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The management of hepatitis B virus infection in relation with human reproduction

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ABSTRACT

The HBV infection is a major public health problem. It is estimated that worldwide around 2 billion people have markers that show they passed through this infection.

In the majority of the cases of HBV infection occurring in adult patients, the natural evolution is towards HBs Ag clearance (which is though not equivalent in all the cases with complete healing as the virus may persist in the hepatocytes in the form of cccDNA). The situation is completely different when the infection is contracted perinatal (intra- or post- partum or more rarely intrauterine) as in these patients, most frequently the infection evolves to chronic forms, either active or inactive or in the form of immune-tolerance. The vertical transmission of HBV infection represents the main problem regarding the management of this infection in pregnancy.

In the following review of the literature I synthetized the data regarding the impact of HBV infection on pregnancy as well as the impact of pregnancy on HBV infection. I also searched data regarding the possibility of father-to-offspring transmission and I tried to clarify the mechanisms that make the transmission possible. Finally, I synthetized the existing recommendations in algorithms that are dedicated to ease the activity of the infectious diseases specialist that has to deal with such cases.

Keywords: hepatitis B, pregnancy, vertical transmission, HBV, prevention

ETIOLOGY

Hepatitis B virus (HBV) is a member of Hepadnaviridae family. 10 genotypes have been described to date (A to J) (1).

HBV is a DNA enveloped virus, hepatotropic, non-cytopatic. HBV virion (the Dane particle) measures 42 nm. The virion contains the nucleocapsid which contains the genetic material of the virus (partially double strained DNA and the viral polymerase. Outside the Dane particle, the HBs antigen (HBs Ag) can be found, a lipoprotein that can be identified in the blood of the infected patients and that is used as a laboratory marker for the infection.

EPIDEMIOLOGY

Chronic B hepatitis is an important public health problem. It is estimated that globally 2 billion people have serological markers that prove past HBV infection, and around 248 million people are chronically infected (2,3). HBV infection prevalence varies from below 2% in USA, Canada and western Europe, 2-7% in the Mediterranean countries, Japan, central Asia, Middle East, south America and over 8% in west Africa and Sudan (3). In Romania, the prevalence of the infection has been estimated at 4.4%, with a total percent of 27 of the patients presenting serological markers of past HBV infection (4).

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The virus can be found in the blood of the infected persons as well as in other biological fluids (semen, saliva, vaginal secretion) and can survive for long periods of time on various surfaces outside human body. The transmission of the infection is made by contact (parenteral or mucosal) with the blood or secretions of an infected person (directly or indirectly, through surfaces or objects). The transmission can also occur after sexual contact (hetero- or homosexual). Another mean of transmission is the vertical transmission: from mother to offspring. It is also discussed the possibility of transmission from father to fetus, through infected genetic material (5, 6).

PATHOPHYSIOLOGY

HBV replication starts with the viral adherence to the receptor from the surface of the hepatocyte (recently identified as the co-transporter of sodium taurocholate) to which the virus binds with the pre-S1 portion of the envelope (7). After penetrating in the hepatocyte, the virion is released from the envelope and it penetrates the nucleus. Here, the positive strain of HBV DNA is synthetized and the viral genome is converted into covalently closed circular DNA (ccc ADN). After transcription, pre-genomic RNA is produced, which is then included in the capsid. The negative and positive strains of HBV DNA are then synthetized ant they can be assembled together with envelope proteins and released from the cell as complete virions or can re-enter in the hepatocyte nucleus where ccc DNA will be produced again. ccc DNA is the form in which the virus survives to the existing antiviral treatments, thus making difficult to obtain viral clearance.

The exact pathogenesis of the disease is still subject of discussion. It is considered that the liver suffering is caused by the immune response, with cellular lysis mediated by T cytotoxic lymphocytes. The following arguments support this theory:

- In patients in which HBs Ag clearance occurs, a stronger reaction of T cytotoxic lymphocytes have been shown to occur compared to those in which HBe Ag remains positive (8).
- The events associated with immune clearance, as for example ”e” system conversion are accompanied by an aggravation of the liver damage translated by a higher elevation of the transaminases (9).
- Fulminant forms of HBV hepatitis occur when a large number of hepatocytes are destroyed.

A study that followed patients with HBV infection over a 23 years period showed that the complete eradication of the virus is actually extremely rare (despite frequent HBs Ag loss and of an efficient immune control mediated both by T cytotoxic lymphocytes and humoral immunity (10).

In rarer forms of the disease, as cholestatic fibrosing hepatitis, which occurs after liver transplant, a direct cytotoxic involvement is supposed (11).

MATERNAL INFECTION

Risk factors

Women that present a more elevated risk for contracting HBV infection are: intravenous drug users, persons that have multiple sexual partners, contacts of HBV infected people, healthcare workers or midwives from institution where people with disabilities are taking care of, recipients of plasma derivate products (including those with innate coagulation problems), patients that need hemodialysis, personnel from healthcare services and public health departments that come in contact with blood, as well as persons from high endemicity of HBV.

