PREVENTION OF PERINATAL HEPATITIS B VIRUS INFECTION

Implications for mother and child
Policy for the Netherlands

Talent uit zich in het vermogen met veel bloed, zweet en tranen door te zetten.

PREVENTION OF PERINATAL HEPATITIS B VIRUS INFECTION

Implications for mother and child
Policy for the Netherlands

STELLINGEN

behorende bij het proefschrift
van
P.M. Grosheide

Rotterdam, 22 december 1993

1.
Om de duur van de bescherming na hepatitis B-vaccinatie accuraat te kunnen bepalen, is onderzoek in groepen die niet voortdurend aan infectie worden blootgesteld noodzakelijk.

2.
Indien bij een vrouw tijdens de zwangerschap een 'nieuw type' virus infectie wordt onderkend, wordt in de praktijk veelal tot een electieve keizersnede besloten om virale besmetting van de pasgeborene te voorkomen; deze handelwijze verdient wetenschappelijke onderbouwing.

3.
Het is niet zo zeer onwetendheid over wat de effectieve preventieve maatregelen ter voorkoming van hepatitis B infectie zijn die onnodige gezondheidsschade veroorzaakt als wel het onvermogen tot het treffen van dergelijke maatregelen.

4.
Artsen die de inning van medische facturen door een incassobureau laten uitvoeren, handelen in strijd met de Wet op de Persoonsregistratie.

5.
Screening en preventie worden het best geaccepteerd door de mensen die het het minst nodig hebben.

6.
Het preventief effect van de opsporing van risicofactoren voor hart- en vaatziekten wordt deels teniet gedaan door de stress, veroorzaakt door de bewustwording van de aanwezigheid van een risicofactor, zoals een verhoogd cholesterol gehalte.

7.
Het risico op congenitale syphilis is geen eerstgeboorterecht; de luesserologie dient dan ook tijdens elke zwangerschap te worden herhaald.

8.
Het kunnen verwerken van emoties komt onder andere de immuunreactie na hepatitis B-vaccinatie ten goede. L. Jabaay, et al. J Psychosom Res 1993;37:361-169.

9.
De tendens alle (medisch) handelen te protocolleren leidt ertoe dat elke vorm van creatieve deviatie ogenblikkelijk in de kiem gesmoord dreigt te worden.

10.
De mogelijkheid tot spellingscontrole bij computerprogramma's voor tekstverwerking zal het gebruik van de voorkeursspelling bevorderen.

11.
De geringe aandacht die bij de toediening van de diverse componenten van het nationaal vaccinatieprogramma wordt besteed aan de bijwerkingen staat niet in verhouding tot het klinisch belang hiervan.

12.
De door vaccinatie toegenomen actieve immuniteit van de huidige generatie kinderen tegen mazelen zal de passieve immuniteit van de volgende generatie zuigelingen doen afnemen.

13.
Men dient zich te realiseren dat het gebruik van termen als 'celdeling' en 'celvermeerdering' bij medici en niet-medici verschillende associaties kan oproepen.

14.
De preventie van seksueel overdraagbare aandoeningen is 'a matter of condom sense'.

PREVENTION OF PERINATAL HEPATITIS B VIRUS INFECTION

Implications for mother and child
Policy for the Netherlands

PREVENTIE VAN PERINATALE HEPATITIS B

Implicaties voor moeder en kind
Beleid in Nederland

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof. dr. P.W.C. Akkermans M.Lit.
en volgens besluit van het college van Dekanen.
De openbare verdediging zal plaatsvinden op
woensdag 22 december 1993 om 13.45 uur

door

Pia Maria Grosheide
geboren te 's-Gravenhage

PROMOTIECOMMISSIE

Promotores:	Prof. dr. S.W. Schalm
	Prof. dr. J. Huisman
Overige leden:	Dr. R.A. Heijtink
	Prof. dr. J.W. Wladimiroff

CIP-GEGEVENS KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Grosheide, P.M.

Prevention of perinatal hepatitis B virus infection:
implications for mother and child: policy for the Netherlands / P.M. Grosheide. - [S.l. : s.n.]. - Ill.
Proefschrift Rotterdam. - Met lit. opg.
ISBN 90-9006741-8
Trefw.: hepatitis B.

Illustratie omslag: Kees de Vries, Erasmus Universiteit Rotterdam
Ontwerp: Chris van Lee, bNO

De totstandkoming van dit proefschrift werd financieel mogelijk gemaakt door
Merck Sharp & Dohme Vaccine Division, producente van HB-VAX-DNA®.

CONTENTS

ABBREVIATIONS

antiHBc	antibodies against hepatitis B core antigen
antiHBe	antibodies against hepatitis B e antigen
antiHBs	antibodies against hepatitis B surface antigen
antiHBcIgG	antibodies against hepatitis B core antigen type IgG
antiHBcIgM	antibodies against hepatitis B core antigen type IgM
CDC	Centers for Disease Control
CHC	Child Health Clinic
CI	confidence interval
CIE	Center of Infectious Disease Epidemiology
CLB	Central Laboratory of the Netherlands Red Cross Blood Transfusion Service
CMI	cell-mediated immunity
CVS	chorion villus sampling
DTP	diphtheria-tetanus-pertussis
DNA	deoxyribonucleic acid
EPI	Expanded Program on Immunization
GHI	Chief Inspectorate for Public Health
GMT	geometric mean titer
HBcAg	hepatitis B core antigen
HBeAg	hepatitis B e antigen
HBsAg	hepatitis B surface antigen
HBIg	hepatitis B immune globulin
HBV	hepatitis B virus
HIV	human immunodeficiency virus
IU/l	international units per liter
IVF	in vitro fertilization
MSD	Merck Sharp & Dohme
PER	protective efficacy rate
PIA	Provincial Immunization Administration
RIA	radioimmunoassay
RPHL	Regional Public Health Laboratory
RIVM	Rijksinstituut voor Volksgezondheid en Milieuhygiëne
SKB	SmithKline & Beecham
TGA	Twente-Gelderse Achterhoek
WHO	World Health Organization

CHAPTER I

GENERAL INTRODUCTION

Background

Perinatal transmission of hepatitis B virus (HBV) from mothers who are carriers of the hepatitis B surface antigen (HBsAg) to their newborn infants is an important cause for the development of hepatitis B infections and for the maintenance of the hepatitis B virus reservoir in the world (1-3). Immunization of infants born to HBsAg-positive women has been shown to almost completely prevent perinatal infection (4-6).

In 1982, a study was initiated in three test areas in the Netherlands to determine whether screening to identify HBsAg carriers among pregnant women could be successfully introduced in prenatal care and whether the newborns of these HBsAg carriers could be protected from perinatal infection of combined passive and active immunization. Hepatitis B vaccination, given concomitantly with the diphtheria-tetanus-pertussis-poliomyelitis (DTP-polio) vaccination, was compared to hepatitis B vaccination initiated immediately after birth as far as compliance, immunogenicity and protective efficacy are concerned. Initial results of the study, completed in December 1992, were described by Mazel (7-8). On the basis of the preliminary results, a national program for the prevention of perinatal hepatitis B infection was launched earlier, in October 1989.

Scope of the thesis

This thesis focuses on three elements in the prevention of hepatitis B infection in newborns:

1) Preventive measures on HBV transmission in pregnancy. The literature on the possible effects of the hepatitis B virus on pregnant women and their offspring is reviewed (chapter 2.1). Secondly, the outcome of a seven-year screening of pregnant women for HBsAg in the three test areas, resulting in a policy proposal for the Netherlands is described (chapter 2.2). Thirdly, we investigated whether early invasive prenatal diagnosis in HBsAg-positive women carries a risk for intrauterine transmission of HBV (chapter 2.3).

In 1988, a hepatitis B epidemic broke out among women receiving in vitro fertilization treatment. This event provided an opportunity to study the immune response in pregnant women receiving post-exposure prophylaxis with hepatitis B immune globulin and hepatitis B vaccine (chapter 2.4).

2) Efficacy and long-term immunogenicity of passive-active immunization in infants of HBsAg carrier mothers. A review of passive-active immunization in infants of HBsAg carrier mothers is given (chapter 3.1). The protective efficacy of passive-active immunization either starting at birth or at the age of three months in infants of HBsAg- and HBeAg-positive mothers is described after five-year follow-up (chapter 3.2). The need for a second dose of hepatitis B immune globulin in infants receiving passive immunization at birth that is followed by delayed active immunization with recombinant hepatitis B vaccine at three months of age is evaluated (chapter 3.3). The protective efficacy and long-term immunogenicity of six passive-active immunization schedules in infants of HBsAg carrier mothers, varying in time of administration, and dose, number of doses and type of vaccine

are assessed (chapter 3.4).

3) Evaluation of the national program for the prevention of perinatal hepatitis B infection. The road to the introduction of routine prenatal screening of pregnant women for HBsAg followed by the passive-active immunization of infants of HBsAg-positive women in the Netherlands is described (chapter 4.1). The implementation of nationwide screening and immunization between October 1, 1989 and December 31, 1992 is reported (chapter 4.2). Factors associated with non-compliance with the national guidelines are assessed and recommendations for improvement of the program are formulated (chapter 4.3).

References

1. Schweitzer IL. Vertical transmission of the hepatitis B surface antigen. Am J Med Sci 1975;270:287-291.
2. Stevens CE, Beasley RP, Tsui J, Lee WC. Vertical transmission of hepatitis B antigen in Taiwan. N Engl J Med 1975;292:771-774.
3. Margolis HS, Alter MJ, Hadler SC. Hepatitis B: evolving epidemiology and implication for control. Semin Liver Dis 1991;11:84-92.
4. Reesink HW, Reerink-Brongers EE, Lafeber-Schut BJTh, Kalshoven-Benschop J, Brummelhuis HGJ. Prevention of chronic HBsAg carrier state in infants of HBsAg-positive mothers by hepatitis B immunoglobulin. Lancet 1979;2:436-437.
5. Maupas P, Chiron JP, Barin F, Coursaget P, Goudeau A, Perrin J, Denis F, Diop Mar I. Efficacy of hepatitis B vaccine in prevention of early HBsAg carrier state in children. Controlled trial in an endemic area (Senegal). Lancet 1981;1:289-292.
6. Tada H, Yanagida M, Mishina J, Fujii T, Baba K, Ishikawa S, Aihara S, Tsuda F, Miyakawa Y, Mayumi M. Combined passive-active immunization for preventing perinatal transmission of hepatitis B virus carrier state. Pediatrics 1982;70:613-619.
7. Mazel JA, Schalm SW, Gast GC de, Nuijten ASM, Heijtink RA, Botman MJ, Bänffer JRJ, Gerards LJ, Zwijnenberg J, Mettau J, Wladimiroff JW, Fetter WPF. Passive-active immunization of neonates of HBsAg-positive carrier mothers: preliminary observations. Br Med J 1984;288:513-515.
8. Mazel JA, Heijtink RA, Schalm SW, Gerards LJ, Botman MJ, Bänffer JRJ, Gast de GC, Nuijten ASM, Wladimiroff JW, Zwijnenberg J, Mettau J, Fetter WPF. Gecombineerde passieve en actieve immunisatie van zuigelingen van HBsAg-positieve moeders. Ned Tijdschr Geneeskd 1985;129:590-594 (in Dutch).

CHAPTER 2.1

HEPATITIS B VIRUS INFECTION AND PREGNANCY

Epidemiology of HBV infection

The geographic patterns of HBV prevalence vary greatly. In areas of low endemicity, e.g. Western Europe and North America, less than 1% of adults are HBV carriers and less than 10% will experience infection (1). In areas of intermediate (East- and South Europe) and high endemicity (Africa and Asia), between 2% and 15% of adults are HBV carriers and between 30% and 100% of the population will experience infection. In areas of low endemicity, infection occurs predominantly in adults and largely as a result of sexual contact, while in areas of intermediate and high endemicity, infection occurs predominantly among infants as a result of mother-to-infant transmission or close contact early in life (1).

HBV can be transmitted to infants from pregnant women who are either HBV carriers or who are acutely infected with HBV. For the Netherlands, only limited data on the prevalence of HBV carriers among selected pregnant populations is available. In 1979 a screening of 2,009 pregnant women who attended an academic obstetric unit in Utrecht showed a 0.4% prevalence of HBsAg in Dutch-Caucasian women and 4.7% in women originating from the Mediterranean (4.3%) and Asia (10%) (2). An overall prevalence for HBsAg of 0.8% amongst 28.412 pregnant women examined between 1982 and 1984 in three centers in the Netherlands was described (3); the prevalence was 0.3% in the predominantly rural area and 2.2% for the participating hospitals in the two urban areas. Over 90% of the HBsAg-positive women belonged to ethnic groups. Data on acute hepatitis B infection during pregnancy is not available but acute disease is considered relatively uncommon in the Netherlands. The frequency of the reported acute HBV infections is low: 48 females of childbearing age were notified to the Chief Inspectorate for Public Health in 1992.

Effects of pregnancy on HBV infection

Prior to the availability of serologic tests for the diagnosis of hepatitis B, an increased severity of viral hepatitis during pregnancy, primarily in the last trimester and an increased maternal mortality were reported (4-6). Other investigators concluded that the course of viral hepatitis is not altered by pregnancy (7-8). The poor prognosis previously noted was ascribed to patient selection or inadequate prenatal care and maternal malnutrition in the underdeveloped areas of the world where these studies were done (8). With the general availability of diagnostic tests for viral hepatitis it was determined that acute hepatitis B infection in pregnant women is no more severe than that in nonpregnant women, and it is self-limited, rarely resulting in chronic disease (8-11). An accidental hepatitis B infection caused by an HBV contaminated human serum pool in 79 women who received in vitro fertilization (IVF), showed that 29% of the pregnant women (7/24) were icteric and 8% (2/24) remained HBsAg-positive for a long period of time (30-35 weeks) but did not develop the HBV carrier state. In the nonpregnant group 43% (24/55) were icteric and 4% (2/55) became HBV carriers (11).

Little is known of the effects of pregnancy on the hepatitis B carrier state. Reactivation with jaundice was reported in the third trimester of pregnancy in one woman known as an

HBsAg carrier (12). Patients presenting with signs and symptoms of acute hepatitis may well be misdiagnosed as having an acute infection rather than reactivation if their previous serological status is not known. To what extent asymptomatic reactivations occur in pregnancy is unknown. On the other hand, subsidence of viral replication as a positive effect of pregnancy and delivery was described in Taiwan (13). HBeAg clearance or seroconversion to antiHBe occurred in 17% of 30 HBeAg-positive HBsAg carrier mothers, frequently 1-2 months postpartum. In addition, in another five women the HBV DNA fell to an undetectable level (< 0.04 ng/ml). In the control group of 30 nonpregnant women HBeAg clearance or seroconversion to antiHBe did not occur and HBV DNA levels merely fluctuated.

Effects of HBV infection on pregnancy

Early reports suggested that pregnancies complicated by maternal hepatitis, presumed to be type B, were associated with an increased frequency of abortions, congenital malformations and stillbirths (5,14). Others did not find such a relationship (7-8,15). One study found that acute hepatitis B infection during pregnancy had no effect on the risk of congenital malformation, stillbirths, abortions, or intrauterine growth retardation, but that it did increase the risk of prematurity, especially if the mother was infected in the last trimester (8). Recently the inadvertent HBV exposure of pre-embryos with contaminated IVF culture medium did not result in any demonstrable damage (11). The pregnancy rate per embryo transfer and the rate of first-trimester abortion did not differ from those without HBV contamination of the culture medium. The occurrence of maternal hepatitis B infection during pregnancy also did not seem to harm the developing fetus and the rate of immature and premature birth did not differ significantly from those in mothers without hepatitis B infection during their pregnancy (11).

