Review article: direct-acting antivirals for the treatment of HCV during pregnancy and lactation - implications for maternal dosing, foetal exposure, and safety for mother and child

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Summary

Background: With the global efforts to eradicate hepatitis C virus (HCV), treatment during pregnancy is becoming a priority for research as this, and maternal cure should reduce vertical transmission. However, as information on the efficacy and safety of direct-acting antivirals (DAAs) in pregnancy is generally lacking, treatment of HCV infection during pregnancy is not currently recommended.

Aim: To provide an overview of current knowledge regarding maternal exposure, placental handling and safety of DAAs during pregnancy and lactation

Methods: A literature search was performed focusing on the effect of pregnancy on maternal exposure to DAAs, the placental handling of DAAs, the safety of DAAs for mother and child during pregnancy and the safety of DAAs during lactation.

Results: Exposure to all DAAs studied is likely to be altered during pregnancy, mostly related to pregnancy-induced effects on drug absorption and metabolism. Although animal studies show that most DAAs are reported to cross the placenta and transfer into breast milk, most DAA combinations show a favourable safety profile. Because of the rapid viral decline after treatment initiation, and to avoid the critical period of organogenesis, treatment may be started at the end of the second trimester or early third trimester.

Conclusions: Treatment of HCV infection during pregnancy is realistic, as DAAs are highly effective and treatment duration is relatively short. There is an urgent need to study DAAs during pregnancy and lactation to contribute to the goal of HCV elimination.

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1  |  INTRODUCTION

In 2015, there were an estimated 71 million persons chronically infected with hepatitis C virus (HCV), resulting in 1.34 million deaths that year.1 With the global efforts of the World Health Organization to eradicate HCV by 2030, it is important to identify individuals at risk and provide treatment as soon as possible after diagnosis.2 While the majority of infections among adults in many settings are linked to injection drug use, the most important source of paediatric HCV infection is vertical transmission of the virus, responsible for approximately 60% of paediatric HCV cases globally.3,4 Among pregnant women, estimates of HCV prevalence have ranged from 0.1% to 8% from different countries and settings.5-8

1.1  |  Vertical transmission of HCV

A large meta-analysis performed by Benova et al reported a 5.8% risk of vertical transmission of HCV, resulting in 1700 HCV-infected newborns in the USA yearly.9,10 In addition to high maternal HCV viral load, the review states that maternal HIV co-infection increased the risk of vertical transmission of HCV to around 10%.9,11,12 However, the majority of the co-infected pregnant women included were not taking antiretroviral therapy, and a recent study suggested that those on antiretroviral therapy have a similar risk of transmitting HCV to those with HIV mono-infection.13 The prevalence of HCV and HIV co-infection among pregnant women varies across settings. One study in Rwanda reported that 3.9% of pregnant women with HCV were co-infected with HIV.14 In a Western/Central European cohort of pregnant women living with HIV, 12% were co-infected with HCV.15-16 In addition to high maternal HCV viral load and having HIV, membrane rupture for more than 6 hours before delivery and internal foetal monitoring (uterine or foetal scalp) have been reported to contribute to an increased risk of vertical HCV transmission.16 Currently, most studies suggest that the risk of transmission may be reduced by elective caesarean section, particularly in the case of HCV/HIV co-infection.17,18 Based on timing of positive HCV RNA test results in the newborn, timing of transmission is thought to occur during intrauterine, peripartum and postpartum periods.19 Transmission may be most frequent during the peripartum period (estimated 40%-50%) when there is blood-blood contact during delivery. In this case, the child will be born HCV-RNA negative, but detectable RNA levels are expected after the first 3 days of life. It is estimated that intrauterine transmission accounts for approximately 30% of the cases, based on HCV-RNA positivity at or shortly after delivery.21 The exact mechanisms by which intrauterine transmission occurs are not well understood, but may include trophoblast-mediated endocytosis of HCV and/or transcytosis of viral particles.22 Postpartum transmission via breastfeeding is rare, as the proportion of children acquiring HCV is similar among those who were breastfed compared to those who were not.17

1.2  |  Testing and consequences of HCV in the newborn

Although vertical transmission seems to be the most important source of paediatric HCV infection, a retrospective cohort study showed that many children of women with chronic HCV are not screened and therefore many paediatric HCV cases will be missed.23 A critical reason for the low proportion of exposed infants who are screened is related to the delay in diagnosis, as HCV antibody testing can only be performed at or after 18 months of age, and the chance of loss to follow-up in the intervening period is high.24 Testing for HCV-RNA positivity in the newborn is generally not routinely performed, but should be recommended in infants born to HCV-RNA positive mothers.25 The majority (80%) of children acquiring HCV through vertical transmission do not clear the infection spontaneously, resulting in chronic paediatric HCV infection.26 Although liver injury from chronic HCV infection generally progresses slowly early in life, serious liver damage can occur during childhood and beyond.27-29 One centre reported five children (out of 91 included patients, mean age: 9 years) with an accelerated course of HCV and early development of decompensated liver disease requiring liver transplantation, two of whom subsequently died.30 In addition to concerns about the physical health of the child with HCV, there may be high levels of distress in the family.31 Treatment regimens based on direct-acting antivirals (DAAs) for children with chronic HCV ≥ 12 years of age or ≥ 35 kilograms were approved by the FDA in April 2017.32 Phase I and II trials of several DAA combinations for children aged 3-12 years are currently ongoing [ClinicalTrials.gov: NCT03067129, NCT03080415, NCT03487848, NCT03022981]. Unfortunately, there will be no treatment available prior to the age of 3 in the near future as this is not requested by the European Medicines Agency (EMA). Therefore, treatment of children with vertically acquired HCV may be complicated as loss to follow-up in healthcare later in life is likely to be high, as is the case in some settings for HIV.33,34