Clinical manifestations

In the acute phase of the disease, the clinical manifestations can vary ranging from sub-clinical, non-icteric hepatitis and icteric hepatitis. In

the chronic phase, the manifestations can range from asymptomatic carrier state to tardive complications of the infection (cirrhosis and/or hepatocellular carcinoma). The extra hepatic manifestations may appear either in the acute phase or in the chronic phase of the B virus hepatitis.

Symptomatic acute viral hepatitis can be encountered in about 30-80% of the patients of adult age (12) but frequently the manifestations are non-specific (pseudo-influenza syndrome, nausea, vomiting, anorexia). The probability to develop jaundice is decreasing with age (13). Pale colored stools, dark urine and tender hepatomegaly are also part of the classic clinical picture of acute B hepatitis. Fatigue is a non-specific element described by the majority of the patients with acute B hepatitis. Clinical manifestations of acute B hepatitis in pregnant woman are no different than those encountered in the general population (14).

Persistent HBV infection (defined as the persistence of HBs Ag 6 months after the time of infection) is most frequently sub-clinical. If symptoms appear, these are most frequently non-specific: fatigue, lack of appetite, nausea, slight pain in the right hypochondria, myalgia and arthralgia. Jaundice, splenomegaly, ascites, encephalopathy are signs of complications (cirrhosis or hepatocellular carcinoma).

The impact of HBV infection on pregnancy

Few populational studies indicated that there is an increased risk for premature birth, low weight at birth, premature rupture of the membranes and gestational diabetes in pregnancies in HBV positive mothers (15). In mothers in which liver function is severely impaired, postpartum hemorrhages and puerperal infections can occur and the pregnancy can end with spontaneous abortion or neonatal (16).

The impact of pregnancy on HBV infection

The pregnancy is generally well tolerated by the mothers with HBV infection. HBV DNA levels can increase during the pregnancy in associations with a decrease of transaminase level, probably due the immune tolerance that is characteristic for the pregnancy, and this situation can be sometimes followed by the decrease of HBV DNA and increase of ALT after the pregnancy, as a consequence of reconstitution of the immune system. This post-partum reactivation of the B hepatitis can be associated with e system seroconversion (HBe antigen clearance and HBe antibodies appearance) in 12.5-17% of the patients (15).

Workup and diagnosis

HBV infection diagnostic is established essentially by the presence of HBs antigen. Acute B hepatitis is diagnosed when HBc IGM antibodies are present. The elevation of the transaminase of over 5-6 times upper value of normal is suggestive for an acute hepatitis, but flares during chronic B infection can produce also such aspect. For disease staging purposes and in order to establish the risk of transmission and the opportunity to initiate antiviral therapy, HBV DNA, HBe Ag and HDV Ab are also necessary (see below).

HBV infection evolves in three phases:

1. The immune-tolerance phase

Is characterized by an almost null response of the immune system to the presence of the virus. Paraclinically is translated by HBs Ag positive, HBe Ag positive and very high levels of viral load without transaminase increase.

In adult, this phase corresponds to the incubation period. In patients that acquire the infection in the perinatal period, this phase can extend over decades.

2. The immune active phase

Is characterized by the response of the immune system which leads to inflammation with liver cytolysis, decrease of HBV DNA levels with HBe Ag positive. There are also patients in which despite the disappearance of HBe Ag (and appearance of HBe Ab) HBV DNA and ALT levels remain high.

In adult this phase corresponds with the symptomatic phase of HBV acute hepatitis, but can extend over decades if the immune response is not powerful enough.

3. Inactive HBV infection (inactive carrier status)

Is characterized by e system seroconversion, transaminase levels normalization and viral load decrease. From this phase, the patient can return to the immune active phase.

HBs Ag testing: ideally before pregnancy. Pregnant women are routinely tested around 8 weeks of pregnancy.

FETAL INFECTION

Risk factors. Maternal-fetal transmission frequency

The most important risk factor for the transmission of the infection from mother to fetus is DNA level over 200.000 IU/ml (15).

Another important risk is the presence of HBe Ag. A study performed before the use of immune globulin prophylaxis showed that the transmission occurred in 85% of the children born from HBe Ag positive mothers, while in children born from mothers with negative HBe Ag the transmission occurred only in 31% of cases (15,17).

In mothers in immune tolerant phase, the risk of transmission is up to 30% even if specific immune globulin and vaccine prophylaxis is correctly applied (18). If the mother is HBe negative and the prophylaxis is correctly done, the risk of transmission is below 10% (15).

Other risk factors that may increase the rate of transmission are: premature birth, prolonged labor and previous transmission despite correct prophylaxis (18).

