferon and ribavirin in treatment-naive genotype 1 hepatitis C: the randomized PILLAR study. Hepatology **2013**; 58:1918–29.
17. Lawitz E, Mangia A, Wyles D, et al. Sofosbuvir for previously untreated chronic hepatitis C infection. N Engl J Med **2013**; 368: 1878–87.
18. Mack CL, Gonzalez-Peralta RP, Gupta N, et al. NASPGHAN practice guidelines: diagnosis and management of hepatitis C infection in infants, children, and adolescents. J Pediatr Gastroenterol Nutr **2012**; 54:838–55.
19. Ceci O, Margiotta M, Marello F, et al. Vertical transmission of hepatitis C virus in a cohort of 2,447 HIV-seronegative pregnant women: a 24-month prospective study. J Pediatr Gastroenterol Nutr **2001**; 33:570–5.
20. Mast EE, Hwang LY, Seto DS, et al. Risk factors for perinatal transmission of HCV and the natural history of HCV infection acquired in infancy. J Infect Dis **2005**; 192:1880–9.
21. Shebl FM, El-Kamary SS, Saleh DA, et al. Prospective cohort study of mother-to-infant infection and clearance of hepatitis C in rural Egyptian villages. J Med Virol **2009**; 81:1024–31.
22. 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.
23. Delamare C, Carbonne B, Heim N, et al. Detection of HCV RNA in amniotic fluid: a prospective study. J Hepatol **1999**; 31: 416–20.
24. 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.
25. 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.
26. Checa Cabot C, Stoszek S, Quarleri J, et al. Mother-to-child transmission of hepatitis c virus (HCV) among HIV/HCV-coinfected women. J Ped Infect Dis **2013**; 2:126–35.
27. Mostafavi A, Arshad M, Qiang G, et al. Examining cells of trophoblastic origin for permissiveness for hepatitis c virus replication. *ID Week*, San Diego, CA: Abstract #1338; 2012.
28. Nie QH, Gao LH, Cheng YQ, et al. Hepatitis C virus infection of human cytotrophoblasts cultured in vitro. J Med Virol **2012**; 84: 1586–92.
29. Fletcher NF, Wilson GK, Murray J, et al. Hepatitis C virus infects the endothelial cells of the blood-brain barrier. Gastroenterology **2012**; 142:634–43.e6.
30. Indolfi G, Azzari C, Resti M. Perinatal transmission of hepatitis C virus. J Pediatr **2013**; 163:1549–52.e1.
31. Conte D, Fraquelli M, Prati D, et al. 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.
32. Gervais A, Bacq Y, Bernuau J, et al. Decrease in serum ALT and increase in serum HCV RNA during pregnancy in women with chronic hepatitis C. J Hepatol **2000**; 32:293–9.
33. Fontaine H, Nalpas B, Carnot F, et al. Effect of pregnancy on chronic hepatitis C: a case-control study. Lancet **2000**; 356:1328–9.
34. Di Martino V, Lebray P, Myers RP, et al. Progression of liver fibrosis in women infected with hepatitis C: long-term benefit of estrogen exposure. Hepatology **2004**; 40:1426–33.
35. Pergam SA, Wang CC, Gardella CM, et al. Pregnancy complications associated with hepatitis C: data from a 2003–2005 Washington state birth cohort. Am J Obstet Gynecol **2008**; 199:38.e1–9.
36. 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.
37. Granovsky MO, Minkoff HL, Tess BH, et al. Hepatitis C virus infection in the mothers and infants cohort study. Pediatrics **1998**; 102:355–9.
38. Cottrell EB, Chou R, Wasson N, et al. Reducing risk for mother-to-infant transmission of hepatitis C virus: a systematic review for the U.S. Preventive Services Task Force. Ann Intern Med **2013**; 158:109–13.
39. Boxall E, Baumann K, Price N, et al. Discordant outcome of perinatal transmission of hepatitis C in twin pregnancies. J Clin Virol **2007**; 38:91–5.
40. Inui A, Fujisawa T, Sogo T, et al. Different outcomes of vertical transmission of hepatitis C virus in a twin pregnancy. J Gastroenterol Hepatol **2002**; 17:617–9.
41. Barlow KM, Mok JY. Dizygotic twins discordant for HIV and hepatitis C virus. Arch Dis Child **1993**; 68:507.
42. Goncales FL Jr, Stucchi RS, Pavan MH, et al. Hepatitis C virus in monozygotic twins. Rev Inst Med Trop Sao Paulo **2000**; 42:163–5.
43. Steininger C, Kundi M, Jatzko G, et al. 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.
44. European Paediatric Hepatitis C Virus Network. A significant sex--but not elective cesarean section–effect on mother-to-child transmission of hepatitis C virus infection. J Infect Dis **2005**; 192:1872–9.
45. Azzari C, Moriondo M, Indolfi G, et al. Higher risk of hepatitis C virus perinatal transmission from drug user mothers is mediated by peripheral blood mononuclear cell infection. J Med Virol **2008**; 80:65–71.
46. Azzari C, Resti M, Moriondo M, et al. Vertical transmission of HCV is related to maternal peripheral blood mononuclear cell infection. Blood **2000**; 96:2045–8.
47. Yeung LT, King SM, Roberts EA. Mother-to-infant transmission of hepatitis C virus. Hepatology **2001**; 34:223–9.
48. Kumar RM, Shahul S. Role of breast-feeding in transmission of hepatitis C virus to infants of HCV-infected mothers. J Hepatol **1998**; 29:191–7.
49. Lin HH, Kao JH, Hsu HY, et al. Absence of infection in breast-fed infants born to hepatitis C virus-infected mothers. J Pediatr **1995**; 126:589–91.
50. American Academy of Pediatrics, Section on Breastfeeding. Breastfeeding and the use of human milk. Pediatrics **2012**; 129: e827–41.

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