1.3  |  Effect of HCV on pregnancy outcome

In addition to the risk of vertical HCV transmission, maternal HCV infection may increase the risk of adverse pregnancy outcomes. Recently, a protocol has been published for a study which will undertake an extensive systematic review of pregnancy outcomes in women with HCV; results are expected soon.35 Only a small proportion of available studies have sufficient power to adjust for potential confounding variables such as tobacco, alcohol and drug use. These studies report an association between maternal HCV infection and the risk of gestational diabetes and intrahepatic cholestasis of pregnancy.36,37 In addition, an increased risk of preterm birth was reported and children born to women living with HCV were more likely to have a lower birth weight and to be small for gestational age.38-41
1.4 | Considerations for HCV treatment during pregnancy

Diagnosis and treatment of HCV in pregnant women is becoming a priority area of research as this has potential, not only to cure the mother, but also to prevent vertical transmission.\(^42,43\) Until recently, ribavirin was a cornerstone of HCV therapy. As this drug is known to be teratogenic and embryotoxic in all animal species studied, its use—and consequently all classical forms of HCV therapy—during pregnancy has been contra-indicated.\(^44\) However, new, potentially much safer combinations of DAAs drugs are now available that are ribavirin free. The relative short duration of treatment (8-12 weeks) and the rapid viral load decline following treatment initiation makes treatment and cure of women with chronic HCV late in pregnancy realistic.\(^24,45\) Testing and treatment during pregnancy seems a unique opportunity, as loss to follow-up of mother and child postpartum is high in many low and middle income country settings, and pregnant women are engaged in healthcare during this period, being highly motivated to take actions to ensure their own health and the health of their unborn child.\(^23,46,47\)

In 2018, the American Association for Maternal-Fetal Medicine, have not yet adopted the recommendation. We identify research gaps on the potential use of DAAs in pregnancy which we review here. These are: (a) The effect of pregnancy on maternal exposure to DAAs; (b) The placental handling of DAAs; (c) Safety of DAAs for mother and child during pregnancy; (d) Safety of DAAs during the lactation period. Implementation and costs of antenatal HCV screening are also important but will not be considered here. We identify research gaps on the potential use of DAAs in pregnant and breastfeeding women living with HCV.

### TABLE 1 Overview safety and pharmacokinetic data of DAAs in pregnancy

<table>
<thead>
<tr>
<th>DAA combination</th>
<th>Genotype</th>
<th>Hypothetical change in maternal exposure (mechanism)</th>
<th>Safety concerns based on animal data</th>
<th>Priority to be studied in clinical trials in pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOF/DAC</td>
<td>Genotype 1-4</td>
<td>↓ DAC exposure (CYP3A4 induction)</td>
<td>Yes</td>
<td>High</td>
</tr>
<tr>
<td>SOF/LDV</td>
<td>Genotype 1, 4-6</td>
<td>↓ LDV exposure (gastric pH increase)</td>
<td>No</td>
<td>Moderate</td>
</tr>
<tr>
<td>SOF/VEL</td>
<td>Pan-genotypic</td>
<td>↓ VEL exposure (gastric pH increase)</td>
<td>Uncertain</td>
<td>High</td>
</tr>
<tr>
<td>SOF/VEL/VOX</td>
<td>Pan-genotypic</td>
<td>↓ VEL/VOX exposure (CYP3A4 induction)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GZR/ELB</td>
<td>Genotype 1,4</td>
<td>↓ GZR/ ELB exposure (CYP3A4 induction)</td>
<td>No</td>
<td>Moderate</td>
</tr>
<tr>
<td>GLE/PIB</td>
<td>Pan-genotypic</td>
<td>↓ GLE exposure (CYP3A4 induction)</td>
<td>Uncertain</td>
<td>High</td>
</tr>
</tbody>
</table>

Abbreviations: DAC, daclatasvir; ELB, elbasvir; GLE, glecaprevir; GZR, grazoprevir; LDV, ledipasvir; PIB, pibrentasvir; SOF, sofosbuvir; VEL, velpatasvir; VOX, voxilaprevir.
acidity during pregnancy may therefore result in lower exposure.\textsuperscript{57,58} During pregnancy, women often suffer from nausea and heartburn for which antacids and/or proton pump inhibitors (PPIs) may be prescribed.\textsuperscript{59} It has been shown that velpatasvir and ledipasvir exposure in healthy volunteers treated with PPIs such as omeprazole, is reduced up to 40%.\textsuperscript{60,61} Therefore, co-administration of PPIs with velpatasvir or ledipasvir during pregnancy should be avoided if possible.\textsuperscript{57,58}