Mother to fetus transmission may occur in 3 moments: intrauterine, during birth or post-partum. Because post-partum prophylaxis is very efficient, it is believed that transmission occurs mostly intra or post-partum. Intrauterine transmission could occur in 5-16% of the pregnancies (15,19,20) and is supposed to be responsible for the cases of transmission despite correct prophylaxis.

The risk of transmission during amniocentesis is influenced by the level of HBV DNA. Levels higher than 10^7 copies/ml were the only factor that influenced statistically significant the rate of HBV transmission in patients in whom amniocentesis was performed (21). It is recommended to avoid passing with the needle through the placenta when this maneuver must be performed in a HBs Ag positive mother. The risk in the case of other invasive maneuvers (like chorionic villi biopsy, cordocentesis or intrauterine fetal surgery) was not sufficiently studied to have a clear conclusion yet.

The type of birth (vaginal or cesarean) is still a reason for controversy. A recent metaanalysis (22) that included 430 articles on this subject (of which in the final only 10 corresponded to the imposed criteria) concluded that cesarean birth could have a benefit compared to vaginal birth regarding the rate of HBV transmission especially in mothers with HBe Ag positive and high HBV DNA levels despite nucleosidic/nucleotidic treatment.

Mother-to-child transmission pathogenesis

The maternal-fetal transmission of HBV infection in newborns is defined as the presence of HBsAg after 9 months from birth (23). HBs Ag or HBV DNA presence immediately after birth is usually transitory and does not imply necessary chronic infection (24). HBs Ag can also be detected transitory as a consequence of vaccination. HBe or HBc antibodies can be found in newborns and children until 12 respectively 24 months as a consequence of their passage through the placenta and are not necessarily markers of infection (25).

Knowing the mechanisms involved in the transmission is important because prevention methods are addressed to these mechanisms.

The intra-uterine transmission is rare as the virus crosses rarely the placenta and probably just as a consequence of some incidents. High viral load and HBe AG presence are the factors incriminated in this way of transmission (26). A possible explanation of the transmission from mothers with HBe Ag positive is that HBe Ag is capable to cross the placenta and can induce immune tolerance in-utero. The intra-uterine transmission can occur in two circumstances: either the virus crosses the placenta, or some "leakage" may appear from maternal to fetal circulation (23).

The transmission most frequently appears during birth and is due the contact of the newborn's blood with the blood or maternal secretions.

Some of the newborns can be infected by intimate contact with their mothers (27). Still, breastfeeding is not considered a risk factor for transmission and therefore is not discouraged (23, 28).

Transmission from mother to child through infected oocyte should be not neglected, and this mean of transmission has been demonstrated by a study on embryos obtained by in-vitro fertilization that have not been transferred intrauterine (29)

Clinical manifestations. Sequels

HBV infected newborns present rarely with any clinical signs or even suggestive biochemical modifications from birth. They remain frequently asymptomatic and develop chronic infection which may remain in the immune tolerance phase months or sometimes even years (sometimes decades) (30). A small number of children can develop an acute hepatitis around the second month of life, which can sometimes evolve as a fulminant hepatitis (31). Most frequently, these are limited and not severe forms.

Some authors described the possibility that HBV infection to be associated with some non-specific congenital malformations (32, 33). Their findings is not sustained by other researchers, which did not find any statistical significant difference between malformations in children from HBs positive mothers when compared to those from uninfected mothers (34).

Antenatal diagnosis

Given that intrauterine transmission of the infection is rare and the potential of the virus to produce congenital malformations is arguable, prenatal diagnosis is useless. Even more, it is possible that the maneuvers necessary for collecting samples for tests to be themselves a risk factor for transmitting the disease to the fetus (35).

Postnatal diagnosis

Children from mothers with HBs Ag positive or HBc Ab positive and HBs Ag negative must be tested between 9 to 18 months of life for HBs Ab and HBs Ag in order to verify if they became immune after vaccination and to exclude the presence of the infection. Until this age, HBs Ag can be transiently present as a vaccination consequence or sometimes even post-partum, without necessary indicating the presence of the infection, and HBs Ab can be present as a consequence of the specific immune-globulin administration (23, 25, 36). Detection of HBs Ag after 9 months (or after 3 months from the last dose of vaccine) indicates the presence of the infection.

THE MANAGEMENT OF THE INFECTION DIAGNOSED BEFORE PREGNANCY

Acute infection

In case of an acute HBV infection in a patient that whishes to become pregnant, it is advisable to wait for 6 months, period in which most of the patients will lose HBs Ag. The patients in which HBs Ag is still positive after the acute infection are considered chronically infected and treated as written below. The persons that obtain HBs

Ag loss will be treated as mentioned in the case of HBc Ab positive and HBs Ag negative patients.

Chronic infection

In a patient with known chronic HBV infection before pregnancy, the management is different for different stages of the disease.