In a retrospective study of 60 pregnant HBsAg carrier women and an equal number of controls without HBV infection, it was shown that both pregnancy course and pregnancy outcome were unaffected by antigenemia (16). Maternal factors as well as fetal factors such as gestational age at birth, birth weight, Apgar scores and neonatal morbidity were similar in the two groups.

Mother-to-infant transmission of HBV

As early as 1951, Stokes demonstrated the transmission of serum hepatitis from an asymptomatic carrier mother to her baby who developed neonatal hepatitis (17). Serum samples from both mother and baby produced hepatitis in volunteers.

Factors of importance for HBV transmission that were subsequently noted included timing of onset of hepatitis during pregnancy, acute versus chronic hepatitis B infection in the mother, the presence of serologic markers of high infectivity (HBeAg, HBV DNA) and geographic origin of the pregnant woman. Women who have acute hepatitis B in the first or

second trimester rarely transmit the HBV to their neonates (18-19). However, if the mother suffers from acute hepatitis B during the last trimester of pregnancy, the risk of transmission is about 70% (18-19). The frequency of HBV infections in infants born to mothers with acute infection was reported much higher (48%) than that (5%) in infants born to asymptomatic carrier mothers (20).

The risk of transmission is directly related to the presence of the hepatitis B e antigen, associated with viral replication, in the mother's serum (21-23). Among mothers who are HBeAg-positive the risk of transmitting the HBV to their offspring is 80 to 90% and more than 85 per cent of their infected infants will become chronic carriers of HBsAg (22-23). The rate of transmission is about 15% when the mother is HBsAg-positive and HBeAg-negative; in almost all of these infants the HBsAg-positive state was transient (21,23). The risk of infection in the latter group appeared to be related to the HBV DNA status of the mother: all HBV DNA positive carrier women were reported to infect their infants with HBV and approximately 10% of the HBeAg-negative women have circulating HBV DNA (24). It became apparent that the risk of mother-to-infant transmission of HBV was primarily related to the levels of HBV DNA in the sera of carrier mothers (24-25). Infants of HBsAg- and HBeAg-positive mothers with high levels of serum HBV DNA ($\geq$ 5 pg/ml) had a significantly higher risk of acquiring the HBV carrier state than infants of mothers with low levels of HBV DNA (< 5 pg/ml) (25). The incidence of the HBV carrier state at the age of 3 years was 79% (30/38) in infants of mothers with high HBV DNA levels versus 0% (0/9) in infants of mothers with low HBV DNA levels.
Infection of infants born to HBeAg-negative mothers, on the other hand, has also been associated with fulminant disease; this outcome has been linked to an HBV variant that has a mutation in the precore region of the genome (26-28).

Finally, the transmission rate was found to be much higher in Asian (40%) or Japanese (48%) women than in European women (< 5%); a fact probably explained by the higher rate of HBeAg seropositivity observed in Oriental HBsAg carriers than in Caucasians (2,20-21,29-30).

The moment at which HBV transmission occurs could be either during the gestational period (in utero), labour and delivery or in the postnatal period. Indirect evidence suggests that mother-to-infant transmission occurs mainly (> 90%) during or directly after delivery (29,31-32). Most infants become HBsAg-positive between one and six months of age pointing to labour and delivery as the time of transmission of HBV based on the incubation period of HBV infection (29,33). Mechanisms for the proposed transmission of HBV at or shortly after birth include mixing of maternal and fetal blood during labour, trauma associated with the birth process and minor skin abrasions of the fetus and ingestion of contaminated fluid during labour.

Maternal-fetal transfusion at the time of delivery could explain the presence of HBsAg in cord blood: the presence of HBsAg in cord blood was shown to be associated with the length of labour (34-35). Although they have clearly been exposed, some of the cord blood positive infants, however, produced no subsequent evidence of HBV infection and some of

the cord blood negative infants developed antigenemia (18,31). Contamination by maternal blood during sample collection or the use of relatively insensitive test methods to detect the low concentrations of HBsAg in cord blood could have influenced these findings (18,31). The chance of avoiding contact with maternal blood during delivery, however is so remote that, in principle, it is possible that all infants are exposed at that time and that failure to transmit HBV infection is related to the mother's low level of infectivity (23-25). Prevention of the chronic carrier state with hepatitis B immune globulin was later shown to be effective in infants with and without HBsAg-positive cord blood. This fact also indicates that transmission of HBV did occur around delivery because antibodies do not influence an established (in utero) infection (36).

Chaudhary suggested that the fetus may ingest contaminated materials during delivery or may become infected through minor skin abrasions (37). It has been shown that HBV infection can be transmitted by the oral route although the oral dose needs to be 50 times as high as that required for the parenteral route (33). The presence of HBsAg in the vaginal fluid in 96% of HBsAg- and HBeAg-positive mothers during labour and in 90% of the gastric aspirate, obtained from their infants during resuscitation, indicated ingestion as a probable mechanism of transmission (31).

That in utero transmission occurs, was first reported by Schweitzer who described a woman who was seropositive for HBsAg in the 6th month of pregnancy but was negative at birth (18). The cord blood was HBsAg-positive and her baby has been HBsAg-positive since birth. The extent to which infection before birth occurs is not exactly known. The small percentage of infants not protected by immunoprophylaxis at birth indicates that in utero transmission of HBV is rare; the rate of transmission across the placenta is estimated 2 to 10% (38-40). Some authors believe that the infants who cannot be protected by immunoprophylaxis after delivery have an already established infection at birth (36,41). The presence of HBsAg in cord blood per se is not considered a marker of in utero infection; it may reflect transient, passively acquired maternal antigenemia (20,42). In utero transmission has been inferred from studies showing that some infants are HBsAg-positive at birth and on the 3rd day after birth or when consumption of passively administered antiHBs antibodies had occurred (39). Others suggested that in utero infection might account for those babies who had HBsAg in blood taken from the femoral artery at birth (24).

Several investigators have come to believe that antenatal infection of newborns takes place considerably more often than previously thought. HBsAg is considered not to be able to pass the placenta but HBeAg, bound to IgG, was found capable of passing the placenta (43). Whether HBV is capable of passing the placenta when bound to IgG deserves further consideration as a mechanism of the intrauterine infection of the fetus. However, contamination by transplacental leakage of HBeAg-positive maternal blood induced by uterine contractions during pregnancy in case of threatened abortion or premature labour was suggested to have an important role in the occurrence of intrauterine infections (44). In three out of five cases with probable infection in utero, signs and symptoms associated with premature labour were noted. The contraction of uterine muscles could cause partial

breakdown of placental villi in threatened abortion or premature labour and may result in microhaematologic leaks across the placenta (44-45).

Goudeau concluded that in utero transmission seldom occurred since antiHBcIgM, a marker of recent infection which cannot pass through the placenta, was not found in the serum of 51 infants of HBsAg-positive mothers within six days of birth (46). However, in one out of five infants infected in utero, increase of antiHBcIgM, lasting less than four weeks, was observed one month after birth even though a sample taken at delivery was negative (45). As a possible reason for the absence of antiHBcIgM in infected infants it was suggested that the production lasts for too short a time to be detected (45). Perinatally acquired HBV infections were also demonstrated to rarely elicit a virus-specific IgM response in infants, in contrast to other perinatally acquired infections (47-48). Alexander et al. have proposed that HBV transmission during pregnancy does occur in a high proportion of infants but that maternally acquired antiHBc suppresses the expression and replication of the virus in the fetus (49). Only after birth, when maternal antibodies decline, infection may develop and immunoprophylaxis will delay viral replication rather than prevent initial infection (49). It was also suggested that maturation of the fetal liver is required before viral replication can occur (50). Both studies assumed that in infants born to HBsAg carrier mothers some hepatocytes become infected in utero and note that the effect of passive or active immunization in these infants is to prevent cell-to-cell transmission of newly synthesized virus rather than to prevent initial infection. However, these hypotheses have been questioned by other investigators (40,51-52).
If maternal antiHBc suppressed HBV infection, the peak of HBsAg seroconversion in infected infants would be expected to occur more than six months after birth, when serum levels of maternal antibody drop. In infants born to HBeAg-positive HBsAg carrier mothers, HBsAg antigenemia mostly occurred within 3 to 5 months after birth at which time 72% of infants still had high levels of passively acquired antiHBc in their serum (51). It was concluded that infection seems to depend on the maternal antigen status rather than on the presence or absence of maternal antiHBc.

Direct investigation of 48 aborted fetuses of HBsAg-positive women after 20 to 32 weeks of gestation showed evidence of transplacental infection in four fetuses but no integration of HBV DNA in the fetal livers (40). In a later study 44% (12/27) of fetal livers were found positive for HBV DNA (53).

Even when not infected during the perinatal period, infants of HBV infected mothers remain at risk of acquiring HBV infection by horizontal transmission during the first years of life (54). The breast milk of HBsAg-positive mothers is often found to be positive for HBsAg but it does not seem to play an important role in the transmission of HBV. Apparently no differences in the rate of antigenemia between infants of carrier mothers that were breast-fed or bottle-fed were found (55) possibly because most infants were already infected perinatally.

Prevention of HBV infection during pregnancy

The heterosexual transmission of HBV has long been acknowledged and there are reports that asymptomatic HBsAg carriers have transmitted acute hepatitis to their female sexual partners (56-57). In general, however, no special measures to limit the risk of HBV infection in pregnant women need to be taken. Factors that influence transmission and measures to reduce spread may be presented in the context of educational programs that cover several infectious diseases. Post-exposure prophylaxis with HBIg and/or hepatitis B vaccine was shown to either prevent HBV infection or to attenuate clinical illness and should, therefore, be considered for use in unvaccinated pregnant women after accidental exposure to HBV (58). On the basis of limited experience there is no apparent risk of adverse effects to developing fetuses when hepatitis B vaccine is administered to pregnant women (59-60).

Prevention of mother-to-infant transmission

Prenatal testing of pregnant women for HBsAg makes it possible to identify in advance those infants at risk for HBV infection, and consequently to pay special attention to the immunoprophylaxis and follow-up of these infants. However, in those parts of the world where the rate of perinatal transmission is high or where maternal HBsAg screening is not feasible, universal hepatitis B vaccination of newborns without prior screening may be considered.

What consequences have to be drawn if an HBV infection is diagnosed during pregnancy? In HBeAg-positive carrier mothers a positive association was found between the presence of HBsAg in the amniotic fluid after amniocentesis was performed at 36 to 37 weeks of gestation and the presence of antigen in the babies at one month of age (35). Invasive prenatal procedures like amniocentesis are inadvisable even though the risk of infection in infants of mothers with acute hepatitis B before the third trimester of pregnancy was reported to be small (18-19). If performed, these procedures should be carried out with extra care in women who are HBeAg-positive in order not to provide an avenue for spread of infection to the fetus.

Prolonged labour should be avoided and it may be questioned whether treatment of premature labour with tocolytic agent in HBeAg-positive women is advisable since uterine contractions are a prerequisite for maternal-fetal transfusion (34,44). Delivery by elective Caesarian section has been suggested for infants of HBeAg-positive mothers to reduce the amount of maternal-fetal transfusion (35,61). However, elective Caesarian section for 93 infants in Taiwan could not prevent chronic hepatitis B infection in 25 (27%) of the infants (62). At present, Caesarian section is not advised as a means of preventing maternal-fetal transmission of HBV. Instead, it has recently been suggested that HBIg in higher doses than the 0.5 ml doses of HBIg generally given directly after birth may also effectively prevent infection in infants of HBeAg-positive mothers (63). Breast feeding need not be discouraged (55).

References

1. Maynard JE, Kane MA, Alter MJ, Hadler SC. Control of hepatitis B by immunization: global perpectives. In: Zuckerman AJ, (ed). Viral Hepatitis and Liver Disease. New York, Alan R. Liss. Inc., 1988:967-969.
2. Ypma TjD, Kater L, Gerards LJ, Heiden van der C, Heijtink RA, Haspels AA. Perinataal verworven hepatitis B. Ned Tijdschr Geneesk 1979;123:1820-1822 (in Dutch).
3. Schalm SW, Mazel JA, Gast GC de, Heijtink RA, Botman MJ, Bänffer JRJ, Gerards LJ, Zwijnenberg J, Fetter WPF, Nuijten ASM, Wladimiroff JW, Christiaens GCML. Prevention of hepatitis B infection in newborns through mass screening and delayed vaccination of all infants of mothers with hepatitis B surface antigen. Pediatrics 1989;83:1041-1047.
4. Frucht HL, Metclafe J. Mortality and late results of infectious hepatitis in pregnant women. N Engl J Med 1954;251:1094-1096.
5. Siegel M, Fuerst HT, Peress NS. Comparative fetal mortality in maternal virus diseases. A prospective study of rubella, measles, mumps, chicken pox, and hepatitis. N Engl J Med 1966;274:768-771.
6. Bomhanmanesh F, Haghighi P, Hekmat K, Rezaizadeh K, Ghavami AG. Viral hepatitis during pregnancy: severity and effect on gestation. Gastroenterology 1973;64:304-312.
7. Adams R, Combes B. Viral hepatitis during pregnancy. JAMA 1965;192:95-98.
8. Hieber JP, Dalton D, Shorey J, Combes B. Hepatitis and pregnancy. J Pediatr 1977;91:545-549.
9. Snydman DR. Hepatitis in pregnancy. N Engl J Med 1985;313:1398-1401.
10. Rustgi VK, Hoofnagle JH. Viral hepatitis during pregnancy. Semin Liver Dis 1987;7:40-46
11. Os van HC, Drogendijk AC, Fetter WPF, Heijtink RA, Zeilmaker GH. The influence of contamination of culture medium with hepatitis B virus on the outcome of in vitro fertilization pregnancies. Am J Obstet Gynecol 1991;165:152-159.
12. Rawal BK, Parida S, Watkins RPF, Ghosh P, Smith H. Symptomatic reactivation of hepatitis B in pregnancy. Lancet 1991;1:364.
13. Lin HH, Chen PJ, Chen DS, Sung JL, Yang KH, Young YC, Liou YS, Chen YP, Lee TY. Postpartum subsidence of hepatitis B viral replication in HBeAg-positive carrier mothers. J Med Virol 1989;29:1-6.
14. Cossart YE. The outcome of hepatitis B virus infection in pregnancy. Postgrad Med J 1977;53:610-613.
15. Siegel M. Congenital malformations following chickenpox, measles, mumps and hepatitis. JAMA 1973;226:1521-1525.
16. Pastorek JG, Miller JM, Summer PR. The effect of hepatitis B antigenemia on pregnancy outcome. Am J Obstet Gynecol 1988;158:486-489.
17. Stokes J, Wolman IJ, Blanchard MC, Farquhar JD. Viral hepatitis in the newborn: clinical features, epidemiology and pathology. Am J Dis Child 1951;82:213-216.
18. Schweitzer IL. Vertical transmission of the hepatitis B surface antigen. Am J Med Sci 1975;270:287-291.
19. Tong MJ, Thursby M, Rakela J, McPeak C, Edwards VM, Mosley JW. Studies on the maternal-infant transmission of the viruses which cause acute hepatitis. Gastroenterology 1981;80:999-1004.
20. Schweitzer IL, Mosley JW, Ashcavai M, Edwards VM, Overby LB. Factors influencing neonatal infection by hepatitis B virus. Gastroenterology 1973;65:277-283.
21. Okada K, Kamiyama I, Inomata M, Mitsunobu M, Imasi BS, Miyakawa Y, Mayumi M. E antigen and anti-e in the serum of asymptomatic carrier mothers as indicators of positive and negative transmission of hepatitis B virus to their infants. N Engl J Med 1976;294:746-749.
22. Beasley RP, Trepo C, Stevens CE, Szmuness W. The e antigen and vertical transmission of hepatitis B surface antigen. Am J Epidemiol 1977;105:94-98.
23. Stevens CE, Neurath RA, Beasley RP, Szmuness W. HBeAg and anti-HBe detection by radioimmunoassay: correlation with vertical transmission of hepatitis B virus in Taiwan. J Med Virol 1979;3:237-241.
24. Lee SD, Lo KJ, Wu JC, Tsai YT, Wang JY, Ting LP, Tong MJ. Prevention of maternal-infant hepatitis B virus transmission by immunization: the role of serum hepatitis B virus DNA. Hepatology 1986;6:369-373.
25. Ip HMH, Lelie PN, Wong VCW, Kuhns MC, Reesink HW. Prevention of hepatitis B virus carrier state in infants according to maternal serum levels of HBV DNA. Lancet 1989;1:406-410.