2.2 Distribution

Apart from physiological changes (eg body volume and tissue perfusion), physicochemical factors (eg drug lipophilicity and molecular weight) determine drug distribution. For example, the increase in body fat during pregnancy is likely to increase the volume of distribution of highly lipophilic drugs, such as DAAs, which in turn may result in lower peak plasma levels, prolonged half-life and lower amplitude of plasma concentrations at steady state. However, little information is available to estimate the contribution of the increased fat mass to the decrease in plasma levels often observed during pregnancy.\textsuperscript{55,62} All DAAs, except sofosbuvir, show a high degree of plasma protein binding.\textsuperscript{63} Particularly for these highly protein bound drugs, an increase in the unbound fraction may be expected due to decreased plasma levels of the main drug-binding plasma proteins albumin and α1-acid glycoprotein during pregnancy. For most drugs, the total concentration decreases during pregnancy while the unbound concentrations are unaffected. Therefore, it is crucial to measure the unbound plasma concentration in pregnancy, next to total plasma concentrations, to reliably identify the effect of pregnancy on the pharmacologically active unbound concentration. This should be taken into account in future studies on DAA pharmacokinetics in pregnancy.\textsuperscript{64}

2.3 Metabolism

Pregnancy-induced changes in the activity of drug-metabolising enzymes including cytochrome P450 (CYP) and the uridine diphosphate glucuronosyltransferase (UGT) family have been observed. While the mechanism of the observed changes has not been identified, accumulated data suggest that the changes are regulated by rising concentrations of hormones.\textsuperscript{55} Increased CYP3A4 capacity in liver and/or intestine is expected to have the most profound impact on DAA pharmacokinetics compared to other metabolising enzymes.\textsuperscript{65} Tracy et al reported that CYP3A activity increased by 35%-38% during pregnancy\textsuperscript{66} and, except for sofosbuvir/ledipasvir, exposure to all DAA combinations may be affected to some extent by induction of CYP3A-mediated metabolism. The use of potent CYP3A inducers in combination with DAAs that are metabolised by CYP3A4 is currently contra-indicated, as this may reduce DAA efficacy, possibly resulting in virological failure.\textsuperscript{65} The moderate pregnancy-induced increase in CYP3A4 activity may have less profound effects compared to the effects of concomitant use of strong CYP3A inducers on DAA exposure, but dose adjustments of DAAs during pregnancy may have to be considered. For some DAA combinations, only exposure to a single component may be affected by pregnancy-induced changes. For example, exposure to velpatasvir and voxilaprevir, but not to sofosbuvir, is influenced by CYP3A activity. However, a dose adjustment of individual components is complicated since all DAA combination treatments are available as fixed-dose combinations, except for sofosbuvir and daclatasvir, which are available as separate formulations. Data from phase IV trials suggest that sustained virological response is not related to plasma concentrations of glecaprevir in nonpregnant patients on high-dose proton pump inhibitors (PPIs), implying that the levels were well above the therapeutic threshold. A slight decrease in glecaprevir exposure, caused by moderate CYP3A4 induction due to pregnancy, is therefore expected to be of less clinical relevance.\textsuperscript{67,68} Regarding daclatasvir, the recommended daclatasvir dose of 60 mg QD is increased to 90 mg QD when co-administered with moderate inducers of CYP3A4A, as reductions in exposure of 25% have been observed frequently for CYP3A4 substrates.\textsuperscript{62} A moderate increase in CYP3A activity due to pregnancy may also require an increase in daclatasvir dose. However, the clinical relevance of increased CYP3A4 metabolism for daclatasvir in pregnancy is unknown as its oral clearance is significantly lower in women than in men, resulting in higher exposure in women.\textsuperscript{69}

2.4 Excretion

Elimination of DAAs (except for sofosbuvir) occurs mainly via biliary excretion as parent drug. Pregnancy may alter the expression of drug transporters in metabolising and eliminating organs, but there is little quantitative information on the influence of pregnancy on transporter activity in the basolateral and canalicular membrane of hepatocytes, and the resulting effect on DAA excretion. Due to this lack of knowledge, no pregnancy physiologically-based pharmacokinetic (PBPK) models have been published which simulate the role of biliary drug excretion.\textsuperscript{70} Sofosbuvir is mainly renally eliminated as its pharmacologically inactive metabolite GS-331007 (78%) and to a lesser extent as unchanged sofosbuvir (3.5%). Because pregnancy leads to an increase in renal blood flow and glomerular filtration rate, renal clearance of sofosbuvir may be increased during pregnancy. It is, however, unclear whether this could influence plasma levels to the point of requiring dose adjustment.\textsuperscript{55,71}

2.5 Drug-drug interactions (DDIs) in case of maternal viral co-infection

For the treatment of maternal hepatitis B co-infection, tenofovir disoproxil fumarate (TDF) is preferred.\textsuperscript{72} As TDF may also be part of combination antiretroviral therapy (cART) for treatment of HIV, literature on DDIs between cART and DAAs can be consulted.\textsuperscript{65} DDIs between HCV and cART are an important consideration in the treatment of (pregnant) HCV/HIV co-infected women,\textsuperscript{14,15} and are well described in the literature.\textsuperscript{65,73} However pregnancy-induced alterations could also influence either HCV and/or HIV drug
therefore increased (90 mg instead of 60 mg once daily) in patients daily, is recommended and therefore concomitant use of darunavir/ritonavir and sofosbuvir/velpatasvir/voxilaprevir should be avoided, 

3.1 | Placental handling in human pregnancy

For ethical reasons, pregnant women have historically often been excluded from clinical trials in the drug development process, resulting in a gap of knowledge regarding both maternal and foetal drug exposure throughout gestation. However, recent reports on the importance of conducting pharmacological research in pregnancy and possible research strategies highlight that times are changing. To our knowledge, information on DAA exposure during pregnancy is currently limited to three conference abstracts. However, none of these studies collected data on foetal exposure (eg as umbilical cord blood concentrations) as treatment was either discontinued or completed during pregnancy and it is therefore not known whether or how well these DAAs cross the human placental barrier in vivo.