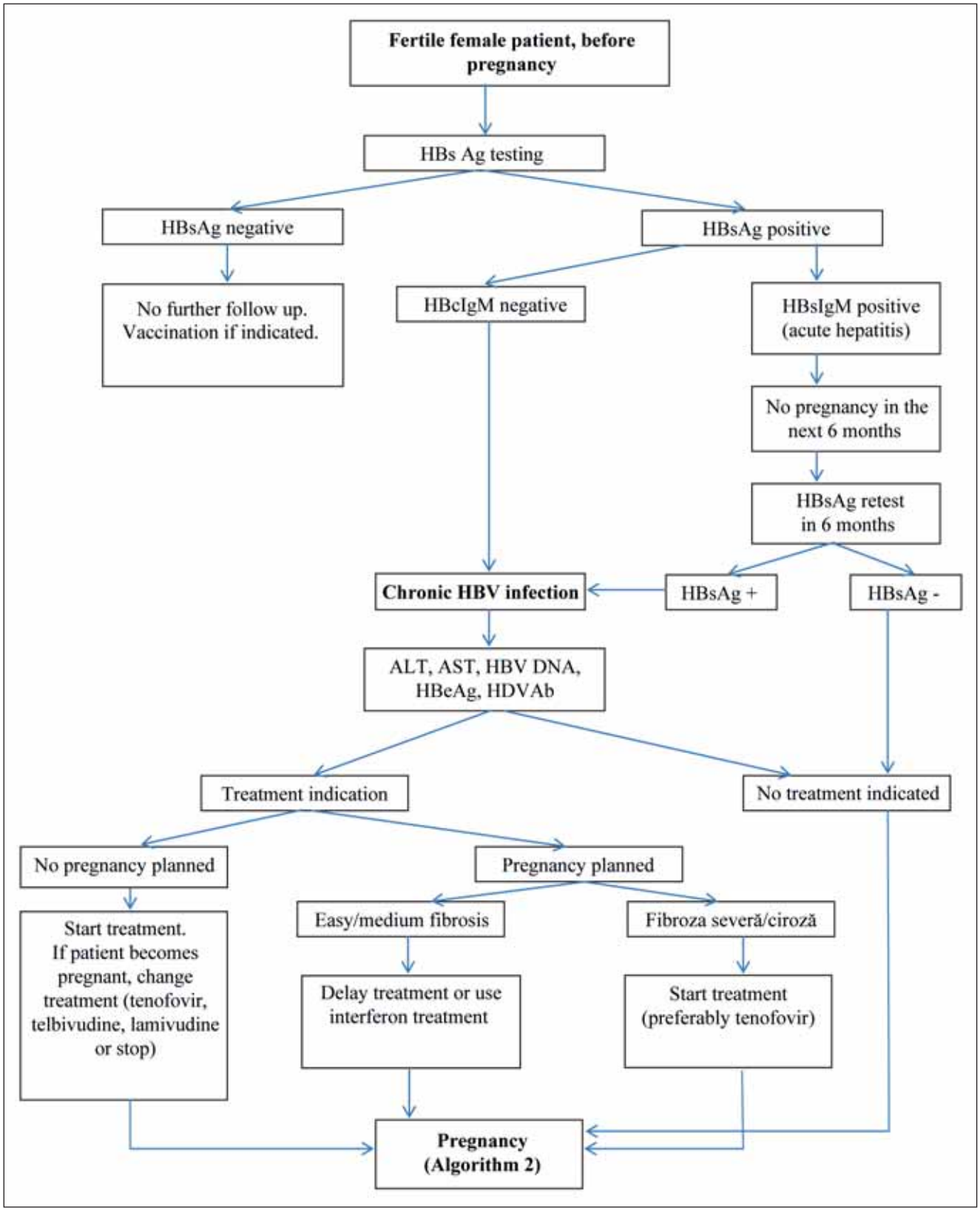
HBV patients in the immune-tolerance phase have high viral loads and HBe Ag present and therefore the risk of transmission is high. These patients do not have an indication for antiviral treatment unless hepatic histological changes are shown (this involves liver biopsy) or if family medical history includes cirrhosis or hepatocellular carcinoma (37). As the transmission occurs most frequently perinatally and because none of the currently available medicines are considered completely safe in pregnancy, the treatment of these patients in the first two trimesters of their pregnancies (besides the above mentioned indications) is not justified (37-39). In the third trimester the treatment is indicated and reduces significantly the risk of transmission of HBV infection from mother to child (38). Newborns need to receive active immunization (vaccine) as well as passive immunization (HBIG) (37-39).

Immune active HBV infection patients have persistently increased transaminase values, HBe Ag can be negative or positive and viral load has values usually over 20000 IU/ml. In these patients, unless an advanced fibrosis stage is proved it is considered more safe to delay antiviral treatment until after the pregnancy and that treatment should be instituted only in the last trimester of pregnancy if the viral load is higher than 10^6 IU/ml (or 200.000 IU/ml according AASLD) (37, 38). In patients with advanced liver fibrosis a therapy with PEG-interferon can be carried before pregnancy, using contraceptive methods during the treatment (37). If this treatment has no results or cannot be done, it is necessary to start oral treatment which has to be maintained during pregnancy. The preferred drug is tenofovir (37, 38).