26. Sinatra FR, Shah P, Weissman JY, Thomas DW, Merritt RJ, Tong MJ. Perinatal transmitted acute icteric hepatitis B in infants born to hepatitis B surface antigen-positive and anti-hepatitis B e-positive carrier mothers. Pediatrics 1982;70:557-559.
27. Delaplane D, Yogev R, Crussi F, Shulman ST. Fatal hepatitis B in early infancy: the importance of identifying HBsAg-positive pregnant women and providing immunoprophylaxis to their newborns. Pediatrics 1983;72:176-180.
28. Terawaza S, Kojima M, Yamanaka T, Yotsumoto S, Okamoto H, Tsuda F, Miyakawa Y, Mayumi M. Hepatitis B virus mutants with pre-core region defects in two babies with fulminant hepatitis and their mothers positive for antibody to hepatitis B e antigen. Pediatr Res 1991;29:5-9.
29. Stevens CE, Beasley RP, Tsui J, Lee WC. Vertical transmission of hepatitis B antigen in Taiwan. N Engl J Med 1975;292:771-774.
30. Derso A, Boxall EH, Tarlow MJ, Flewett TH. Transmission of HBsAg from mother to infant in four ethnic groups. Br Med J 1978;1:949-952.
31. Lee AK, Ip HM, Wong VC. Mechanisms of maternal-fetal transmission of hepatitis B virus. J Infect Dis 1978;138:668-671.
32. Papaevangelou G, Hoofnagle JH. Transmission of hepatitis B virus infection by asymptomatic chronic HBsAg carrier mothers. Pediatrics 1979;63:602-605.
33. Krugman S, Giles JP. Viral hepatitis - new light on an old disease. JAMA 1970;212:1019-1029.
34. Eimer H. Untersuchungen zur maternofetalen Transfusion bei geburtshilflichen Operationen. Geburtsh u Frauenheilk 1972;32:657-661.
35. Wong VCW, Lee AKY, Ip HMH. Transmission of hepatitis B antigens from symptom free carrier mothers to the fetus and the infant. Br J Obstet Gynaecol 1980;87:958-965.
36. Beasley RP, Hwang LY, Lin CC, Stevens CE, Wang KY, Sun TS, Hsieh FJ, Szmuness W. Hepatitis B immune globulin (HBIG) efficacy in the interruption of perinatal transmission of hepatitis B virus carrier state. Lancet 1981;2:388-393.
37. Chaudhary RK. Perinatal transmission of hepatitis B virus. Can Med Ass J 1983;28:664-666.
38. Beasley RP, Hwang Y, Lee GCY, Lan CC, Roan CH, Huang FY, Chen CL. Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immunoglobulin and hepatitis B vaccine. Lancet 1983;2:1099-1102.
39. Wong VCW, Ip HMH, Reesink HW, Lelie PN, Reerink-Brongers EE, Yeung CY, Ma HK. Prevention of the HBsAg carrier state in newborn infants of mothers who are chronic carriers of HBsAg and HBeAg by administration of hepatitis-B vaccine and hepatitis-B immunoglobulin. Lancet 1984;1:921-926.
40. Li L, Sheng MH, Tong SP, Chen HZ, Wen YM. Transplacental transmission of hepatitis B virus. Lancet 1986;2:872.
41. Stevens CE, Toy PT, Tong MJ, Taylor PE, Vyas GN, Nair PV, Gudavalli M, Krugman S. Perinatal hepatitis B virus transmission in the United States. Prevention by passive-active immunization. JAMA 1985;253:1740-1745.
42. Kohler PF, Dubois RS, Merill DA, Bowes WA. Prevention of chronic neonatal hepatitis B virus infection with antibody to the hepatitis B surface antigen. N Engl J Med 1974;291:1378-1380.
43. Arakawa K, Tsuda F, Takahashi K, Ise I, Naito S, Kosugi E, Miyaiawa Y, Mayumi M. Maternofetal transmission of IgG-bound hepatitis B e antigen. Pediatr Res 1982;16:247-250.
44. Lin HH, Lee TY, Chen DS, Sung JL, Ohto H, Etoh T, Kawana T, Mizuno M. Transplacental leakage of HBeAg-positive maternal blood as the most likely route in causing intrauterine infection with hepatitis B virus. J Pediatr 1987;111:877-881.
45. Ohto H, Lin HH, Kawana T, Etoh T, Tohyama H. Intrauterine transmission of hepatitis B virus is closely related to placental leakage. J Med Virol 1987;21:1-6.
46. Goudeau A, Yvonnet B, Lesage G, Barin F, Denis F, Coursaget P, Chiron JP, Diop Mar ID. Lack of anti-HBc IgM in neonates with HBsAg carrier mothers argues against transplacental transmission of hepatitis B virus infection. Lancet 1983;2:1103-1104.

47. Panda SK, Bhan MK, Guha DK, Gupta A, Datta R, Zuckerman AJ, Nayak NC. Significance of maternal and infant serum antibodies to hepatitis B core antigen in hepatitis B virus infection of infancy. J Med Virol 1988;24:343-349.
48. Margolis S, Xu Z, Nainan OV, PY Ou-Yang, Duan SC, Zhuang YL. Poor IgM antibody response to hepatitis B core antigen in infants with hepatitis B virus infection. J Pediatrics 1989;115:609-611.
49. Alexander GJ, Eddleston AL. Does maternal antibody to core antigen prevent recognition of transplacental transmission of hepatitis-B-virus infection? Lancet 1986;1:296-297.
50. London WT, O'Connell AP. Transplacental transmission of hepatitis B virus. Lancet 1986;1:1037-1038.
51. Panda SK, Gupta A, Datta R, Nayak NC. Transplacental transmission of hepatitis B virus. Lancet 1986;2:919-920.
52. Milne A. Transplacental transmission of hepatitis B virus. Lancet 1986;1:860-861.
53. Tang SX, Yu GL. Intrauterine infection with hepatitis B virus. Lancet 1990;2:302.
54. Beasley RP, Hwang LY. Postnatal infectivity of hepatitis B surface antigen-carrier mothers. J Infect Dis 1983;147:185-190.
55. Beasley RP, Stevens CE, Shiao IS, Meng HC. Evidence against breast-feeding as a mechanism for vertical transmission of hepatitis B. Lancet 1975;2:740-741.
56. Fagan EA, Smith PM, Davison F, Williams R. Fulminant hepatitis B in successive female sexual partners of two antiHBe-positive males. Lancet 1986;2:538-540.
57. Tassopoulos NE, Papaevangelou GJ, Roumeliotou-Karayannis A, Richardson SC. Heterosexual transmission of heptitis B virus from symptomless HBsAg carriers positive for antiHBe. Lancet 1986;2:972.
58. Mitsui T, Iwano K, Suzuki S, Yamazaki C, Masuko K, Tsuda F, Aihara S, Akahane Y, Miyakawa Y, Mayumi M. Combined hepatitis B immune globulin and vaccine for postexposure prophylaxis of accidental hepatitis B virus infection in hemodialysis staff members: comparison with immune globulin without vaccine in historical controls. Hepatology 1989;10:324-327.
59. Ayoola EA, Johnson AOK. Hepatitis B in pregnancy: immunogenicity, safety and transfer of antibodies to infants. Int J Gynecol 1987;25:297-301.
60. Levy M, Koren G. Hepatitis B vaccine in pregnancy: maternal and fetal safety. Am J Perinatol 1991;8:227-232.
61. Lee SD, Lo KJ, Tsai YT, Wu JC, Wu TC, Yang ZL, Ng HT. Role of Caesarian section in prevention of mother-infant transmission of hepatitis B virus. Lancet 1988;2:833-834.
62. Beasley RP, Stevens CE. Vertical transmission of HBV and interruption with globulin. In: Vyas G, Cohen SN, Schmid R (eds). Viral hepatitis. Philadelphia, Franklin Institute Press 1978:333-345.
63. Schalm SW, Pit-Grosheide PM. Prevention of hepatitis B transmission at birth. Lancet 1989;1:44.

CHAPTER 2.2

ANTENATAL SCREENING FOR HEPATITIS B SURFACE ANTIGEN

P.M. Grosheide[1], J.W. Wladimiroff[2], R.A. Heijtink[3], J.A. Mazel[4], G.C.M.L. Christiaens[5], A.S.M. Nuijten[6], S.W. Schalm[1] and the Dutch Study Group Prevention Neonatal Hepatitis B

Department of Internal Medicine II[1] and Department of Obstetrics and Gynaecology[2], University Hospital Dijkzigt, Rotterdam; Department of Virology[3], Erasmus University, Rotterdam; General Practitioner[4], Borne; Department of Obstetrics and Gynaecology[5], Academic Hospital Utrecht, Utrecht; State School of Training for Midwives[6], Rotterdam, the Netherlands.

Accepted for publication in British Medical Journal.

SUMMARY

This study was organized to design a practical screening program to identify HBsAg carriers among pregnant women in the Netherlands. A second aim was to determine whether screening of all pregnant women in a country with low prevalence for hepatitis B would be advised. From July 1982 to October 1989, it was directed to include HBsAg screening as part of routine prenatal laboratory testing at 14 weeks of gestation in three large city hospitals and in one rural area where the number of home deliveries is high. In addition, women coming to the delivery unit without test result underwent rapid screening on admission in two hospitals. Coverage of screening was checked through the delivery records or birth notifications. Information regarding risk factors for hepatitis B infection and country of origin was obtained from each HBsAg-positive patient.

The rate of screening reached 97%, including 10% achieved by rapid screening, in the hospital setting. The estimated coverage in the rural area was > 90%. The prevalence of HBsAg among 99,706 gravidae was 0.7%, ranging from 0.3% in the rural area to 1.8% in the urban areas. Screening at delivery was associated with a HBsAg positivity rate that was 2.5 times higher ($p << 0.001$). The most common risk factor for infection was geographic origin. Only 12% of the HBsAg carriers were of Dutch origin; 48% of them had no identifiable risk factor.

It is concluded that universal screening for HBsAg is advised and is practically feasible. For countries like the Netherlands with its routine antenatal care, incorporation of HBsAg testing in the laboratory package and screening at delivery for those women escaping prenatal control, achieves high compliance.

INTRODUCTION

Antenatal care aims at the prevention of maternal and fetal morbidity. In the Netherlands antenatal care is performed by a range of professionals: midwives, general practitioners and hospital doctors. In many areas up to 50% of antenatal care is in the hands of midwives who have their own clinics outside hospital. At 14 weeks of gestation venous blood is routinely checked for rhesus blood groups and syphilis. If relevant, antibodies against rubella, toxoplasmosis and cytomegalic virus are tested.

Since the availability of hepatitis B vaccine the prevention of perinatal transmission of hepatitis B virus has come into reach. Pregnant women who are hepatitis B surface antigen (HBsAg)-positive should be identified prior to delivery in order to prevent hepatitis B infection in their neonates by passive-active immunization. Direct evidence indicates that screening for HBsAg should reduce the incidence and mortality of perinatal hepatitis B infection by about 90% (1-3).

Therefore, a multicenter study was designed in 1982 to determine whether an efficient organization of screening for hepatitis B could be set up by adding HBsAg testing to the

already accepted and widely used protocol for routine antenatal screening at 14 weeks of gestation, in and outside the hospital setting.
The second aim was to determine if a policy of selective serotesting of pregnant women with risk factors for hepatitis B virus infection was effective in detecting women who are antigenemic or whether universal screening should be recommended. The study lasted seven years and preliminary results were reported earlier
(4-5). The feasibility of routine antenal screening for HBsAg in pregnant women and the various factors influencing compliance are reported here. A policy proposal for universal screening on a national base is also formulated.

PATIENTS AND METHODS

Three large city hospitals in Rotterdam (n=2) and Utrecht (n=1) and one rural area Twente-Gelderse Achterhoek (TGA region) where the number of home deliveries is high (≥ 50%) participated in the study. The survey was conducted from July 1982 to October 1989. Permission was obtained from each of the local Ethics Review Boards involved. All participating midwives, obstetricians and general practitioners received written and oral information explaining the purpose of the screening.
At each first visit general practitioners, midwives and obstetricians asked the pregnant women for informed consent to screen for HBsAg. Each patient had blood collected by venipuncture around the 14th week of pregnancy. The serum was submitted for routine prenatal serologic testing for rhesus blood groups, syphilis and HBsAg. HBsAg was tested either by radioimmunoassay (Ausria II, Abbott Laboratories, Ill, USA) and enzymeimmunoassay (Auszyme, Abbott) in the laboratories associated with the hospitals or by the reversed passive hemagglutination assay (Auscell, Abbott) in the regional laboratory. All reactive specimens were confirmed by retesting. All HBsAg-positive blood samples were also tested for e antigen (HBeAg) and antiHBe (Abbott-e, Abbott anti-e). Until January 1988, a positive test result was always followed by a second blood sample for HBsAg testing at 28 weeks of gestation to confirm the HBsAg carrier state.
During the study period an additional blood sample of the HBsAg-positive women was obtained at delivery to verify the eligibility of her infant for one of our passive-active immunization trials (4-5.) The HBsAg-status of the expectant mothers was checked in the delivery rooms in Rotterdam. Whenever a test result was missing, blood was obtained and tested with the rapid hemagglutination HBsAg test (rapid screening). In the TGA region and in Utrecht rapid screening was not implemented. Questionnaires on country of origin, parity and history of hepatitis were obtained from all HBsAg-positive women at delivery.

Notation on the HBsAg-status of the patients was introduced in the hospital parturition books. Each month, coverage of screening was monitored by a trial assistant through a check of the parturition books. The number of screening tests of pregnant women that was reported during the same period, was also recorded. In the TGA region the number of births

that were reported from all municipalities (n=30) involved were checked against the list of screenings by the regional laboratory.