3.2 | Placental handling in pre-clinical research

Studies on placental handling of DAAs are solely based on developmental toxicology studies in animal models. As shown in Table 2, placental transfer of sofosbuvir, daclatasvir, glecaprevir, pibrentasvir, grazoprevir and elbasvir has been observed in rats. Placental transfer of grazoprevir and elbasvir has, in addition to rats, also been observed in rabbits, but to a minimal extent. However, because of interspecies differences in placental anatomy, placental transfer data from animal studies is of poor translational value. Compared to the placenta of rabbit, rat and mouse, the structure of the human placenta differs in gross shape, histology of the maternofoetal interface and type of maternofoetal interdigitation, which may all affect placental drug transfer.

3.3 | Prediction of placental drug handling ex vivo

To predict transfer of drugs across the human placenta in vivo, data from animal studies may be combined with information on drug-specific physicochemical characteristics. Comparing DAAs based on their specific physicochemical properties provides information on potential changes in their pharmacokinetics during pregnancy (Chapter 2) and a similar approach can yield estimates of placental transfer, and hence provides a rough estimate of foetal exposure. A review by Giagninis et al summarised the factors affecting transport of drugs across the placental barrier. In general, maternofoetal exchange increases with gestational age because of physiological changes, eg reduced membrane thickness and increased uterine blood flow, inherent to the increased foetal demand of oxygen and nutrients. Passive diffusion is the major route of placental transport and is responsible for rapid transfer of lipophilic drugs with a molecular weight of <500 Da. Larger molecules may also be subjected to passive diffusion, which is a relatively slow process; all DAAs are highly lipophilic, indicated by a log P >2.5 (except for sofosbuvir, log P = 1.62), favouring effective passive diffusion. On the other hand, their high molecular weight (all >500 Da) may hamper or at least slow the process. In addition, the degree of ionisation and protein binding also influence the rate and extent of placental transfer.

3 | PLACENTAL HANDLING OF DIRECT-ACTING ANTIVIRALS

Drug transport across the placental barrier is a major determinant of foetal exposure and toxicity. However, for some conditions foetal exposure to maternally administered drugs could potentially provide pre-exposure prophylaxis and thereby reduce the chance of vertical transmission, as has been hypothesised for HIV.
Protein binding will contribute to trapping of drug in the foetal or maternal circulation, and as maternal and foetal plasma protein concentrations differ and change with advancing gestational age, the maternal-to-foetal ratio of total drug plasma concentration may vary accordingly.

Next to passive diffusion, drug transport across the placental barrier may also be carrier-mediated, either as facilitated diffusion or via active transporters. All DAAs included in this review are ATP-binding cassette (ABC) transporter substrates of P-gp (ABCB1) and/or Breast Cancer Resistance Protein (BCRP/ABCG2). As these efflux transporters are expressed at the apical side of the syncytiotrophoblast layer, they possibly play a role in reducing foetal exposure. Xenobiotics interacting with these transporters may also influence placental transfer of DAAs. Since placental transporter expression changes during pregnancy, placental transfer may be also dependent on timing of treatment during pregnancy. A reduction in P-gp mRNA and protein levels from first trimester towards term has been reported, likely related to the general decrease in foetal protection after the critical period of organogenesis. Therefore, there is a greater potential of P-gp substrates (eg most DAAs), to reach the unborn child with advancing gestational age. Literature on BCRP expression throughout gestation is inconsistent.

As outlined above, different factors either facilitate or impede drug transport across the placental barrier. Hence, it is difficult to estimate the extent of placental transport of a specific drug at a specific time point during pregnancy. In addition to in vitro techniques using immortalised cell lines or tissue explants, computer-assisted modelling attempts are useful to explore the contribution of the physicochemical properties to placental transport. The dual side placental perfusion model has proven to be a valid experimental method to study the transport of xenobiotics ex vivo and is currently used extensively to investigate placental passage. As stated before, it is hypothesised that the extent of placental drug transfer