In a patient hat is on therapy for HBV infection and becomes pregnant, the therapy has to be reconsidered. The medication shown to be safe in pregnancy are lamivudine, telbivudine and tenofovir, but even these must be used with caution as they are classified as B class medication (existing animal studies have not shown risk, but there are not sufficient data in pregnant woman) (37, 38). If the patient is under interferon treatment, this has to be stopped and changed on oral medication, preferably with tenofovir (37). If the pregnancy is planned, the treatment can be completely stopped in a patient without advanced fibrosis or can be changed on tenofovir if the patient has advanced fibrosis or cirrhosis (37). Newborns must receive both active and passive immunization (37-39).

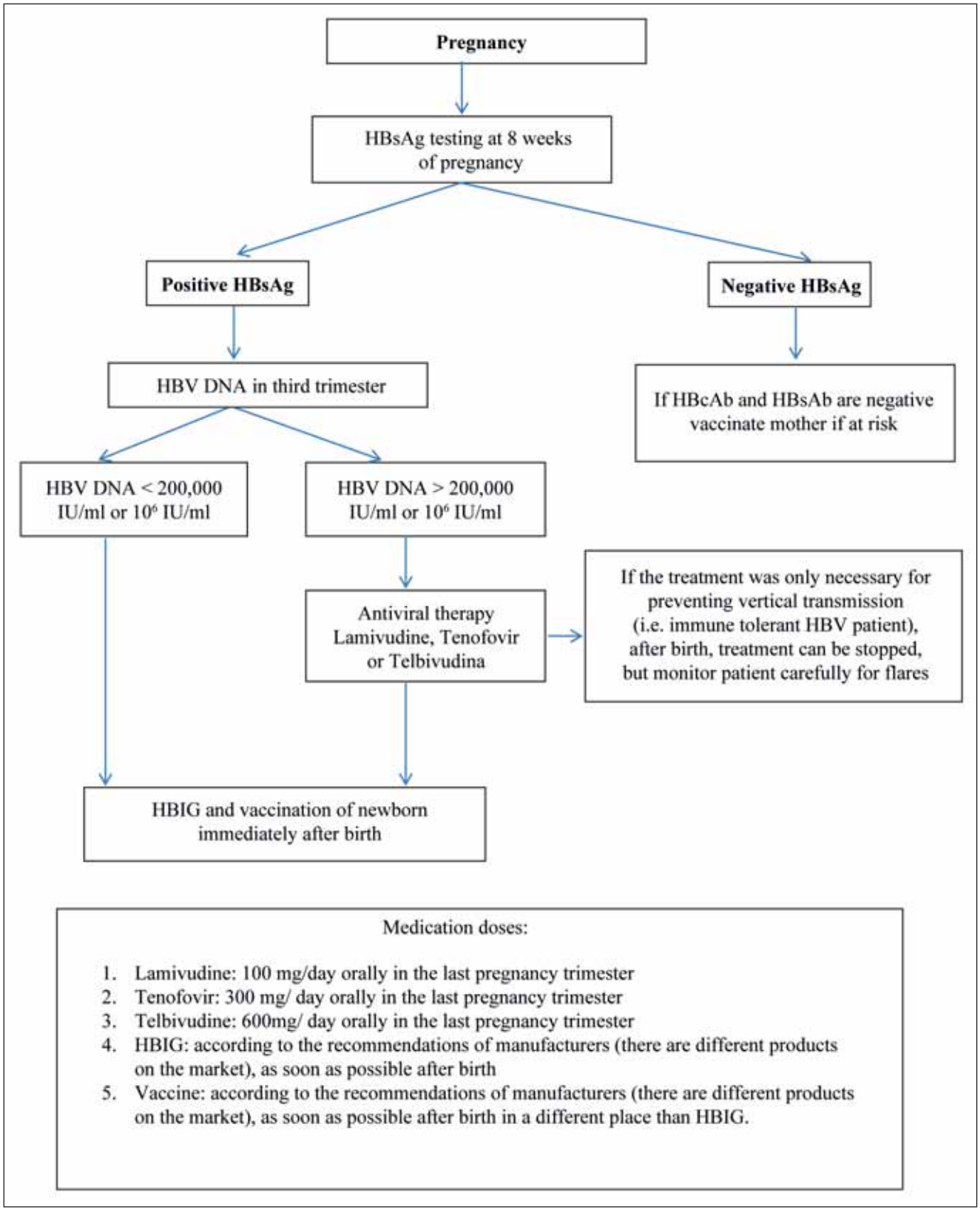
Patients with HBV infection in inactive phase have normal values of transaminase, HBe Ag negative and low HBV DNA levels (usually below 2000 IU/ml). A patient in this phase does not necessitate a special preparation for the pregnancy. These patients do not necessitate antiviral therapy outside pregnancy, they only need to be monitored periodically (37, 38). In weeks 24-28, the HBV DNA must be monitored in order to establish if there will be necessary to institute antiviral therapy during the last trimester of the pregnancy. Newborns must receive both active and passive immunization (37-39).