Statistics

Differences in discrete variables were analyzed by Fisher's exact test for small numbers and Chi-square test. Differences in proportions were calculated using 95% confidence intervals (95% CI). Continuous variables were analyzed by the Wilcoxon two sample rank sum test.

RESULTS

Since the results in the two participating hospitals in Rotterdam were similar they are considered as one center. The number of women tested and the number found seropositive per center are given in Table 1. Screening for HBsAg in Rotterdam reached an overall coverage of 97% with 10% of women screened at delivery. In the first year of screening it reached 95% of the hospital population with 14% screening at delivery. In the final year 98% of pregnant women were screened with 4% undergoing rapid screening on admission to the delivery rooms. In Utrecht where a pre-delivery check of the HBsAg status was not regularly performed, a coverage of 87% was reported. At the end of the study period screening reached 91% of the hospital population including 88% in routine screening and 12% in rapid screening. In the TGA region the estimated coverage at the end of the first year of the study was 85%. Later coverage appeared optimal since more blood samples of

Table 1

Antenatal screening for HBsAg and HBsAg positivity rates in serum samples from pregnant women, per treatment center.

		Pregnant women			
Center	Births	Tested		HBsAg-positive	
	n	n	(%)	n	(%)
Rotterdam	22,255	21,516	(97)	397	(1.8)
Utrecht	8,691	7,597	(87)	107	(1.4)
TGA region	69,530	70,593	(102)	230	(0.3)
Total	100,476	99,706	(99)	734	(0.7)

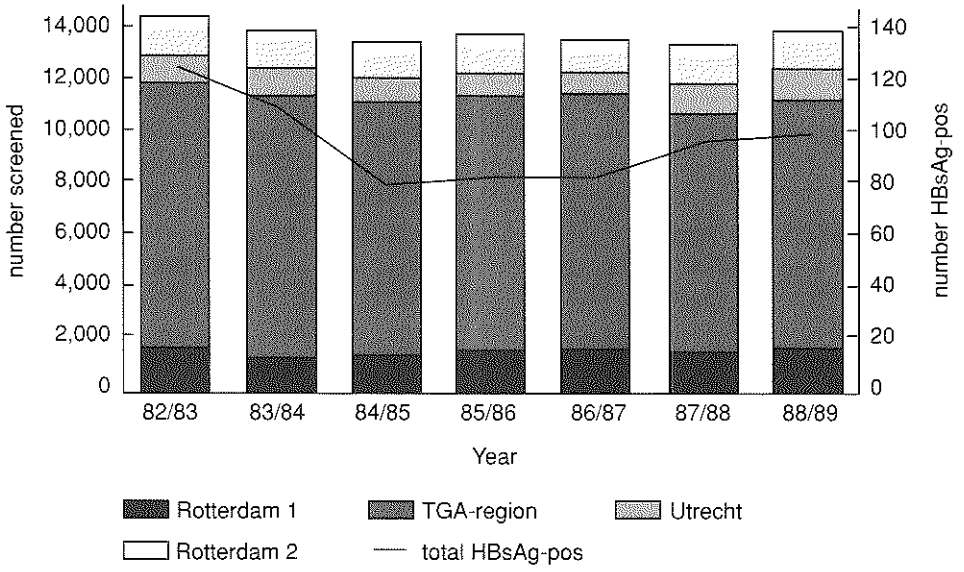


Figure 1
Annual distribution of the number of women screened for HBsAg and the number of HBsAg-positive mothers in four centers in the Netherlands (1982-1989).

pregnant women were tested than births notified (102%) in the study period.
The participating centers screened 99,706 pregnant women for HBsAg; 96,972 (97.3%) registered for routine prenatal screening and 2,734 (2.7%) women came to the delivery unit without prenatal screening for HBsAg. 734 HBsAg-positive women were identified during the study period. The number of women tested in relation to the number found HBsAg-positive throughout the study period is shown in Figure 1. The overall prevalence of HBsAg in the treatment centers was 0.74%, with some variation throughout the years.
The prevalence of HBsAg per treatment center varied from 1.8% (95% CI: 1.7-2.0) in Rotterdam to 1.4% (95% CI: 1.1-1.7) in Utrecht and 0.3% (95%CI: 0.26-0.34) in the TGA region.

During the study period 705 infants of HBsAg-positive mothers were born. 617 (87.5%) were detected at the time of the first visit to the antenatal clinic. The remaining 88 (12.5%) presented to the delivery unit without prenatal screening with 5 apparently detected outside Rotterdam. The HBsAg positivity rates for the routinely screened women and for the women screened at delivery in Rotterdam were 1.5% (294/19,420) and 4.0% (83/2,096) respectively (Chi-square test; $p << 0.001$). In case of rapid screening the prevalence appeared to be more than a 2.5 times higher. Characteristics of the women found HBsAg-positive by prenatal or rapid screening in Rotterdam are presented in Table 2. Except for

Table 2

Characteristics of the total number of HBsAg-positive mothers (N=705) and those found HBsAg-positive by prenatal (n=294) or rapid screening (n=83) in Rotterdam.

Mothers	Total		Screening: Prenatal		Screening: Rapid		P-value
	n	(%)	n	(%)	n	(%)	
Age in years							
< 20	63	(9)	23	(8)	11	(13)	
20 - 24	236	(33)	102	(35)	28	(34)	
25 - 29	187	(27)	75	(26)	22	(27)	
30 - 34	131	(19)	55	(19)	11	(13)	
35 - 39	69	(10)	31	(11)	8	(10)	
≥ 40	19	(3)	8	(3)	3	(4)	
range	15 - 49		15 - 47		16 - 47		
mean age ± SD	26.9 ± 6.2		27.0 ± 6.2		26.5 ± 6.7		0.38†
HBe-status							0.54§
HBeAg-positive	118	(17)	43	(15)	10	(12)	
HBeAg-negative	586	(83)	250	(85)	73	(88)	
unknown	1		1		-		
Country of origin							0.78§
Netherlands	83	(12)	21	(7)	6	(7)	
Mediterranean	377	(53)	131	(45)	38	(46)	
Surinam	74	(10)	52	(18)	13	(16)	
Asian	102	(14)	36	(12)	14	(17)	
other	69	(10)	54	(18)	12	(14)	
Parity							0.03§
primiparae	226	(32)	97	(33)	38	(46)	
multiparae	476	(68)	197	(67)	45	(54)	
unknown	3	(1)	-		-		

† Wilcoxon Mann Whitney
§ χ^2

parity there were no statistically significant differences between the two groups. Only 11.8% (n=83) of women found HBsAg-positive were of Dutch Caucasian origin.

Of the 705 HBsAg-positive women, 118 (16.7%) were also HBeAg-positive. The HBeAg positivity rate was higher for the non-Dutch mothers (Chi-square test; $p << 0.001$), being highest for mothers of Asian origin (Table 3).

Questionnaires on risk factors for HBV were available in 55% (n=46) of the Dutch women. It became clear that 48% (22/46) of these subjects would have escaped identification without routine screening (Table 4).

All but 2 HBsAg-positive women appeared to be chronic carriers as characterized by the presence of HBsAg in serum for at least 6 months. One woman had lost HBsAg by the time she delivered and was antiHBc-positive with 11 IU antiHBs/l. The other woman may have been false positive at the first screening (Ausria) since both HBsAg and antiHBc were negative at delivery.

Most of the HBsAg-positive women had one child during the study period but 120 had two, 23 had three and 2 women had four infants. All but 12 of the 145 women who delivered more than once kept the same HBe-status; nine women seroconverted from HBeAg-positive to antiHBe-positive and 3 women were HBeAg-positive in their next pregnancy. The 95.07 years of HBeAg-positive follow-up resulted in an annual seroconversion rate to antiHBe of 0.10.

Table 3

Country of birth and HBe-status of the HBsAg-positive mothers in the study period July 1982 through October 1989.

			HBsAg-positive pregnant women			
Country of origin	Total n	(%)	HBeAg-negative n	(%)	HBeAg-positive n	(%)
Netherlands	83	(12)	75	(90)	8	(10)
Mediterranean	377	(54)	330	(88)	47	(12)
Surinam	74	(11)	55	(74)	19	(26)
East Asian	102	(14)	64	(63)	38	(37)
other	69*	(10)	62	(90)	6	(9)
Total	704*	(100)	586	(83)	118	(17)

* The HBe-status is unknown in one case.

The HBeAg positivity rate in Asian women differs statistically from the rate in women of Dutch, Mediterranean and other origin ($p < 0.001$; $\chi^2_{[1]}$) but is not different from the HBeAg positivity rate in Surinam women ($p = 0.10$).

Table 4
Positive responses to risk factor questionnaires in the Dutch obstetric population found to be HBsAg-positive (n=46).

Risk factor	Number HBsAg-positive	Risk factor (%)
Health care employee	7	15
History of transfusions	5	11
Endemic area	4	9
HBsAg-positive family	3	6.5
Intravenous drug use	3	6.5
Iatrogenic	2	4
Unknown	22	48
Total	46	100

DISCUSSION

Our study clearly shows the potential effectiveness of universal antenatal screening of pregnant women for HBsAg. Two pertinent observations can be made. First, the acceptance of routine antenatal screening was excellent; the compliance rate in the hospital setting in Rotterdam was 97%. In the rural region indirect evidence suggests that also a very high rate is reached since there were more pregnant women screened than births, in the same period. Second, about half of the Dutch HBsAg carriers did not have identifiable risk factors for HBV and would not have been identified in a screening program aimed at risk groups.

Ideally, the identification of HBsAg carriers should occur late in the third trimester or at the time of delivery. In the Netherlands, identification of women at high risk at the time of delivery is not an easy task because of the high number of home deliveries. Instead, an effective and thorough screening program was implemented in routine antenatal care at 14 weeks, making use of the existing protocol of blood testing for ABO and syphilis. A column for the notation of the HBsAg status of the patient in the parturition books compels the professionals to check the HBsAg status.

Initially a considerable effort was placed into educational campaigns that were aimed at the professionals in the participating centers. Little effort was needed to maintain compliance thereafter; one person responsible for a regular check of the coverage of screening in each hopital is sufficient. More effort was needed in the TGA region where many professionals were involved. We observed that rapid screening was necessary in about 10% of the population in Rotterdam which may partly be caused by the fact that the study was done in large hospitals where many patients are medically indigent. This may explain

why more primiparae, planning to deliver at home, were admitted without HBsAg test result (Table 2).

The relative risk for HBV infection was about 2.5 times higher in women not receiving routine prenatal care. A relation between country of origin with possibly language or cultural barriers and limited access to prenatal care was not likely (Table 2). Rapid screening was always performed in Rotterdam when indicated (about 1% of the hospital deliveries involved stillborn infants and rapid screening was not performed in these cases). Rapid screening appeared difficult to implement in Utrecht and in the TGA region. Provisions for the above scenario were not readily made by the professionals involved nor accepted as part of the obstetric laboratory screening package. These laboratory tests are now more widely in use. It is important that the results of rapid screening are available before the woman leaves the hospital; only then will it be possible to administer passive immunization to the newborn within the recommended time interval (3).

The HBsAg positivity rate was 0.7% in the pregnant population; the initial higher prevalence was likely caused by "catch up screening" of women later in pregnancy. The assembled study population was not weighted for age, education and country of origin, but the average number of 14,000 recruited annually, constituted 7.5 percent of the estimated total number of pregnant women each year (190,000). Obviously, the geographic location of the centers may have influenced the numbers and origin of the immigrant population served. The urban hospitals with high prevalence rates serve a population that includes high risk ethnic groups or low socioeconomic groups or both, whereas the TGA region serves a suburban, socio-economically more privileged population. The prevalence of 0.3% in the TGA region could be an underestimate because of the use of the hemagglutination assay. 98 of 100 samples from HBsAg carriers detected by radioimmunoassay were also detected by the hemagglutination assay used in the TGA region. Both false negative samples had low levels of HBsAg. The test system was considered adequate as it was expected that low level HBsAg carriers have a reduced chance to transmit HBV to their offspring (6-7). At present, however, the more sensitive third generation assays (e.g. Ausria) are preferred for routine screening or diagnostic purposes (8).

Only one woman appeared to be false positive and one HBsAg-positive woman had lost HBsAg at the time of delivery. Therefore, it was decided in 1988 that testing of a second blood sample during gestation was no longer useful in the study nor in general practice. The seroconversion rate from HBeAg to antiHBe was not influenced by pregnancy as has been postulated by Lin et al. (9). We found a lower annual seroconversion rate than the spontaneous seroconversion rates (5-15% per year) that were reported in the literature (10).

Ethnic origin was the major risk factor for HBV in our study. Asian and Mediterranean countries are considerable geographic risk locations (11). The HBeAg prevalence in young adulthood (age 15-45) is 30-50% in HBsAg-positives from Asia and less than 20% in adults from the Mediterranean (12-14). Our findings are in agreement with these reports. We concentrated on recognizing risk factors in the Dutch study population. From the

completed questionnaires, in 48% of cases, it could not be predicted which women should undergo screening. Other studies also revealed a considerable proportion of patients with HBsAg who did not have identifiable, CDC defined (15) risk factors (16-17). Questionnaires, therefore, have a low predictive value. Even in low endemic areas it is more efficient to serotest every woman in the obstetric population as is already accepted for blood donors. The overall prevalence of 0.7% is more than ten times the level considered as the cut-off (0.06%) for cost-effective screening in the US (18).

Based on these results, the National Health Authorities formulated an official policy regarding the prevention of perinatal HBV infection by routine screening of every woman around 14 weeks of gestation. Laboratories should add the HBsAg assay to their prenatal screening package for a nominal sum. Since new infections or spontaneous clearance of HBsAg occur, women need to undergo repeat testing in subsequent pregnancies. If a woman tests positive, the entire HBV panel and liver enzymes should be tested to determine whether she has an acute or chronic infection and to ascertain hepatic function. Serologic testing should be possible on demand for women who deliver without an HBsAg test result. If the laboratory results cannot be available within 12 hours after delivery, the administration of hepatitis B immune globulin to the newborn may be considered. Appointment of persons responsible for the implementation and coverage of screening is advised.

Acknowledgments

This study was supported by a grant from the Praeventiefonds, the Netherlands. We are grateful to J Boot and WCJ Hop for their statistical analysis and continuing support. Special thanks go to YAM Weber and C Kerkhof for performing the laboratory tests.

The Dutch Study Group consisted of: A Deurloo-Volaard, State School of Training for Midwives, Rotterdam; J Zwijnenberg and Th van der Laar, Department of Paediatrics, Ruwaard van Putten Hospital, Spijkenisse; JRJ Bänffer, Regional Public Health Laboratory, Rotterdam; M Bakker-Bendik, Department of Internal Medicine II, Academic Hospital Dijkzigt, Rotterdam;
MJ Botman, Regional Laboratory of Pathology and Microbiology, Enschede; H Ribbers-Smeenk, Borne; GC de Gast, Blood Transfusion Laboratory, Utrecht; LJ Gerards and H van Dijk, Wilhelmina Childrens Hospital, Utrecht;
WPF Fetter and W Baerts, Department of Paediatrics, St Sophia Hospital, Zwolle, the Netherlands.