<table>
<thead>
<tr>
<th>DAA therapy</th>
<th>Preclinical and postnatal development</th>
<th>Placental transfer</th>
<th>Lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Safety concerns?</strong></td>
<td><strong>Tested animal species (Dose and duration)</strong></td>
<td><strong>Transfer across placenta</strong></td>
<td><strong>Tested animal species (% of maternal plasma levels)</strong></td>
</tr>
<tr>
<td><strong>DAA combination</strong></td>
<td><strong>Drug</strong></td>
<td><strong>Safety concerns?</strong></td>
<td><strong>Rats: 10x RHD, GD6-18, GD6-LD20 Rabbits: 28x RHD, GD6-19</strong></td>
</tr>
<tr>
<td>SOF/DAC</td>
<td>SOF&lt;sup&gt;b&lt;/sup&gt;</td>
<td>No</td>
<td><strong>Yes</strong></td>
</tr>
<tr>
<td>DAC</td>
<td>Yes&lt;sup&gt;c&lt;/sup&gt;</td>
<td><strong>Rats: 4x RHD, GD7-19 Rabbits: 16x RHD, GD6-15</strong></td>
<td><strong>Yes</strong></td>
</tr>
<tr>
<td><strong>SOF/LDV</strong></td>
<td><strong>SOF&lt;sup&gt;b&lt;/sup&gt;</strong></td>
<td>No</td>
<td><strong>Rats: 10x RHD, GD6-18, GD6-LD20 Rabbits: 28x RHD, GD6-19</strong></td>
</tr>
<tr>
<td>LDV</td>
<td>Possible&lt;sup&gt;c&lt;/sup&gt;</td>
<td><strong>Rats: 4x RHD, GD6-18 Rabbits: 2x RHD, GD7-20</strong></td>
<td>Unknown</td>
</tr>
<tr>
<td><strong>SOF/VEL</strong></td>
<td><strong>SOF&lt;sup&gt;b&lt;/sup&gt;</strong></td>
<td>No</td>
<td><strong>Rats: 10x RHD, GD6-18, GD6-LD20 Rabbits: 28x RHD, GD6-19</strong></td>
</tr>
<tr>
<td><strong>SOF/VEL/VOX</strong></td>
<td>VEL</td>
<td>Possible&lt;sup&gt;d&lt;/sup&gt;</td>
<td><strong>Rats: 6x RHD, GD6-17, GD6-LD20 Rabbits: 0.5-0.7x RHD, GD7-20 Mice: 31x RHD, GD6-15</strong></td>
</tr>
<tr>
<td>VOX</td>
<td>No</td>
<td><strong>Rats: 141x RHD, GD6-LD20 Rabbits: 4x RHD, GD7-19</strong></td>
<td>Unknown</td>
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<tr>
<td><strong>GZR/ELB</strong></td>
<td>GZR</td>
<td>No</td>
<td><strong>Rats: 117x RHD, GD6-20, GD6-LD20 Rabbits: 41x RHD, GD7-20</strong></td>
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<td>ELB</td>
<td>No</td>
<td><strong>Rats: 10x RHD, GD6-20, GD6-LD20 Rabbits: 18x RHD, GD7-20</strong></td>
<td><strong>Yes</strong></td>
</tr>
<tr>
<td><strong>GLE/PIB</strong></td>
<td>GLE</td>
<td>Possible&lt;sup&gt;e&lt;/sup&gt;</td>
<td><strong>Rats: 53x RHD, GD6-18, GD6-LD20 Rabbits: 0.07x RHD, GD7-19</strong></td>
</tr>
<tr>
<td>PIB</td>
<td>No</td>
<td><strong>Rats: 1.5x RHD GD7-19 Mice: 51x RHD GD6-15, GD6-LD20</strong></td>
<td><strong>Yes</strong></td>
</tr>
</tbody>
</table>

**TABLE 2** Safety data from reproductive teratogenicity studies of DAAs in pregnancy

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**Abbreviations:** DAC, daclatasvir; ELB, elbasvir; GD, gestation day; GLE, glecaprevir; GZR, grazoprevir; LD, lactation day; LDV, ledipasvir; PIB, pibrentasvir; RHD, recommended human dose; SOF, sofosbuvir; VEL, velpatasvir; VOX, voxilaprevir.

<sup>a</sup>Transfer into milk was studied in rats.
<sup>b</sup>Exposure to predominant circulating metabolite of sofosbuvir (GS-331007).
<sup>c</sup>At a high dose (4.6-fold RHD), an increased incidence of skeletal variations (vertebrae, sternea, ribs) in rats was observed. These effects are likely related to a decrease in maternal body weight gain and decreased food intake.
<sup>d</sup>A possible teratogenic effect was indicated in rabbits where an increase in total visceral malformations was seen in exposed animals at AUC exposures up to 0.7-fold RHD for SOF/VEL and 0.5-fold RHD for SOF/VEL/VOX.
<sup>e</sup>Maternal toxicity with some embryofoetal toxicity precluded the ability to evaluate glecaprevir in the rabbit at human clinical exposures.
increases towards term. Therefore, data from ex vivo placental perfusion experiments using term placentas potentially overestimate foetal exposure during earlier phases of pregnancy. For a variety of drugs studied, transfer across term placentas ex vivo shows good correlations with in vivo maternal and cord blood concentration. Hence, data from ex vivo placental perfusion experiments may also be used to rank the various DAA s with regard to their potential to cross the placenta.

4 | SAFETY OF DIRECT-ACTING ANTIVIRALS DURING PREGNANCY

In the absence of conclusive evidence from DAA exposure during human pregnancy, assessments of DAA safety are based on data derived from animal reproduction toxicology studies. Table 2 summarises the results of these pre-clinical studies on embryofoetal toxicity, teratogenicity, placenta transfer and breast milk. The literature search strategy can be found in Appendix S1. Although the majority of the adverse effects of drugs are related to direct foetal exposure because of placental transfer, drugs may also affect the developing foetus indirectly by disturbing placental function. However, the species-specific placental physiology, as well as immunological and endocrinological differences hamper the translation of placental drug effects from animal studies. A large registry of infants intrauterine-exposed to DAA s is needed to assess long-term effects in humans. Such an approach has been undertaken for HIV, the Antiretroviral Pregnancy Registry, and has been proven to be successful in monitoring effects of intrauterine exposure to antiretrovirals. Adding HCV to an existing pregnancy registry may be an option worth considering.