HBc Ab positive, HBs Ag negative patients. This serologic aspect may indicate either past infection under immune control or naturally resolved infection, or occult HBV infection. Patients with past HBV infection that obtained serologic conversion in s system must not be considered completely healed as they can intermittently have detectable HBV DNA in their blood (13, 40). This aspect is actually the cause of most occult HBV infections defined as the persistence of HBV genome inside the hepatocytes in HBs Ag negative patients (usually also with HBV DNA undetectable). Another possible cause



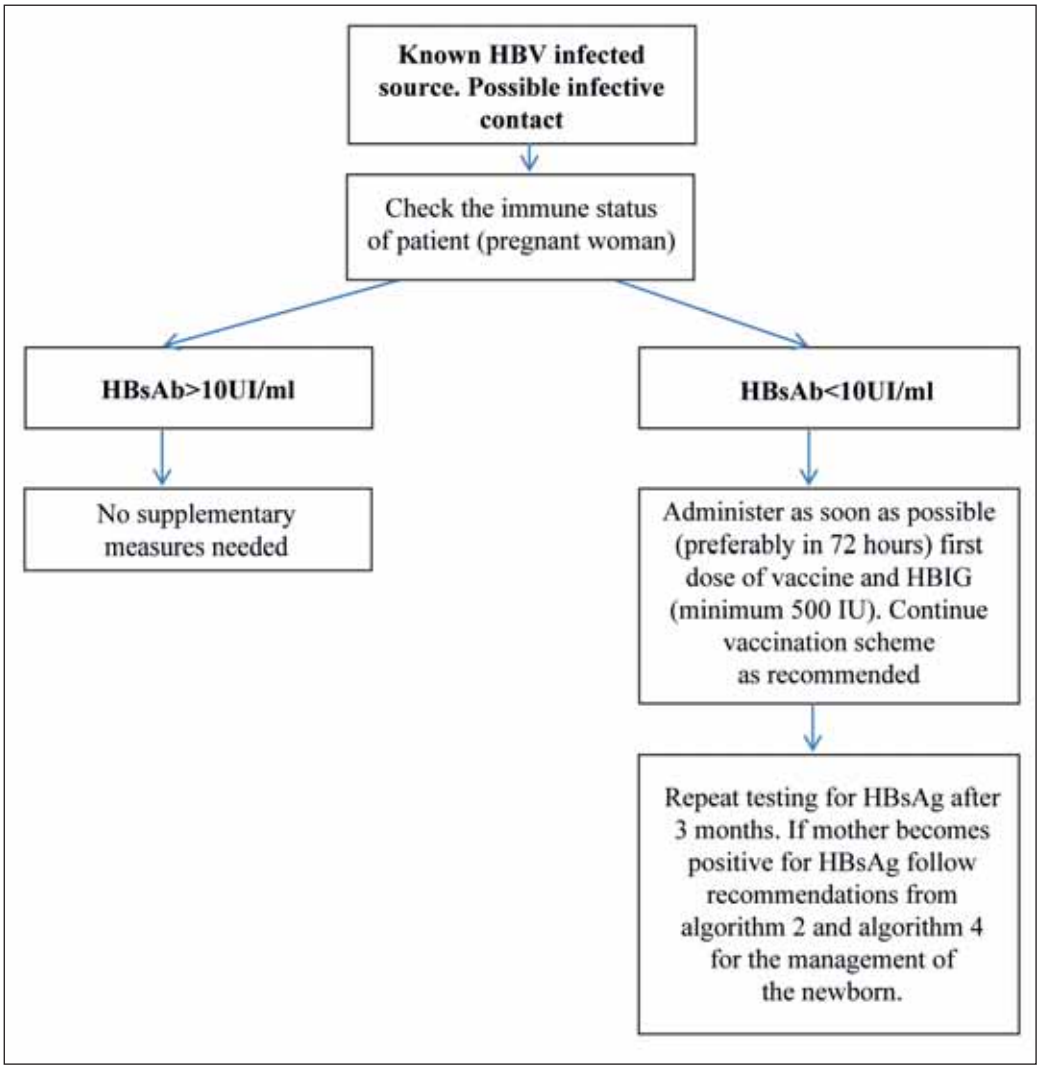
Algorithm 1. *Management of women before pregnancy (in relation with HBV infection)*

for occult HBV infection is the mutation of HBV virus which leads to forms with HBs Ag that is not recognized by current testing kits. It must be mentioned here that there are forms of HBV occult infection in which all serological markers are negative (41). A study on 105 mothers with HBc Ab positive and HBs Ag negative proved the possibility of transmission of HBV infection as 7



Algorithm 2. *Management of HBV infection during pregnancy*

of the newborns presented markers of infection, 5 of them with detectable HBV DNA. Still, none of these children developed in the end chronic HBV infection (42). American and European guidelines recommendations on the management of chronic HBV in pregnancy does not include this category in the indications for HBIG administration (37, 38).