References

1. Maupas P, Chiron P, Barin F, Coursaget P, Goudeau A, Perrin J, Denis F, Diop Mar I. Efficacy of hepatitis B vaccine in prevention of early HBsAg carrier state in children: controlled trial in an endemic area (Senegal). Lancet 1981;1:289-292.
2. Tada H, Yanagida M, Mishina J, Fujii T, Baba K, Ishikawa S, Aihara S, Tsuda F, Miyakawa Y, Mayumi M. Combined passive and active immunization for preventing perinatal transmission of hepatitis B virus carrier state. Paediatrics 1982;70:613-619.
3. Beasley RP, Hwang LY, Stevens CE, Lin CC, Hsieh FJ, Wang KY, Sun TS, Szmuness W. Efficacy of hepatitis B immune globulin (HBIG) for prevention of perinatal transmission of the HBV carrier state: Final report of a randomized double-blind, placebo-controlled trial. Hepatology 1983;3:135-141.

4. Mazel JA, Schalm SW, de Gast GC, Nuijten ASM, Heijtink RA, Botman MJ, Bänffer JRJ, Gerards LJ, Zwijnenberg J, Mettau J, Wladimiroff JW, Fetter WPF. Passive-active immunization in neonates of HBsAg-positive carrier mothers. Preliminary observations. Br Med J. 1984;288:513-515.
5. Schalm SW, Mazel JA, de Gast GC, Heijtink RA, Botman MJ, Bänffer JRJ, Gerards LJ, Zwijnenberg J, Fetter WPF, Nuijten ASM, Wladimiroff JW, Christiaens GCML. Prevention of hepatitis B infection in newborns through mass screening and delayed vaccination of all infants of mothers with hepatitis B surface antigen. Paediatrics 1989;83:1041-1048.
6. Lee SD, Lo KJ, Wu JC, Tsai YT, Wang JY, Ting LP, Tong MJ. Prevention of maternal infant hepatitis B virus transmission by immunization: the role of serum hepatitis B virus DNA. Hepatology 1986;6:369-373.
7. Ip HMH, Lelie PN, Wong VCW, Mimms L, Reesink HW. Prevention of the HBV-carrier state in infants of HBsAg- and HBeAg-positive mothers with high and low serum levels of HBV DNA. A 3-year placebo-controlled study comparing the efficacy of hepatitis B vaccine with and without hepatitis-B immunoglobulin. Lancet 1989;1:406-410.
8. Ronalds CJ, Grint PCA, Hardiman, Heath RB. Testing for hepatitis B surface antigen-a critical review. Serodiag Immunother Infec Dis 1989;3:293-297.
9. Lin HH, Chen PJ, Chen DS, Sung JL, Yang KH, Young YC, Liou YS, Chen YP, Lee TY. Postpartum subsidence of hepatitis B viral replication in HBeAg-positive carrier mothers. J Med Virol 1989;29:1-6.
10. Hoofnagle JH, Dusheiko GM, Seeff LB, Jones A, Waggoner JG, Bales ZB. Seroconversion from hepatitis B e antigen to antibody in chronic type B hepatitis. Ann Intern Med 1981;94:744-748.
11. World Health Organization. Prevention of hepatocellular carcinoma by immunization: a WHO meeting. Bull WHO 1983;61:731-744.
12. Maynard JE. Worldwide control of hepatitis B. Int J Epidemiol 1984;13:406-407.
13. Francis DP. Worldwide control of hepatitis B virus; an approaching reality? Paediatrics 1985;76:851-852.
14. Wainwright RB, McMahon BJ, Bender TR, Heyward WL, Nakanishi S, Wainwright KY, Foliaki S, Erickson SL, Fields HA. Prevalence of hepatitis B virus infection in Tonga: identifying high risk groups for immunisation with hepatitis B vaccine. Int J Epidemiol 1986;15:567-71.
15. Centers for Disease Control. Postexposure prophylaxis of hepatitis B. Recommendations of the Immunization Practices Advisory Committee. MMWR 1984;33:285-290.
16. Greenspoon JS, Martin J, Greenspoon RL, McNamara BT. Necessity for routine obstetric screening for hepatitis B surface antigen. J Reprod Med 1989;34:655-658.
17. Jonas MM, Schiff ER, O'Sullivan MJ, Medina de M, Reddy KR, Jeffers LJ, Fayne T, Roach KC, Steele BW. Failure of Centers of Disease Control Criteria to identify hepatitis B infection in a large municipal obstetrical population. Ann Intern Med 1987;107:335-337.
18. Arevalo JA, Washington AE. Cost-effectiveness of prenatal screening and immunization for hepatitis B. JAMA 1988;259:365-369.

CHAPTER 2.3

EARLY INVASIVE PRENATAL DIAGNOSIS IN HBsAg-POSITIVE WOMEN

P.M. Grosheide[1], H.W.P. Quartero[2], S.W. Schalm[1], R.A. Heijtink[3], G.C.M.L. Christiaens[4]

Department of Internal Medicine II[1] and Department of Obstetrics and Gynaecology[2], University Hospital Dijkzigt, Rotterdam; Department of Virology[3], Erasmus University, Rotterdam; Department of Obstetrics and Gynaecology[4], Academic Hospital Utrecht, Utrecht, the Netherlands.

Accepted for publication in Prenatal Diagnosis.

SUMMARY

From 1982 to 1989 pregnant women in two large city hospitals in the Netherlands had serum samples screened for hepatitis B surface antigen. Infants of mothers found HBsAg-positive received hepatitis B immune globulin immediately after birth and hepatitis B vaccine in the first year of life. Blood samples of infants were regularly tested for HBsAg and antibodies directed against HBsAg.

A retrospective analysis of the pregnancy outcome in HBsAg-positive women who had invasive tests for prenatal diagnosis was carried out to determine whether amniocentesis or chorion villus sampling are risk factors for the intrauterine transmission of the hepatitis B virus.

Amniocentesis was carried out in 17 HBsAg-positive women and chorion villus sampling in 1 case. Only two women were HBsAg- and HBeAg-positive. Prenatal diagnosis led to the termination of pregnancy for fetal chromosome abnormality in three cases. The remaining 15 pregnancies were uneventful; all infants were negative for HBsAg and developed an active immune response to the vaccine. These data suggest that amniocentesis in HBsAg-positive women constitutes a low risk for the intrauterine transmission of the hepatitis B virus, but definite conclusions for HBsAg- and HBeAg-positive women cannot be drawn.

INTRODUCTION

With increasing technical capabilities for more and earlier prenatal diagnosis, obstetricians should question the potential risks of invasive tests in hepatitis B surface antigen (HBsAg)-positive women.

Without prophylactic measures, the risk of perinatal infection among infants born to mothers infected with the hepatitis B virus (HBV) ranges from 10% to 90%, depending on the mother's hepatitis B e antigen (HBeAg) status (1-2). Up to 90% of infants infected by their mothers at birth develop chronic HBV infection (3).

Most HBV infections occur perinatally; infants are exposed to the virus during labour (3-5). Mother-to-infant transmission presumably occurs from haematologic leaks across the placenta and/or birth canal exposure to maternal secretions or blood (6-7).

Early administration of hepatitis B immune globulin (HBIg) to the newborn infant is, therefore, important to protect the infant from perinatal HBV infection. Passive-active immunization, consisting of HBIg and hepatitis B vaccine has been very effective (> 90%) in preventing HBV infection among infants born to HBsAg-positive mothers (8-9). However, some HBeAg-positive HBsAg carrier mothers infect their infants before labour and delivery, i.e. during premature labour or threatened abortion (10). These infants will not be amenable to immunoprophylaxis and any immunization program is likely to fail. Hepatitis B virus does not appear to pass the intact placenta. In addition to placental leakage

due to uterine contractions, disruption of the placental barriers may also result from invasive tests like chorionic villus sampling and amniocentesis for early prenatal diagnosis of fetal genetic disease. The question is raised whether health care professionals should offer an HBsAg-positive woman prenatal genetic testing where there is a chance of infecting the infant with HBV in utero.

Because data in literature are scarce, we retrospectively investigated the impact of early chorionic villus sampling (CVS) and amniocentesis on intrauterine transmission of HBV from HBsAg-positive women to their offspring.

PATIENTS AND METHODS

In 1982 a multicenter trial was started in the Netherlands to detect HBsAg carrier mothers and to immunize their offspring (11). Obstetricians in four treatment centers were asked to screen all pregnant women for HBsAg at the first antenatal visit. All confirmed HBsAg-positive samples were tested for HBeAg. The serum samples were assayed by radioimmunoassay (Abbott Laboratories, Chicago, Ill, USA).

Infants of HBsAg-positive mothers who agreed to passive-active immunization of their newborns received HBIg (100 to 150 IU antiHBs/ml or 300 IU antiHBs/ml, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service) at birth and active immunization according to several dose and time schedules. Cord blood and blood samples from infants were regularly tested for HBsAg (Ausria II, Abbott) and antiHBs (Ausab, Abbott) until at least 12 months of age.

A number of 302 HBsAg-positive women was identified and their infants delivered over a seven year period in the two university hospitals participating in the vaccination trial. Both hospitals act as centers for prenatal diagnosis. Amniocentesis or CVS are among others that are recommended for women of advanced maternal age ($\geq$ 36 years) or a history of fetal chromosomal anomaly or neural tube defects.

At the start of the vaccination program the potential risks of transmitting the hepatitis B virus through transplacental leakage caused by invasive diagnostic procedures were unknown. Since no records were kept on invasive testing for prenatal diagnosis in HBsAg-positive pregnant women, we performed a retrospective study into the files of HBsAg-positive women who had their newborn infants immunized within the trial. If prenatal genetic testing had been performed this was followed by data collection on the sampling procedure and pregnancy outcome. The HBV DNA levels of the HBsAg carrier mothers who had prenatal diagnosis performed were also assayed quantitatively by HBV DNA assay (Abbott) since at present it is believed that the HBV DNA level of the HBsAg carrier mothers is a better marker for infectivity than HBeAg (12). Intrauterine HBV infection was defined as the presence of HBsAg in cord blood and in the subsequent blood samples of infants.

RESULTS

Invasive prenatal diagnosis was carried out in 18 of the 302 women who were found to be HBsAg-positive. Only six (15%) of 40 HBsAg-positive women aged 36 years or older had undergone prenatal diagnosis. Prenatal diagnosis was not carried out in another two women over 36 years of age because they were HBsAg-positive. In the other 12 women prenatal diagnosis was performed for reasons other than maternal age. Two of the 18 HBsAg-positive women were also HBeAg-positive. One HBeAg-positive woman had high levels of HBV DNA (61 pg/ml). The other HBeAg-positive woman had an acute HBV infection at the time she underwent prenatal diagnosis but her blood sample was not available for additional HBV DNA testing. All other women were HBV DNA negative. Amniocentesis was performed in 17 cases and CVS in the remaining case.
Indications for prenatal diagnosis, HBe-status of the women, maternal and gestational age and pregnancy outcome are presented in Table 1.
Amniocentesis was performed at 15.4-18 weeks of gestation; one woman, however, underwent amniocentesis at 30 weeks of gestation because multiple malformations were identified by ultrasound. CVS was performed at 10 weeks of gestation. No transplacental amniocentesis took place and the liquor samples showed no signs of blood staining.

Table 1
Characteristics of the HBsAg-positive women and the invasive prenatal procedures carried out.

	Amniocentesis* n=17	(%)	Chorionic villus sampling n=1
Indication			
maternal age	6	(35)	-
other	11	(65)	1
HBe-status			
HBeAg-positive	2	(12)	-
HBeAg-negative	13	(76)	1
unknown	2	(12)	-
Duration of gestation			
1st trimester	-		1
2nd trimester	16		-
3rd trimester	1		-
Pregnancy outcome			
termination	3	(18)	-
liveborn	14	(82)	1

* not transplacental

The woman who had an acute hepatitis B infection and was both HBsAg- and HBeAg-positive at the time of amniocentesis displayed a low HBsAg-positive liquor (13). In all other cases amniotic fluid was not tested for HBsAg.

Pregnancy was terminated in three cases because of anencephaly (14.6 weeks) or multiple malformations (18.4 and 31 weeks). These women were all HBsAg-positive but HBeAg-negative. The remaining 15 pregnancies were uneventful and resulted in the delivery of 15 healthy infants. Mean gestational age for live births was 39.1 ± 2.3 weeks (range 32 to 42 weeks). The median birth weight was 3558 ± 722.6 grams (range 1660 to 4920 grams). None of the neonatal blood samples were HBsAg-positive (0/15; 95% confidence interval: 0-21.8). All infants were HBsAg-negative at the age of 3 months and thereafter. At the age of 12 months they had developed protective levels of antiHBs following active immunization.

DISCUSSION

We presented the outcome of invasive prenatal diagnosis in 15 women who were HBsAg-positive at the time of the procedure. All women, including those highly infective during invasive testing, gave birth to a healthy infant without signs of intrauterine HBV infection. HBsAg positivity within the first months of life despite immunoprophylaxis at birth, may be due to infection acquired in utero (10). The patient population, however, is selected and numbers may be too small to declare invasive prenatal diagnosis in HBsAg-positive women to be completely safe. The upper limit of the 95% confidence limit on a stroke rate of 0% among 15 patients is 22%.

The exact mode of intrauterine transmission of HBV from mother to infant is unknown. In case of invasive testing involving the chorion or the placenta, HBV may be directly transmitted from maternal to fetal blood. Another possible route of HBV transmission may be the swallowing of HBsAg contaminated amniotic fluid by the fetus. On a theoretical basis it seems safe to avoid transplacental procedures and/or CVS in HBsAg carrier pregnant women and certainly so in the more infectious subgroup which is also HBeAg-positive or has high levels of HBV DNA. Any vascular area in the uterine wall should be avoided as to limit the contamination of the amniotic cavity with maternal blood.

Several important factors, associated with maternal-fetal transmission of HBV, need further discussion. A likely explanation for the absence of HBV infections in our study is that direct blood to blood contact did not take place. HBV transmission by needle-stick in comparison, has been shown to vary between 5% and 11% (14-15). The risk of infection depends on the level of infectivity of the injected material (16). After a percutaneous exposure to HBeAg-positive blood the risk of transmission increases to approximately 30% (14-15). Since only two women in our study may be considered as highly infective, the risk for the development of HBV infection through microleakage of maternal blood after invasive prenatal diagnostic techniques may have been negligible. The only woman who

underwent CVS was HBeAg-negative so no conclusions can be drawn as to the safety of this procedure in HBeAg-positive women.

Wong et al. performed third-trimester amniocentesis in 61 HBsAg-positive women and found HBsAg in 16 (26%) liquor samples (17). Amniocentesis in HBsAg-positive women did not affect the incidence of HBsAg in one month old babies (14.3%) in comparison to a control group (5%) who did not have amniocentesis ($p > 0.1$). However, a positive correlation ($p = 0.04$) was reported in HBeAg-positive mothers between the presence of HBsAg in amniotic fluid and the finding of antigen in newborns at the age of one month. The authors conclude that amniocentesis should be discouraged if mothers are HBsAg- and HBeAg-positive.

The risk of infection also depends on the quantity of injected material. Introduction of an amount as small as 10^{-8} ml of HBeAg-positive blood is sufficient for contamination (18). The incidence of maternal-fetal transfusion in literature varies from 2% to over 50%, depending on complications during pregnancy and the methods of testing (19). The overall risk of fetal red cells entering the maternal circulation following amniocentesis was 10 percent according to Sebring et al. (20). Considerable fetal-maternal haemorrhage after CVS was described by Mantingh who found increased concentrations of maternal serum alphafetoprotein in 60% of the blood samples (21). However, the degree of maternal-fetal transfusion and disruption of placental barriers as a result of invasive testing for prenatal diagnosis is not known.