4.1 | Sofosbuvir/daclatasvir

The only data available on sofosbuvir/daclatasvir in human pregnancy include one study on accidental sofosbuvir/daclatasvir exposure around the time of conception (n = 7). No adverse birth outcomes were reported but one infant tested HCV positive at 18 months with low viral load, which is not unexpected as all women discontinued therapy early, before week 9 of gestation.

Sofosbuvir administration showed no adverse effects in preclinical reproduction toxicology studies using rabbits at exposure levels comparable to 10-fold the recommended human dose (RHD). As sofosbuvir could not be detected in rodent plasma probably due to high esterase activity, assessment of reproductive toxicity tests in rats are based on exposure to the major (inactive) metabolite GS-331007. At GS-331007 exposure levels following a 10-fold RHD, no effect on intrauterine development or any malformations were seen in rats. As sofosbuvir was detectable in human plasma, data from rodent studies regarding sofosbuvir exposure should be interpreted with caution. For daclatasvir, embryofoetal toxicity (external and/or visceral malformations) in rabbits and rats was reported by the EMA. However, exposure in rats and rabbits was 4.6 and 16-fold RHD respectively. Remarkably, the Food and Drug Administration (FDA) reported no concerns for embryofoetal toxicity in rats when exposed to 6x RHD and in rabbits when exposed to 22-fold RHD.

In the general patient population, this DAA combination has a favourable safety profile. The most frequently reported adverse reactions were fatigue, headache and nausea. A special warning for the use of sofosbuvir (in combination with either daclatasvir or ledipasvir) and amiodarone was based on observed bradycardia in several patients.

4.2 | Sofosbuvir/ledipasvir

Data on sofosbuvir/ledipasvir use during pregnancy are limited to two conference abstracts: one study performed in India included pregnant women living with HCV who requested treatment because of anxiety about vertical transmission (n = 15), and DAA s were started during the second and early third trimester, and the other, previously mentioned, is a phase I trial of sofosbuvir/ledipasvir started during the second trimester of pregnancy (n = 8) from the USA. Both studies reported no safety concerns; there were no cases of vertical transmission to date in the USA study, but the number is yet to be reported for the Indian study. Considering the small number of women in the two studies, a lack of vertical transmission would not be unusual even in the absence of treatment.

The decision to study sofosbuvir/ledipasvir in a clinical setting is supported by a favourable embryofoetal safety profile based on preclinical studies. As stated earlier in this review, sofosbuvir use seems safe for the developing offspring in rats and rabbits. At exposure levels of 3.4-fold RHD, minor effects of ledipasvir on fertility of female rats were reported. However, this was not seen at 2-fold RHD and effects were likely related to non-adverse maternal toxicity. The FDA reported that there are no clear adverse effects on foetal development in rats and rabbits at 4-fold and 2.3-fold RHD respectively. However, a lower body weight of the offspring in rats at an exposure level of 4-fold RHD is reported by the EMA and at slightly higher exposure levels (4.6-fold RHD), an increased incidence of skeletal variations was observed. In the absence of other toxicity findings, this effect on the offspring may be related to non-adverse maternal toxicity.

In terms of adverse events, fatigue and headache were reported in adult patients treated with ledipasvir/sofosbuvir.

4.3 | Sofosbuvir/velpatasvir (tvxolaprevir)

The EMA reports a possible teratogenic effect (visceral malformations) due to velpatasvir exposure of 0.7-fold RHD in pregnant rabbits; however, according to the FDA label, this is written as being of “no significant effect”. Mice and rat studies found no embryofoetal adverse effects at 23-fold RHD and 4-fold RHD respectively. There was no evidence of placental transfer of velpatasvir as it could not be detected in litter after a single dose of 30 mg/kg on gestation day 13 or 18. Maternal voxilaprevir administration did not result in
adverse embryofetal effects in rats (141-fold RHD) and rabbits (4-fold RHD). No data on placental transfer of voxilaprevir in animals have been reported.99-102

The most common adverse effects seen in clinical studies were headache, fatigue and nausea. When combined with voxilaprevir, diarrhoea and nausea were also reported as common adverse events.58,75

### 4.4 | Grazoprevir/elbasvir

Grazoprevir and elbasvir reproduction studies have failed to reveal any adverse effects in rats (10-fold RHD) and rabbits (18-fold RHD).103,104

In patients with HCV, reported adverse reactions were fatigue and headache with a special warning for plasma liver enzyme (ALT) elevations. The rate of late ALT elevations during treatment was directly related to plasma exposure to grazoprevir and generally occurred from approximately 8 weeks after start of treatment. These late ALT elevations were typically asymptomatic and resolved with ongoing therapy with grazoprevir/elbasvir or after completion of therapy.105

### 4.5 | Glecaprevir/pibrentasvir

Registration files state that either glecaprevir administration in rats (63-fold RHD) or pibrentasvir administration in mice (100-fold RHD) did not result in reproductive toxic effects. However, this conclusion was considered questionable because maternal toxicity with some embryofetal toxicity precluded the ability to evaluate glecaprevir in rabbits at human clinical exposures.106,107

In clinical studies, the most commonly reported adverse reactions were fatigue and headache. Occasionally, elevations of total bilirubin levels have been reported in patients using glecaprevir/pibrentasvir. The effect is more pronounced with higher glecaprevir plasma levels and is likely due to glecaprevir-mediated inhibition of bilirubin transport and metabolism.108 Although maternal bilirubin elevations are asymptomatic and transient, the effects on the foetus may be questionable as unconjugated bilirubin can cross the placental barrier.109 Therefore, it may be hypothesised that high maternal bilirubin levels may increase the risk of neonatal jaundice.