Algorithm 3. *Management of a pregnant woman in case of a contact with a possible HBV infective contact*

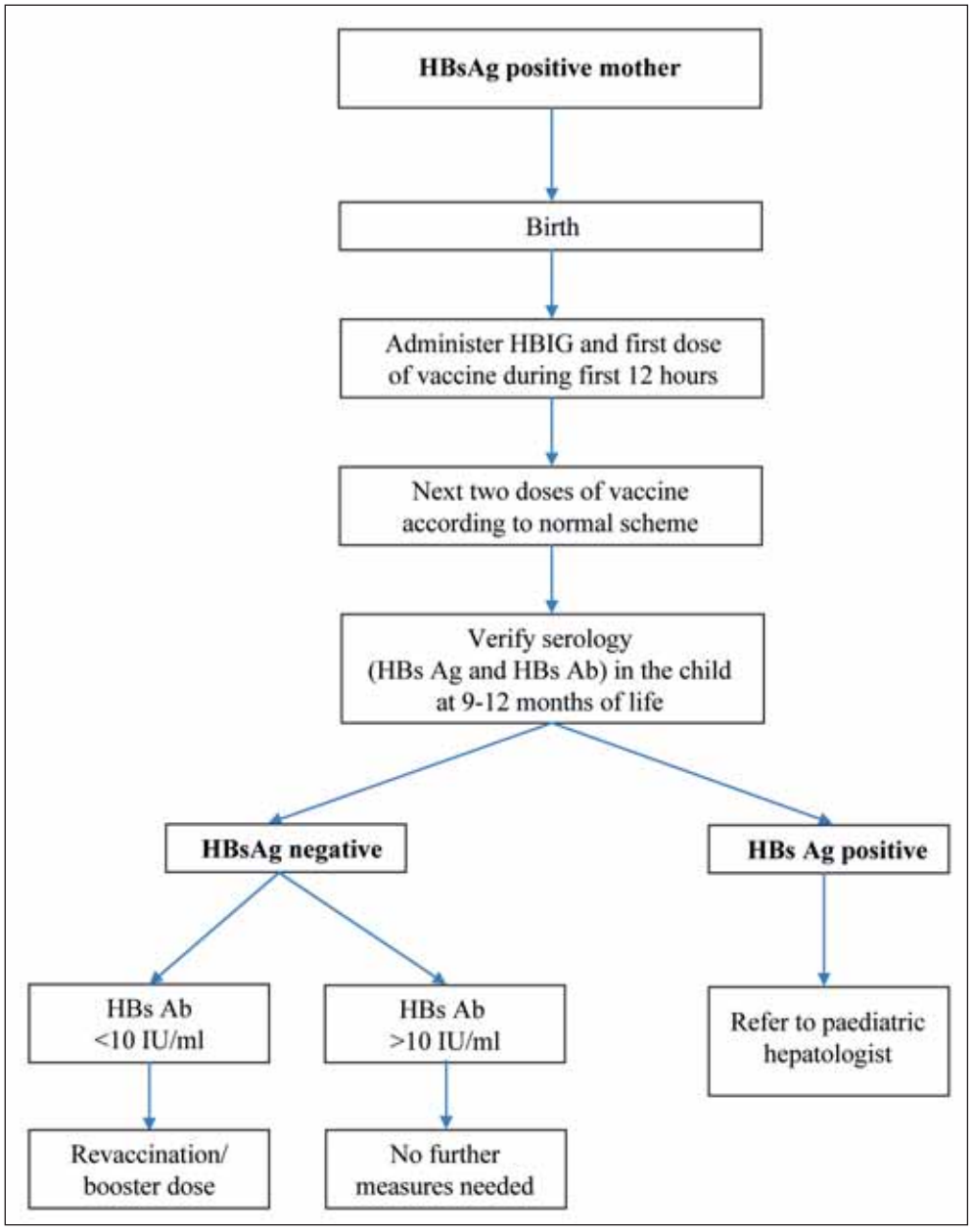
THE MANAGEMENT OF THE INFECTIONS DIAGNOSED DURING PREGNANCY

Acute infection

Acute HBV infection in pregnant patients has a similar evolution with the infection in non-pregnant patients (14). It has not ben proved that HBV acquired during pregnancy would increase fetal mortality or would have teratogenic effects, but acute HBV infection has been associated with an increased rate of pre-term birth and low weight at birth (14). Acute hepatitis in the first part of the pregnancy is associated with a rate of transmission of 10%, but if the infection is acquired during the last trimester of pregnancy, in the absence of prophylactic measures, the rate of transmission is much higher (14). The newborns from HBs Ag positive mothers at the time of birth must receive both active and passive immunization (37, 38). There is no sufficient data in the literature regarding the management of the newborns from mothers that obtain s system seroconversion during pregnancy. They must be definitely vaccinated against HBV, but as of administering HBIG there is no clear recommendation.