Transmission of HBV by oral route has been documented in an experimental setting but appeared to be far less effective than the parenteral route (22). In natural situation the risk of infection is considered very low. This finding is partly based on the experience in endoscopy; HBV infection through endoscopy appeared low or non-existent (23). Although 20% of placental perforations is associated with bloody taps (24), ingestion of HBsAg contaminated amniotic fluid by the fetus is, therefore, not likely to be an important mode of transmission. Especially so, since transplacental procedures were not performed in the HBsAg-positive women studied. In the one woman who had a weakly HBsAg-positive amniotic fluid, contamination with maternal blood is the most likely explanation.

Finally, it is suggested that the fetal organs only become susceptible to HBV during the third trimester of pregnancy (4). The risk of the hepatitis B virus being transmitted from a newly infected mother to her fetus was 70% during the third trimester, but appeared to be virtually non existent in women which were infected at an earlier stage (25). Failure to transmit HBV from mother to fetus before the third trimester may be due to failure of the HBV to pass the placenta or failure of the fetus to support the HBV. For instance, no integration of HBV DNA was detected in fetal liver cells from 48 terminated pregnancies after 20 to 32 weeks of gestation (26). Conception and development of the pre-embryo in an HBsAg-positive woman occurs in a potentially infectious medium and even the accidental HBV exposure of pre-embryos to a contaminated culture medium during in vitro fertilization procedures did not result in intrauterine infections (13).

Although data are scarce, the available information supports the idea that HBV will not

harm the fetal liver early in pregnancy. The present uncertainties, however, point to the need for a more careful and considered approach to the widespread use of invasive prenatal diagnostic techniques in HBsAg-positive women. For highly infectious women (HBeAg-positive or HBV DNA positive) a firm conclusion on the safety of invasive prenatal diagnosis, especially CVS, cannot be drawn. Extensive studies in countries with high endemicity for HBV may perhaps solve the issue of competing considerations.

Acknowledgments

We would like to thank YAM Weber and C Kerkhof (Department of Virology; Erasmus University, Rotterdam) for performing the laboratory tests. We are indebted to JW Wladimiroff and FJ Los (Department of Obstetrics and Gynaecology and Department of Clinical Genetics; Academic Hospital Dijkzigt, Rotterdam) for their helpful comments regarding the manuscript.

References

1. Beasley RP, Trepo C, Stevens CE, Szmuness W. The e antigen and vertical transmission of hepatitis B surface antigen. Am J Epidemiol 1977;105:94-98.
2. Stevens CE, Neurath RA, Beasley RP, Szmuness W. HBeAg and anti-HBe detection by radioimmunoassay: correlation with vertical transmission of hepatitis B virus in Taiwan. J Med Virol 1979;3:237-241.
3. Stevens CE, Beasley RP, Tsui J, Lee WC. Vertical transmission of hepatitis B antigen in Taiwan. N Engl J Med 1975;292:771-774.
4. Gerety RJ, Schweitzer IL. Viral hepatitis type B during pregnancy, the neonatal period, and infancy. J Pediatrics 1977;90:368-374.
5. Lee AKY, Ip HMH, Wong VCW. Mechanisms of maternal-fetal transmission of hepatitis B virus. J Infect Dis 1978;138:668-671.
6. Beasley RP, Hwang LY, Lin CC, Leu ML, Stevens CE, Szmuness W, Chen KP. Incidence of hepatitis B virus infections in preschool children in Taiwan. J Infect Dis 1982;146:198-204.
7. Lin HH, Lee TY, Chen DS, Sung JL, Ohto H, Etoh T, Kawana T, Mizuno M. Transplacental leakage of HBeAg-positive maternal blood as the most likely route in causing intrauterine infection with hepatitis B virus. J Pediatr 1987;111:877-881.
8. Stevens CE, Taylor PE, Tong MJ, Toy PT, Vyas GN, Nair PR, Weissman JY, Krugman S. Yeast recombinant hepatitis B vaccine. Efficacy with hepatitis B immuneglobulin in prevention of perinatal hepatitis B virus transmission. JAMA 1987;257:2612-2616.
9. Schalm SW, Mazel JA, Gast de GC, Heijtink RA, Botman MJ, Bänfffer JRJ, Gerards LJ, Zwijnenberg J, Fetter WPF, Nuijten ASM, Wladimiroff JW, Christiaens GCML. Prevention of hepatitis B infection in newborns through mass screening and delayed vaccination of all infants of mothers with hepatitis B surface antigen. Pediatrics 1989;83:1041-1047.
10. Ohto H, Lin HH, Kawana T, Etoh T, Tohyama H. Intrauterine transmission of hepatitis B virus is closely related to placental leakage. J Med Virol 1987;21:1-6.
11. Mazel JA, Schalm SW, de Gast GC, Nuijten ASM, Heijtink RA, Botman MJ, Bänffer JRJ, Gerards LJ, Zwijnenberg J, Mettau J, Wladimiroff JW, Fetter WPF. Passive-active immunization in neonates of HBsAg-positive carrier mothers. Preliminary observations. Br Med J 1984;288:513-515.
12. Lee SD, Lo KJ, Wu JC, Tsai YT, Wang JY, Ting LP, Tong MJ. Prevention of maternal infant hepatitis B virus transmission by immunization: the role of serum hepatitis B virus DNA. Hepatology 1986;6:369-373.
13. Os van HC, Drogendijk AC, Fetter WPF, Heijtink RA, Zeilmaker GH. The influence of contamination of culture medium with hepatitis B virus on the outcome of in vitro fertilization pregnancies. Am J Obstet Gynecol 1991;165:152-159.

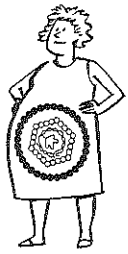
14. Grady GF, Lee VA, Prince AM, Gitnick GL, Fawaz KA, Vyas GN, Levitt MD, Senior JR, Galambos JT, Bynum TE, Singleton JW, Clowdus BF, Akdamar K, Aach RD, Winkelman EI, Schiff GM, Hersch T. Hepatitis B immune globulin for accidental exposures among medical personnel: final report of a multicenter controlled trial. J Infect Dis 1978;138:625-638.
15. Seeff LB, Wright EC, Zimmerman HJ, Alter HJ, Dietz AA, Felsher BF, Finkelstein JD, Garcia-Pont P, Gerin JL, Greenlee HB, Hamilton J, Holland PV, Kaplan PM, Kiernan T, Koff RS, Leevy CM, McAuliffe VJ, Nath N, Purcell RH, Schiff ER, Schwartz CC, Tamburro CH, Vlahcevic Z, Zemel R, Zimmon DS. Type B hepatitis after needlestick exposure: prevention with hepatitis B immune globulin: final report of the Veterans Administration Cooperative Study. Ann Intern Med 1978;88:285-293.
16. Alter HJ, Seeff LB, Kaplan PM, McAuliffe VJ, Wright EC, Gerin JL, Purcell RH, Holland PV, Zimmerman HJ. Type B hepatitis: the infectivity of blood positive for e antigen and DNA polymerase after accidental needlestick exposure. N Engl J Med 1976;295:909-913.
17. Wong VCW, Lee AKY, Ip HMH. Transmission of hepatitis B antigens from symptom free carrier mothers to the fetus and the infant. Br J Obstet Gynecol 1980;87:958-965.
18. Shikata T, Karasawa T, Abe K, Uzawa T, Suzuki H, Oda T, Imai M, Mayumi M, Moritsugu Y. Hepatitis B e antigen and infectivity of hepatitis B virus. J Infect Dis 1977;136:571-576.
19. Schröder J. Transplacental passage of blood cells. J Med Gen 1975;12:230-242.
20. Sebring ES and Polesky HF. Fetomaternal hemorrhage: incidence, risk factors, time of occurrence and clinical effects. Transfusion 1990;30:344-357.
21. Mantingh A. On CVS. Early experience with chorionic villus sampling (CVS) in the north of the Netherlands. Ph.D. Thesis 1988, Groningen.
22. Krugman S, Giles JP. Viral hepatitis - new light on an old disease. JAMA 1970;212:1019-1029.
23. Villa E, Pasqyinelli C, Rigo G, Ferrari A, Perini M, Feretti I, Gandolfo M, Rubbiani L, Antonioli A, Barohi T, Manenti F. Gastrointestinal endoscopy and HBV infection: no evidence for a causal relationship. Gastrointest Endosc 1984;30:15-17.
24. Kappel B, Nielsen J, Brogaard Hansen K, Mikkelsen M, Therkelsen AAJ. Spontaneous abortion following mid-trimester amniocentesis. Clinical significance of placental perforation and blood-stained amniotic fluid. Br J Obstet Gynecol 1987;94:50-54.
25. Schweitzer IL. Vertical transmission of the hepatitis B surface antigen.
Am J Med Sciences 1975;270:287-291.
26. Li L, Sheng MH, Tong SP, Chen HZ, Wen YM. Transplacental transmission of hepatitis B virus.
Lancet 1986;2:872.

CHAPTER 2.4

IMMUNE RESPONSE TO HEPATITIS B VACCINE IN PREGNANT WOMEN RECEIVING POST-EXPOSURE PROPHYLAXIS

P.M. Grosheide[1], S.W. Schalm[1], H.C. van Os[2], W.P.F.Fetter[3], R.A. Heijtink[4]

Department of Internal Medicine II[1] and Department of Obstetrics and Gynaecology[2], University Hospital Dijkzigt, Rotterdam; Department of Paediatrics[3], St Sophia Hospital, Zwolle; Department of Virology[4], Erasmus University, Rotterdam, the Netherlands.



European Journal of Obstetrics and Gynaecology 1993;50:53-58.

SUMMARY

Hepatitis B immune globulin and vaccine were given as post-exposure prophylaxis to 73 women after an outbreak of hepatitis B due to in vitro fertilization treatment.
The immunization schedule consisted of 5 ml of hepatitis B immune globulin (125 IU/ml) at months 0 and 1 and recombinant hepatitis B vaccine (10 µg of HBvaxDNA) at months 0, 1, 2, and 6. The safety and immunogenicity of hepatitis B vaccine were studied in 16 women who became pregnant after in vitro fertilization; 57 nonpregnant women receiving the same treatment served as controls. Blood samples were drawn at 0, 1, 2, 6, and 7 months. One vaccinee had a clinical abortion two days after initial immunization; other side effects of vaccination were not found in vaccinees or in their offspring. All vaccinees exhibited antibodies against hepatitis B surface antigen after vaccination but relatively low peak geometric mean titers of 258 IU/l and 684 IU/l were attained in pregnant and nonpregnant women, respectively. There were no significant differences in seroconversion rates and geometric mean titers between the two groups although the immune response to hepatitis B vaccine was slower and lower in pregnant women at all times.

Our results suggest that when post-exposure prophylaxis for hepatitis B infection is indicated, passive-active immunization can be started safely during pregnancy. The relative weak response to the vaccine calls for monitoring of the antiHBs one month after the initial series of vaccinations.

INTRODUCTION

Many studies have described the safety and immunogenicity of hepatitis B vaccine in neonates of HBsAg-positive mothers (1-2) and women of childbearing age (3-4). Little is known, however, about the effects of hepatitis B vaccine in pregnant women and the fetus. A recent paper by Levy assessed the maternal and fetal safety of hepatitis B vaccination of ten women during the first trimester of pregnancy (5). Ayoola described the results of hepatitis B vaccination of pregnant women in their third trimester in order to provide adequate protection to their offspring by the transfer of antibodies (6).

Since it has been recommended that high-risk pregnant women undergo hepatitis B vaccination if they are HBsAg-negative at prenatal screening (7), more data on the safety of and immune response to hepatitis B vaccine in pregnancy are of importance.

Depression of cell-mediated immunity (CMI) in pregnant women facilitates fetal retention and may very well interfere with the immune response to HBs-antigen.

A hepatitis B epidemic among women undergoing in vitro fertilization treatment allowed us to study the effects of hepatitis B vaccination in pregnant women receiving post-exposure prophylaxis. Nonpregnant women receiving the same prophylactic treatment served as controls.

PATIENTS AND METHODS

In March 1988 a hepatitis B epidemic was discovered in women undergoing in vitro fertilization (IVF). A number of 175 women were possibly exposed to the hepatitis B virus (HBV).

All women, with the exception of pregnant women with a positive rapid HBsAg test (Hepatest; Wellcome Laboratories, Dartford, England) or with clinical signs of infection, were offered immunization during their first visit after discovery of the epidemic; i.e. 2 to 3 months after IVF and the suspected exposure to HBV but several weeks before a clear distinction could be made between women exposed and not exposed.

For the post-exposure situation an intensive immunization schedule was followed (8). Two injections of antiHBs immune globulin (HBIg; 5 ml containing 125 IU/ml, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam) were given intramuscularly at months 0 and 1 and three intramuscular injections of hepatitis B vaccine (HBvaxDNA; 10 µg/ml, Merck Sharp & Dohme, West Point, Pa, USA) at 0, 1, and 2 months. A booster injection was given at 6 months. Vaccinees with evidence of hepatitis B infection (HBsAg-positive or antiHBc-positive) discontinued vaccination as soon as the laboratory results became available.

Blood specimens were obtained at first screening and from vaccinees at 1, 2, 6, and 7 months thereafter and tested for HBsAg, antiHBc by enzymeimmunoassay (Auszyme, Corzyme; Abbott Laboratories, Chicago, Ill, USA) and antiHBs by radioimmunoassay (Ausab; Abbott). AntiHBs was determined quantitatively with the aid of the World Health Organization reference preparation and expressed in international units per liter (IU/l). Vaccinees were regularly informed about their immune status and their risk for hepatitis B. More details on the epidemic, hepatitis B infections and vaccination results among women participating in the IVF program have been given in previous publications (9-10).

Infants were examined at birth by a pediatrician with a follow-up schedule of 1 year. Immediately after birth cord blood was obtained from all infants and follow-up blood samples were obtained at 1, 3, 6, and 12 months of age and tested for HBsAg and antiHBc.

Statistics

Results were analyzed by standard statistical tests. Differences in seroprotection rates between treatment groups were calculated with 95% confidence intervals (CI) and compared using the Fisher's exact test. AntiHBs concentrations in pregnant and nonpregnant women were compared, after logarithmic transformation, by the Student's t-test for unpaired data; a P-value of less than 0.05 was considered significant. The geometric mean titer (GMT) was calculated for those vaccinees who had antiHBs levels ≥ 1.

A $\geq$ 4-fold increase in antibody concentrations, generally accepted in viral serology as a significant increase, was introduced as a parameter of the anamnestic immune response to the vaccine from month 6 to month 7.

RESULTS

The program of HBIg injections and immunization was administered to 16 pregnant women (mean age 32.5) and 57 nonpregnant women (mean age 32.6). HBsAg and antiHBc remained negative in all cases included in this follow-up study.

Immunization during pregnancy

The moment of administration of passive-active immunization during pregnancy is summarized in Table 1. Six women were in their first trimester (< 13 weeks) when they received the first dose of HBIg and vaccine. The booster dose was given after delivery in most cases (9/14).