### 5 | SAFETY OF DIRECT-ACTING ANTIVIRALS DURING THE LACTATION

As studies have shown that avoidance of breastfeeding does not seem to reduce the chance of vertical HCV transmission, breastfeeding is considered safe among women with chronic HCV, except for women with cracked nipples.110 Since breastfeeding is currently not contra-indicated in these patients living with HCV, it is important to gather information of the potential use and safety of DAAs during the lactation period.

To evaluate safety of drug use during the lactation period the following aspects should be considered: the effect of drugs on milk production; drug concentrations in milk; and the effects of exposure on the breastfed child. In the absence of human data, data on DAA exposure in milk are limited to rat studies. They should be interpreted with caution due to species-specific differences in lactation physiology such as mammary gland anatomy, storage and release of milk into ducts, protein and fat composition of milk and the expression of drug transporters in mammary tissue, as these factors play a major role in the extent passage of drugs into milk.111 Data obtained from rat studies showed that DAAs are transferred into milk, but no effects on growth and development were observed in nursing pups exposed to DAAs via milk.97,99,104,107 Maternal plasma-to-milk ratios have been reported to differ considerably between DAAs in rat studies. Whereas glecaprevir concentrations in milk are <8% of maternal plasma levels, grazoprevir milk concentrations exceed maternal plasma concentrations (400%). This disparity in extent of milk transfer is, however, difficult to explain based on the physicochemical differences and transporter profiles of the two compounds.112 Grazoprevir and glecaprevir have a comparable molecular weight with a similar degree of plasma protein binding. Moreover, glecaprevir is known to be a BCRP substrate, whereas grazoprevir is not. As this transporter is considered to contribute to excretion of drugs into milk,113 the proportion of glecaprevir transferred to milk is expected to be high, but extensive plasma protein binding impedes its transfer into breastmilk.

### 6 | DISCUSSION

There are multiple reasons to either consider or defer DAA treatment during pregnancy as reviewed extensively by others.7,114,115 Although the AASLD/IDSA guidelines recommend universal screening of pregnant women,24 no DAA regimen is currently approved for treatment during pregnancy because of insufficient human safety and efficacy data and treatment is therefore delayed until after delivery. The high chance of loss to follow-up of both mother and her HCV-exposed child in many settings, together with loss of healthcare insurance after pregnancy may complicate adequate maternal and paediatric treatment, highlighting the pregnancy period as a unique window of opportunity to both cure the mother and prevent vertical transmission of HCV.46,116

Standard dosing regimens of currently used DAAs may be suboptimal for the pregnant patient population as pregnancy-induced pharmacokinetic changes may influence maternal drug exposure, hence efficacy of the drugs. The expected increased elimination of DAAs, both because of a potentially increased biliary excretion as well as induction of metabolising enzymes, may potentially lead to subtherapeutic levels. This pregnancy-induced effect is expected to be less pronounced for sofosbuvir/ledipasvir and glecaprevir/pibrentasvir compared to other DAA combinations. Instead of adjusting the dose—which is rarely done in clinical practice—a DAA combination less prone to pregnancy-induced effects could be chosen, if available.

Apart from data on maternal exposure to DAAs, early data on placental handling and subsequent foetal exposure are important to study possible placental toxicity of DAAs as well as the potential of
DAAs to provide foetal pre-exposure prophylaxis. Several ex vivo and in vitro models may provide insight into pharmacokinetics, including placental transfer of drugs. The ex vivo placental perfusion model, using human term placentas yet unexposed to drugs, can be helpful in studying the initial phase of placental DAA handling and may provide an estimation of foetal exposure. Subsequently, pregnancy-PBPK modelling can be used as a tool to assess foetal plasma levels upon maternal administration of different dosing regimens. In the view of potential pre-exposure prophylaxis, foetal exposure to DAAs may, next to maternal viral load reduction, contribute to the reduced risk of vertical transmission. In contrast to HIV, research has yet not focused on the use of DAAs for pre-exposure prophylaxis in the general population. Future research is needed to clarify the role of foetal exposure in reducing the chance of vertical transmission.

Most DAAs are reported to cross the placental barrier in either rats and/or rabbits when administered early during pregnancy. In clinical practice, it is more likely that treatment will be started late in pregnancy to avoid the critical period of foetal organogenesis. Therefore, data from animal reproductive toxicity studies are difficult to extrapolate to the human clinical situation as there is a difference in timing of treatment and because of the large interspecies variability in placental anatomy, both affecting the extent of placental passage. Based on animal studies, it is likely that postpartum exposure to DAAs may occur via breastfeeding. Nowadays, breastfeeding is not contra-indicated in women with chronic HCV. However, this advice may change if breastfeeding women are treated with DAAs in the future, given that fact that all DAAs are detected in milk of rats, with grazoprevir levels in milk being 400% of maternal levels.

In order to decide on the timing of treatment, it is not only important to assess the most critical window of possible teratogenic effects of drug exposure (generally the earlier phase of pregnancy), but also to consider the timing of vertical transmission of HCV. As peripartum blood-blood contact may be the most important source of vertical transmission, maternal HCV RNA should be undetectable during delivery. Ideally, treatment would be completed before delivery. In that case, taking into account that women living with HCV may deliver preterm, the optimal time period to start DAA treatment may be at the end of the second trimester (at around week 23/24 of gestation) or early third trimester (at around 27/28 weeks of gestation), in case of a 12- or 8 week treatment period respectively, and may have to be extended until after delivery to complete treatment. For late presenters (>28 weeks of gestation), treatment may still be effective as DAAs cause a rapid viral decline, resulting in undetectable HCV RNA in just 2-4 weeks.