Chronic infection

In the patients diagnosed with chronic infection during pregnancy, the risk of transmission is related to HBV DNA level and the presence or absence of HBe Ag. After identifying a HBs Ag positive patient, the evaluation must contain ALT, AST, HBe Ag, HBe Ab, HDV Ab, HBV DNA. Based on these tests, the infection can be staged.



Algorithm 4. *Diagnostic and management of newborns from HBV infected mothers*

In patients in which viral load exceeds 10^6 IU/ml (or 200.000 IU/ml according to AASLD) antiviral treatment is recommended during the last trimester (37, 38). Newborns must receive both active and passive immunization (37-39).

PRIMARY PROPHYLAXY OF HBV INFECTION

Before pregnancy

In a patient with negative HBs Ag and negative HBs Ab, vaccination can be considered before pregnancy. The vaccination scheme consists of three intramuscularly administered doses over a period of 6 months. With this scheme 90% of the persons become immune (43). The preparation for a future pregnancy does not represent a routine indication for HBV vaccination.

During pregnancy

HBV vaccine is considered safe during pregnancy (44). Pregnancy itself does not represent an indication for vaccination. Pregnant woman that have an increased risk of acquiring the infection can be vaccinated at the indication of their doctors. Vaccinations procedures started before pregnancy can be continued during pregnancy.

POST-EXPOSURE PROPHYLAXIS IN PREGNANCY (PEP)

The management of a pregnant woman exposed to HBV is similar with that of non-pregnant patients. The HBs Ab status of the mother must be immediately tested. If the patient is vaccinated and HBs Ab value is over 10 IU/ml, no supplementary measures are needed. If the pregnant woman is vaccinated but antibody response is inadequate, HBIG should be administered followed by one booster dose of vaccine. In this group, a complete vaccination routine can be taken into account or the administration of two HBIG doses, according to CDC guidelines (45, 46). If the pregnant woman has not been vaccinated, HBIG has to be administered as soon as possible and followed by the first dose of vaccine of a complete routine that will be completed over the next 6 months or 3 months (rapid post-exposure scheme 0,1,2 months) (45, 46). The sooner the institution of PEP, the higher its effectiveness. Studies have shown that PEP initiation must be done in the first 7 days in the case of parenteral inoculum or in 14 days in case of sexual contact in order to be efficient (45). If HBs status and vaccinal situation of the patient are unknown, we consider that if the contact is definite, the patient should be treated as if she was never vaccinated.

INFECTION IN MALE PARTNER

The idea that the infection can be passed to the embryo by the male gametocyte is not new, the first article found in PubMed on this subject being from 1985 (47). In 2002 it has been demonstrated that HBV DNA can be integrated in the genetic material of spermatozoa causing mutations (48). In 2005 the father-to embryo transmission has been demonstrated in hamsters (49). A more recent study (50) also demonstrated the presence of HBV DNA in human embryos from HBs Ag negative mothers and positive HBs Ag fathers. A study on 164 discordant couples (with the father being infected and the mother not) did not reveal transmission to none of the newborns (51). The study from 2015 of Cao (5) still suggests the possibility of such a transmission. Supplementary studies are needed to establish if this route of transmission is or is not really of concern, and if it is, methods to prevent it must be described further.

IMPLICATIONS OF HBV INFECTION IN ASSISTED HUMAN REPRODUCTION

Regardless of the provenience of the oocytes (from mother or donor), the management of a future mother with positive HBsAg is the same as in case of the HBsAg positive women that obtain their pregnancy without assisted reproduction techniques and in which the infection is known from before pregnancy. From the ethic point of view, such a pregnancy is acceptable, the situation being the same as in the case of a naturally obtained pregnancy.

In discordant couples it is recommended that the partner with negative HBs Ag to be vaccinated (if he/she is not already HBs Ab positive). The procedure of fertilization can be started immediately when the uninfected partner starts to produce HBs Ab. In discordant couples, if the male partner is infected and the female partner produces HBs Ab, the American Society for Reproductive Medicine does not recommend the sperm washing procedure to be used. This procedure should be used in the case of a HBs Ab negative female partner (52). In some centers the procedure is still routinely practiced despite the above recommendations (53).

Based on the existing literature, the classical in vitro fertilization (IVF) technique can be considered safe for both variants of discordant couples in case of taking care of the aspects mentioned above. The intra-cytoplasmic sperm injection (ICSI) is considered safe if the male is the infected partner, but it may involve an increased risk of transmission if the female is the infected partner (53).

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