Adherence to the vaccination protocol was far from perfect; 93% of pregnant (14/15) and 88% of nonpregnant (50/57) women received all four vaccine injections. The blood samples of month 7 were missing for 21% (4/14) of pregnant vaccinees and 22% (11/50) of nonpregnant vaccinees.

Table 1

Number of passive and active hepatitis B immunizations given during pregnancy or after delivery.

	Trimester				
HBIg/vaccine	First	Second	Third	After delivery	Total
1st dose	6*	10	-	-	16
2nd dose	4	11	-	-	15
3rd dose	-	14	1	-	15
4th dose	-	-	5	9	14†

* One vaccinee had a spontaneous abortion two days after initial immunization.
† One vaccinee was lost to follow-up after month 2.

Safety

One pregnant vaccinee had a spontaneous abortion at ten weeks, 2 days after initial immunization. No major adverse reactions to HBIg or vaccine were reported in vaccinees during the follow-up period of at least 7 months.

The 14 fully-immunized pregnant women had 19 healthy infants.

Congenital malformation was not noticed in any of the children at birth. At the age of 12 months growth parameters and development were normal in all infants. Blood samples taken from these infants post partum were all negative for HBV.

Table 2

Percentage of protective antibody levels (antiHBs ≥ 10 IU/l) after passive-active hepatitis B immunization in pregnant and nonpregnant women.

	Percentage antiHBs ≥ 10 IU/l at month			
	1	2	6	7
Pregnant	93 (13/14)	100 (15/15)	75* (9/12)	100 (11/11)
Nonpregnant	100 (57/57)	100 (57/57)	92* (46/50)	100 (39/39)

Number of women with antiHBs ≥ 10 IU/l per number of women tested given in parentheses.
* Fisher's exact test (p = 0.12)

Table 3

Concentrations of antiHBs ≥ 1 IU/l, expressed as GMTs, in pregnant and nonpregnant vaccinees after passive-active hepatitis B immunization.

	GMT in IU/l at month			
	1	2	6	7
Pregnant	28 (14)	40 (15)	29 (10)	258 (11)
Nonpregnant	37 (57)	48 (57)	39 (49)	684 (39)
P-value*	p = 0.02	NS†	NS	NS

Number of women with antiHBs ≥ 1 IU/l is given in parentheses.
* Student's t-test
† Not Significant

Immunogenicity

The percentage of vaccinees with seroprotection (≥ 10 IU/l antiHBs) is shown in Table 2 for pregnant and nonpregnant women. The antiHBs levels at month 1 ranged from 8 to 99 IU/l in the pregnant group and from 20 to 97 IU/l in the nonpregnant group. At month 2 all women had antiHBs levels above 10 IU/l, from either passive or active immunization. However, at month 6 only nine (75%) of 12 pregnant women (95% CI: 51-100) compared to forty-six (92%) of 50 nonpregnant women (95% CI: 85-100) had protective antiHBs antibodies after completion of the first series of three vaccinations (Fisher's exact test; p = 0.12). Two pregnant women and one nonpregnant woman had less than 1 IU antiHBs/l at month 6.

From month 1 onwards, the GMT of the antiHBs concentrations appeared to be lower for pregnant women than nonpregnant women (Table 3). A statistically significant difference (Student's t-test; p = 0.02) in GMTs was observed at month 1 only.
The distribution of the individual levels of antiHBs during and after immunization is shown in Figure 1. AntiHBs titers in the pregnant group are within the range of antiHBs in the nonpregnant group.

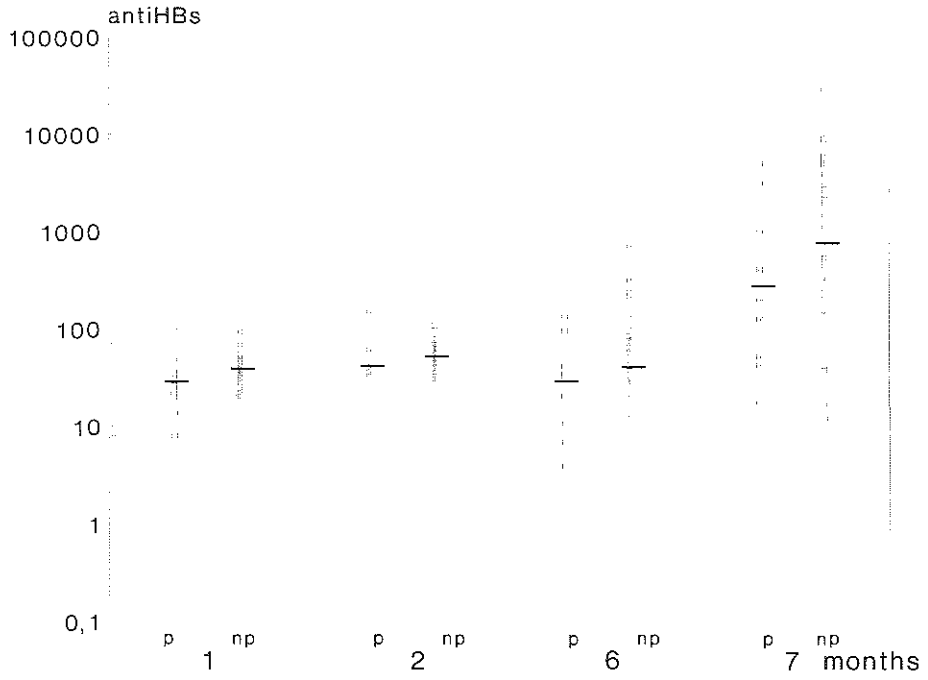


Figure 1
Individual levels of antiHBs and GMT during and after hepatitis B immunization in pregnant (p) and nonpregnant (np) women.

After the booster dose at month 6, all vaccinees had protective antiHBs levels, varying from 18 to 4955 IU/l in pregnant (GMT: 258 IU/l) and from 12 to 28 972 IU/l in nonpregnant women (GMT: 684 IU/l). The two pregnant women with no detectable antiHBs at month 6 responded to the booster injection with antiHBs titers of 18 IU/l and 52 IU/l, respectively, the nonpregnant vaccinee with < 1 IU/l at month 6 had an antiHBs titer of 17 IU/l at month 7. No serum was available at month 6 from the nonpregnant vaccinee who had an antiHBs level of 12 IU/l after complete vaccination.

A ≥ 4-fold increase in antiHBs level between month 6 and month 7 was found for 82% (9/11) of the pregnant women and 90% (35/39) of the nonpregnant women (Fisher's exact test; $p = 0.60$).

DISCUSSION

In this study the safety and immunogenicity of hepatitis B vaccine in pregnant women and nonpregnant women were compared.

Physicians are advised to avoid any type of medication during pregnancy, especially during the first trimester. The recommendation not to administer hepatitis B vaccine during pregnancy is not based on evidence of clinical toxicity but is a matter of principle and legal liability.

No major adverse events were observed in vaccinees receiving passive-active immunization. In none of the infants could a congenital HBV infection or a congenital malformation related to HBV infection or immunization be demonstrated; development and growth were normal during follow-up.

The results of this study support the view that the hepatitis B vaccine has no deleterious effect on fetal organogenesis and fetal development. Most of the initial immunizations in our study took place in the second trimester, a period when the fetal organs are well developed. However, one pregnant woman had a spontaneous abortion 2 days after the first dose of HBIg and vaccine. Whether this adverse pregnancy outcome is related to immunization cannot easily be determined. We did find similar abortion rates after IVF treatment (N=175) in the first trimester for the 128 women ultimately exposed to the hepatitis B virus (24%), the additional 47 women not exposed (21%) and women in a control period (26%) (9). Since HBIg is inactivated and the vaccine is produced synthetically by recombinant DNA techniques a direct relation with the clinical abortion does not seem likely. Although safe use of vaccine early in pregnancy is suggested the number of pregnant vaccinees in the first trimester in our study and the study of Levy are small (5).

Low compliance may have been due to the fact that all participants, whether pregnant or not, were regularly informed about their immune status and its implications by their obstetrician (10).

The immunogenicity, expressed either as the geometric mean titer or as the frequency of

antiHBs responses ≥ 10 IU/l, provided by vaccination, was similar for pregnant and nonpregnant women but antiHBs titers developed more slowly and were lower in pregnant women. Calculations, however, are based on relatively small numbers. The GMTs at months 1 and 2, probably based on passively acquired antiHBs, may be lower in pregnant women as a result of hemodynamic changes since most of the injections were given in the second trimester. The expansion of plasma volume starts early in pregnancy and reaches a plateau by 30-34 weeks (11). At month six, only 75% of pregnant women had protective levels of antiHBs. Compared to the seroprotection rates of more than 90% of vaccinees one month after two initial doses of vaccine (schedule 0, 1, and 6 months) found in several other studies, this is a relatively low percentage for a healthy adult population of women of this age (3-4,12). Likewise 8% of the nonpregnant women were unprotected at month 6 in comparison to none of the vaccinees after short interval vaccination at 0, 2, and 6 weeks in the study of Wahl (8).

The GMTs for pregnant and nonpregnant women were relatively low (< 50 IU/l) at month 6. Interference of HBIg with the response to hepatitis B vaccine is unlikely since it has not been reported in any other study using combined passive-active immunization before (1-2,13-14).

Our results indicate that the degree of protection against hepatitis B achieved in both pregnant and nonpregnant women was suboptimal despite an intensive immunization schedule. The active antiHBs response to the vaccine was ultimately excellent, 100% of the vaccinees having antiHBs ≥ 10 IU/l at month 7. Most pregnant women received the booster dose after delivery (Table 1). The rise in antiHBs after the fourth dose of vaccine clearly represented a booster reaction. A ≥ 4-fold rise in antiHBs titers was found in an equal percentage of pregnant and nonpregnant women and the distribution of antiHBs in pregnant women appeared to run parallel to the antiHBs titers for nonpregnant women but at a lower level (Figure 1). All observations, however, may suffer from sample and selection bias.

Gestation is associated with depression of cell-mediated immunity; the depressive effects of corticosteroid excess on cellular immunity are well known and pregnant women develop weaker CMI (15-16). Humoral immunity generally continues to function normally during pregnancy. The changes in cellular immunity seemed relatively minor since adequate antiHBs titers were observed in the end.

For the post-exposure situation the intensive immunization schedule proved to be safe and adequate for pregnant women as well as nonpregnant women. Therefore, in our opinion for women who are at great risk for HBV infection pregnancy should not be considered a contraindication for hepatitis B vaccine. However, if post-exposure prophylaxis is required and the woman is pregnant concomitant administration of HBIg seems advisable. Regular control in search for active antibody production is also legitimate. As far as the final antibody levels are concerned a booster dose after delivery may give rise to higher and long-term protective levels of antiHBs in most women.

Acknowledgments

We thank the medical and nursing staffs of the Department of Obstetrics and Gynaecology of the University Hospital Dijkzigt for their cooperation and technical assistance. Special thanks goes to WCJ Hop for his technical assistance.

References

1. Beasley RP, Hwang LY, Lee GCY, Lan CC, Roan CH, Huang FY, Chen CL. Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. Lancet 1983;2:1099-1102.
2. Stevens CE, Taylor PE, Tong MJ, Toy PT, Vyas GN, Nair PV, Weissman JY, Krugman S. Yeast-recombinant hepatitis B vaccine. Efficacy with hepatitis B immune globulin in prevention of perinatal hepatitis B virus transmission. JAMA 1987;257:2612-2616.
3. Scheiermann N, Gesemann KM, Kreuzfelder E, Paar D. Effects of a recombinant yeast dried hepatitis B vaccine in healthy adults. Postgrad Med J 1987;63(suppl 2):115-119.
4. Westmoreland D, Player V, Heap DC, Hammond A. Immunization against hepatitis B - what can we expect? Results of a survey of antibody response to immunization in persons 'at risk' of occupational exposure to hepatitis B. Epidemiol Infect 1990;104:499-509.
5. Levy M, Koren G. Hepatitis B vaccine in pregnancy: maternal and fetal safety. Am J Perinatol 1991;8:227-232.
6. Ayoola EA, Johnson AOK. Hepatitis B in pregnancy: immunogenicity, safety and transfer of antibodies to infants. Int J Gynecol Obstet 1987;25:297-301.
7. ACOG Committee Opinion: Committee on Obstetrics. Guidelines for hepatitis B virus screening and vaccination during pregnancy. Int J Gynecol Obstet 1991;35:367-369.
8. Wahl M. Hermodsson S, Iwarson S. Hepatitis B vaccination with short dose intervals-a possible alternative for post-exposure prophylaxis? Infection 1988;16:229-232.
9. Van Os HC, Drogendijk AC, Fetter WPF, Heijtink RA, Zeilmaker GH,. The influence of contamination of the culture-medium with hepatitis B virus on the outcome of IVF-pregnancies. Am J Obstet Gynecol 1991;165:152-159.
10. Grosheide PM, Van Os HC, Schalm SW, Heijtink RA. Immunoprophylaxis to limit a hepatitis B epidemic among women undergoing in vitro fertilization. Vaccine 1991;9:682-687.
11. Hytten F. Blood volume changes in normal pregnancy. Clin Haematol 1985;14:601-612.
12. Dienstag JL, Werner BG, Polk BF, Snydman DR, Craven DE, Platt R, Crumpacker CS, Ouellet-Hellstrom R, Grady GF. Hepatitis B vaccine in health care personnel: safety, immunogenicity and indicators of efficacy. Ann Int Med 1984;101:34-40.
13. Roumeliotou-Karayannis A, Papaevangelou G, Tassopoulos N, Richardson SC, Krugman S. Post-exposure active immunoprophylaxis of spouses of acute viral hepatitis B patients. Vaccine 1985;3:31-34.
14. Celis E, Abraham KG, Miller RW. Modulation of the immunological response to hepatitis B virus by antibodies. Hepatology 1987;7:563-568.
15. Weinberg ED. Pregnancy-associated depression of cell-mediated immunity. Rev Infect Dis 1984;6:814-831.
16. Stirrat GM. Immunology of disease of pregnancy. In: Stern CMH (ed). Immunology of pregnancy and its disorders. Dordrecht, Boston, London: Kluwer Acad. Publ. Group 1989:115-143.

CHAPTER 3.1

HISTORY OF PASSIVE AND ACTIVE IMMUNIZATION IN INFANTS

Hepatitis B immune globulin

Passive immunization with hepatitis B immune globulin (HBIg), a preparation containing high concentrations of antibodies to the hepatitis B surface antigen (antiHBs), was among the first methods used to prevent HBV infection. Several uncontrolled studies with few infants showed evidence on the effect of HBIg in the prevention of perinatal HBV infection (1-3). Kohler et al. reported that the perinatal transmission of HBV from four HBsAg-positive mothers, including two mothers with acute hepatitis B in the third trimester of pregnancy, was prevented by the administration of HBIg to susceptible infants within the first week of life (1). In Reesink's report, 21 infants given HBIg within 48 hours of birth followed by monthly injections for six months did not become HBsAg-positive (3).