The choice for a specific DAA combination should be based on safety data from animal reproducive studies and safety and efficacy data from the nonpregnant patient population. Further research on safety, PK and efficacy in pregnant women is warranted prior to implementation of treatment in this population. Research on the potential effect of maternal DAA use on the state of foetal-directed immune tolerance is of particular importance in this population as DAAs are thought to improve the proliferative potential of HCV-specific T cell response again which may decrease immune tolerance. Furthermore, foetal exposure should be assessed, eg by measuring umbilical cord blood concentrations, when women continue treatment until after delivery. This information is needed to assess the potential of DAAs for pre-exposure prophylaxis. Furthermore, an international registry is needed to provide a safety net to detect adverse effects after intrauterine or postpartum DAA exposure.

Next to safety and efficacy data, various other factors have to be taken into account to decide which HCV treatment regimen should be preferred. Firstly, genotype specificity of DAAs plays an important role as the phase I study on sofosbuvir/ledipasvir in pregnancy had to exclude a third of the pregnant women for participation because of genotype 2 or 3 infection. As genotyping is costly, not feasible in all settings and delays treatment start, future research may focus particularly on the use of the pan-genotypic DAA combinations glecaprevir/pibrentasvir and sofosbuvir/velpatasvir (with or without voxilaprevir) during pregnancy. Furthermore, the use of co-medication may determine the DAA regimen of first choice. In the case of HCV/HIV co-infection, EFV should not be used in combination with any DAA and when boosted PIs are part of ART, only sofosbuvir/ledipasvir or sofosbuvir/velpatasvir should be used concomitantly. If women use proton pump inhibitors (PPIs) during pregnancy, neither sofosbuvir/ledipasvir nor sofosbuvir/velpatasvir should be prescribed. In case of concomitant use of strong CYP3A4 inducers, eg rifampicin, sofosbuvir/ledipasvir should be advised. Lastly, local drug availability and costs may determine which drug will be prescribed, despite the fact that this DAA regimen may not be the regimen of first choice based on animal safety data, genotype specificity or potential for DDIs. An important example that needs to be taken into consideration is the widespread use of sofosbuvir/daclatasvir in Egypt, which is one of the countries with the highest HCV prevalence worldwide. Women becoming pregnant using daclatasvir are advised to stop their treatment because of the lack of knowledge regarding safety and efficacy, despite not knowing whether cessation of treatment during pregnancy, likely resulting in HCV disease relapse, may be less advantageous than continuing treatment. Given the paucity of data and the potential exposure to daclatasvir during conception or early pregnancy, research is needed on daclatasvir efficacy in human pregnancy and global pregnancy registry databases are warranted to assess its safety.

Ideally, a pan-genotypic, safe and effective DAA combination would be available to all pregnant women living with HCV, diagnosed by a (cost-effective) universal screening programme. However, cost-effectiveness of specific DAA combinations, local drug availability and preferences of pregnant women themselves and their treating physicians play a dominant role and these factors have to be taken into consideration.

| CONCLUSION |
| Treatment of HCV with DAAs during pregnancy and breastfeeding is not currently recommended because of lack of data on safety, leaving pregnant women diagnosed with HCV untreated until after |
delivery (which in itself maybe distressing for the mother and deter her from breastfeeding). In our opinion, this window of opportunity to simultaneously improve maternal health and prevent vertical transmission should not be missed. There is an urgent need to study DAAs in pregnant and breastfeeding women to target these patient populations as well as their HCV-exposed children and to contribute to the HCV elimination goal of 2030.

ACKNOWLEDGEMENTS

Declaration of personal interests: A. J. and I. C., reports grants from Abbvie, Bristol Myers Squibb, Gilead, Janssen Pharmaceuticals and Viiv Healthcare through the PENTA Foundation and from the Collaborative Initiative for Paediatric HIV Education and Research, Gilead Sciences, NHS England outside the submitted work and funding from the Medical Research Council [programme number MC_UU_12023/26, grant number MR/R019746/1] and the PENTA Foundation. D. G. reports grants from Janssen Pharmaceuticals and Viiv Healthcare through the PENTA Foundation and from, Medical Research Council, EU, UKAID (was DFID) and Wellcome trust and PENTA Foundation outside the submitted work. A. C. has received research grants to her institution from PENTA, Viiv, Janssen Research, Gilead and Merck. D. B has received research grants to his institution from Merck, BMS, Janssen/Tibotec, Viiv Healthcare, Gilead and has received educational grants from Merck and was speaker a symposia for Merck, is member of the advisory board of Viiv Healthcare and Merck. All other authors have no potential conflicts to declare.

AUTHORSHIP

Author contributions: J.F. and M.S. performed the literature search, collected the data and wrote the paper; A.J., D.G. and I.C. contributed to the design of the review and wrote the paper; R.G., F.R. and J.D. provided feedback during the research and writing process; A.C. and D.B. contributed to the design of the review and provided feedback during the research and writing process. All authors approved the final version of the article.

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SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section at the end of the article.