Since 1974 placebo-controlled studies were conducted to assess the efficacy of interruption of perinatal transmission of HBV with HBIg among infants of HBeAg-positive carrier mothers in Taiwan (4-6). Among 61 placebo recipients the carrier rate was 92% after 15 months of follow-up; compared with 54% among 67 infants who received a single 1 ml dose of HBIg at birth and 26% among 57 infants who received 0.5 ml HBIg at birth, three months and six months (6). The protective efficacy was 42% and 71% respectively for the two treatment schedules. HBIg not only resulted in fewer infections but also prolonged the time of onset of infection: in most cases passive immunization delayed the onset of HBV infection beyond 6 months of age (5-7).

The timing of the initial administration of HBIg may be of importance: limited data suggest that HBIg is most effective if given within the first 2 days of life and the administration of HBIg as soon as possible after birth has been recommended (3-5). The new approach to prophylaxis with hepatitis B vaccine diminished the attention to determine optimal timing, frequency and dose of HBIg administration.

Hepatitis B vaccine

In 1971 Krugman et al. reported the first results of active immunization (8). Known infective serum containing HBV and HBsAg, when diluted 1:10, boiled for 1 minute and injected into humans prior to exposure to the virus, proved immunogenic, partially protective and not infectious. Since then, the way to the manufacturing procedures of the hepatitis B vaccine was paved. The hepatitis B vaccine was prepared from the plasma of chronic asymptomatic HBsAg carriers (9). Inactivation steps insured non-infectivity.

In prelicensure studies the plasma-derived hepatitis B vaccine has been shown to be safe, highly immunogenic and effective in preventing HBV infections in both neonates and infants (10-11). After 12 months of follow-up a 85% protective efficacy rate was established in susceptible infants given three doses of hepatitis B vaccine at one-month intervals (10). Vaccination was without side effects and 95% of infants showed a specific antiHBs response (11). Moreover, it was shown that active immunization against HBV can be induced in infants with passive immunity due to antiHBs of maternal origin (10-11). Adverse reactions after vaccination are minimal, mainly consisting of transient pain at the site of injection

affecting 4% of children or slight elevations of temperature during the first five days after vaccination (12). The plasma vaccine became commercially available in 1982. Presently, the production of hepatitis B vaccine is based upon recombinant DNA technology; baker's yeast (Saccharomyces cerevisiae) is chosen for the expression of HBsAg (13).

Combined passive and active immunization

Efforts to further reduce the chronic carrier rate and to prevent late infections in infants of HBsAg-positive mothers focused on a combination of passive and active immunization (14-15). Previous observations supported the concurrent use of HBIg for the immediate protection and vaccine for the prolonged protection and this approach was favoured by the fact that passively acquired antibody did not interfere with an active immune response to the vaccine (3,6,11).

Beasley and colleagues have reported on the efficacy of varying combinations of HBIg and hepatitis B vaccine (15). Infants of HBeAg-positive HBsAg carrier mothers were given 0.5 ml HBIg at birth. Group A infants were given an additional dose of HBIg at 3 months at which time they were started on vaccine. Group B infants were started on vaccine at 4-7 days and group C at 1 month. Three doses of vaccine were given, the first two a month apart and the third 6 months later. All combinations produced a substantial decrease in the HBsAg carrier rate from 88% in historic controls to between 2% for group A infants and 8% among infants in group C. There was no difference in efficacy of the three schedules; the overall protective efficacy was 94% compared with that of HBIg alone (71%) or of vaccination alone (75%). These results have been confirmed by others (16-17). Multiple doses of HBIg conferred no added advantage over a single dose when both were followed by hepatitis B vaccination and there is no need for second doses of HBIg if vaccination is started at birth (15-16). In combined regimens the timing of the first dose of vaccine does not appear to be critical (15).

Rates of prevention of perinatal transmission of HBV to infants of HBeAg-positive HBsAg carrier mothers by combined use of HBIg and vaccine have ranged from 83% to 95% (16-19). Failure of passive-active immunization to protect against perinatally transmitted HBV may be due to established infection from in utero transmission, high level of maternal HBV DNA or to an inadequate active antibody response to the vaccine (15-16,18,20-21).

The antibody response to the hepatitis B vaccine is dependent on type, dose and schedule of vaccination used, as well as age and status of the immune system of the vaccinee (22). Administration schemes of both plasma-derived and recombinant-derived vaccines feature 2 or 3 intramuscular injections of vaccine at monthly intervals, followed by a booster injection 6 to 12 months after the first dose. Immunogenicity studies have shown that 95-100% of healthy infants respond to the full series of vaccine (16-19,23-25). More than 10 international units (10 IU/l) of antibodies against HBsAg is considered an adequate immune response (26). Testing of the recombinant vaccines for efficacy has shown them

comparable with the plasma vaccine but recipients of the recombinant vaccine developed relatively lower antibody titers at all periods tested than infants receiving the plasma vaccine (27). Additional studies have been undertaken to evaluate reduced dosages and different vaccination schedules in attempts to decrease the high cost of the vaccine or to allow its integration with other vaccines into infant immunization programs. Similar protective efficacy rates but reduced antibody titers were demonstrated by the use of lower doses of vaccine (28-30) and the use of two instead of three initial doses of vaccine (31-33).

Enhancement of the immune response was found if the infant was older at the time of initial vaccine injection or if the booster dose was given later (33-36). Delayed administration of hepatitis B vaccine concomitant with diphtheria-tetanus-pertussis-polio (DTP-polio) vaccine, starting at the age of 3 months, produced higher levels of antiHBs in infants than immunization starting at birth (33-35). Delayed active immunization proved effective for long-lasting protection and practical in reducing the number of doctor visits for infants born to HBsAg-positive mothers in the Netherlands (33-34). No interaction was noticed when hepatitis B vaccine was administered with other childhood vaccines like DTP-polio vaccine (37-39). Recently, encouraging results were reported from clinical trials in infants with a quadrivalent DPT-HB vaccine that is designed to further reduce the number of injections (40).

Premature infants do not have a reduced immune response as compared to term infants (41). An impaired immune response to the hepatitis B vaccine, however, was observed in infants undergoing haemodialysis (42), in infants with malignancies (43), and in HIV infected infants (44). Genetic causes for unresponsiveness to the vaccine in infants have also been described (45). In infants with an inadequate or a weak immune response after primary vaccination and without signs of hepatitis B infection, additional hepatitis B vaccinations may yield protective antibody levels (46).

The protection to HBV infection appears long-lasting, though antiHBs titers decrease with time (30,32). Follow-up studies of vaccinated infants who remained at risk for HBV infection have shown that antibody levels may decline after five years but breakthrough infections are infrequent and are not clinically evident (30,32,47). Most of these infections have been detected only as seroconversions to antiHBc or as increases of antiHBs titers (30,47). Moreover, infants who initially developed a protective level of antiHBs displayed a rapid, anamnestic antibody response when given a single dose of vaccine four years after the primary series of vaccine indicating persistence of immunologic memory (48).
Few HBsAg-positive infections among immunized infants, however, were observed in Senegal, where 4 of 100 infants became carriers during 6 years of follow-up (49). These data suggest that most infants remain protected from symptomatic HBV infection even if antiHBs is no longer detectable. Booster doses of hepatitis B vaccine later in life are currently not recommended but the duration of protection has yet to be established (50).

Taken together, passive-active immunization is highly effective in preventing perinatal HBV infections; the protective efficacy seems not related to the type or dose of vaccine nor to the vaccination schedule used whereas the level of antibody response is dose and vaccine dependent.

Recently, hepatitis B "escape mutants", lacking the "a" epitope on the viral envelope were found in endemic areas in infants with apparently successful vaccination (51). Such variants are capable of replication in the presence of otherwise protective levels of antiHBs. The importance of these variants for vaccination programs on global scale is currently unknown.

References

1. Kohler PF, Dubois RS, Merill DA, Bowes WA. Prevention of chronic neonatal hepatitis B virus infection with antibody to the hepatitis B surface antigen. N Engl J Med 1974;291:1378-1380.
2. Dosik H, Jhaveri R. Prevention of neonatal hepatitis B infection by high-dose hepatitis B immune globulin. N Engl J Med 1978;298:602-603.
3. Reesink HW, Reerink-Brongers EE, Lafeber-Schut BJTh, Kalshoven-Benschop J, Brummelhuis HGJ. Prevention of chronic HBsAg carrier state in infants of HBsAg-positive mothers by hepatitis B immunoglobulin. Lancet 1979;2:436-438.
4. Beasley RP, Stevens CE. Vertical transmission of HBV and interruption with globulin.
In: Vyas GN, Cohen SN, Schmid R, (eds). Viral Hepatitis. Philadelphia, Franklin Institute Press 1978:333-345.
5. Beasley RP, Hwang LY, Lin CC, Stevens CE, Wang KY, Sun TS, Hsieh FJ, Szmuness W. Hepatitis B immune globulin (HBIG) efficacy in the interruption of perinatal transmission of hepatitis B virus carrier state. Lancet 1981;2:388-393.
6. Beasley RP, Hwang LY, Stevens CE, Lin CC, Hsieh FJ, Wang KY, Sun TS, Szmuness W. Efficacy of hepatitis B immune globulin (HBIG) for prevention of perinatal transmission of the HBV carrier state: Final report of a randomized double-blind placebo-controlled trial. Hepatology 1983;3:135-141.
7. Stevens CE, Beasley RP, Lin CC, Hwang LY, Sun TS, Hsieh FJ, Wang KY, Szmuness W. Perinatal hepatitis B virus infection: use of hepatitis B immune globulin. In: Szmuness W, Alter HJ, Maynard JE, (eds). Viral hepatitis. Philadelphia, Franklin Institute Press 1982:527-535.
8. Krugman S, Giles JP, Hammond J. Viral hepatitis, type B (MS-2 strain): studies on active immunization. JAMA 1971;217:41-45.
9. Hilleman MR, Bunyak EG, Roehm RR, Tytell AA, Bertland AU, Lampson GP. Purified and inactivated hepatitis B vaccine: progress report. Am J Med Sci 1975;270:401-404.
10. Maupas P, Chiron JP, Barin F, Coursaget P, Goudeau A, Perrin J, Denis F, Diop Mar I. Efficacy of hepatitis B vaccine in prevention of early HBsAg carrier state in children. Controlled trial in an endemic area (Senegal). Lancet 1981;1:289-292.
11. Barin F, Goudeau A, Denis F, Yvonnet B, Chiron JP, Coursaget P, Diop Mar I. Immune response in neonates to hepatitis B vaccine. Lancet 1982;1:251-253.
12. Krugman S. The newly licensed hepatitis B vaccine. Characteristics and indications for use.
JAMA 1982;247:2012-2015.
13. McAleer WJ, Buynack EB, Maigetter RZ, Wampler DE, Miller WJ, Hilleman MR. Human hepatitis-B vaccine from recombinant yeast. Nature 1984;307:178-180.
14. Tada H, Yanagida M, Mishina J, Fujii T, Baba K, Ishikawa S, Aihara S, Tsuda F, Miyakawa Y, Mayumi M. Combined passive and active immunization for preventing perinatal transmission of hepatitis B virus carrier state. Pediatrics 1982;70:613-619.
15. Beasley RP, Hwang LY, Lee GC, Lan CC, Roan CH, Huang FY, Chen CL. Prevention of perinatally transmitted hepatitis B virus infection with hepatitis B immuno globulin and hepatitis B vaccine.
Lancet 1983;2:1099-1102.
16. Wong VCW, Ip HMH, Reesink HW, Lelie PN, Reerink-Brongers EE, Yeung CY, Ma HK. Prevention of the HBsAg carrier state in newborn infants of mothers who are chronic carriers of HBsAg and HBeAg by administration of hepatitis B vaccine and hepatitis B immunoglobulin. Double-blind placebo-controlled study. Lancet 1984;1:921-926.

17. Xu, ZY, Liu CB, Francis DP, Purcell RH, Gun ZL, Duan SC, Chen RJ, Margolis HS, Huang CH, Maynard JE and the United States-China Cooperative Study Group on Hepatitis B. Prevention of perinatal acquisition of hepatitis B virus carriage using vaccine: preliminary report of a randomized, double-blind placebo-controlled and comparative trial. Pediatrics 1985;76:713-718.
18 Stevens CE, Toy PT, Tong MJ, Taylor PE, Vyas GN, Nair PV, Gudavalli M, Krugman S. Perinatal hepatitis B virus transmission in the United States. Prevention by passive-active immunization. JAMA 1985;253:1740-1745.
19. Lo KJ, Tsai YT, Lee SD, Wu TC, Wang JY, Chen GH, Yeh CL, Chiang BN, Yeh SH, Goudeau A, Coursaget P, Tong MJ. Immunoprophylaxis of infection with hepatitis B virus in infants born to hepatitis B surface antigen-positive carrier mothers. J Infect Dis 1985;152:817-822.
20. Reddy DN, Dilawari JB. Vaccine protection against the neonatal HBsAg carrier state. Lancet 1984;1:68.
21. Ip HMH, Lelie PN, Wong VCW, Kuhns MC, Reesink HW. Prevention of hepatitis B virus carrier state in infants according to maternal serum levels of HBV DNA. Lancet 1989;1:406-410.
22. Hollinger FB. Factors influencing the immune response to hepatitis B vaccine, booster dose guidelines and vaccine protocol recommendations. Am J Med 1989;87(suppl 3A):36-40.
23. McLean AA, Hilleman MR, McAleer WJ, Buynak EB. Summary of world wide experience with H-B-vax (B MSD). J Infect 1983;7(suppl):95-104.
24. Stevens CE, Taylor PE, Tong MJ, Toy PT, Vyas GN, Nair PV, Weissman JY, Krugman S. Yeast-recombinant hepatitis B vaccine: efficacy with hepatitis B immune globulin in prevention of perinatal hepatitis B virus transmission. JAMA 1987;257:2612-2616.
25. Poovorawan Y, Sanpavat S, Pongpunlert W, Chumdermpadetsuk S, Sentrakul P, Safary A. Protective efficacy of a recombinant DNA hepatitis B vaccine in neonates of HBe antigen-positive mothers. JAMA 1989;261:3278-3281.
26. Szmuness W, Stevens CE, Zang EA, Harley EJ, Kellner A. A controlled clinical trial of the efficacy of the hepatitis B vaccine (Hepatavax B): a final report. Hepatology 1981;5:377-385.
27. Panda SK, Ramesh R, Rao KVS, Zuckerman AJ, Nayak NC. Comparative evaluation of the immunogenicity of yeast-derived (recombinant) and plasma-derived hepatitis B vaccine in infants. J Med Virol 1991;35:297-302.
28. Goh KT, Tan KL, Kong KH, Oon CJ, Chan SH. Comparison of the immune response of four different dosages of a yeast-recombinant hepatitis B vaccine in Singapore children: a four-year follow-up study. Bull WHO 1992;70:233-239.
29. Lee CY, Huang LM, Chang MH, Hsu CY, Wu SJ, Sung JL, Safary A. The protective efficacy of recombinant hepatitis B vaccine in newborn infants of hepatitis B e antigen-positive-hepatitis B antigen carrier mothers. Ped Inf Dis J 1991;10:299-303.
30. Lo KJ, Lee SD, Tsai YT, Wu TC, Chan CY, Chen GH, Yeh CL. Long-term immunogenicity and efficacy of hepatitis B vaccine in infants born to HBeAg-positive HBsAg-carrier mothers. Hepatology 1988;8:1647-1650.
31. Piazza M, Picciott L, Villari R, Guadagnino V, Orlando R, Isabella L, Macchia V, Memoli AM, Vegnente A, Borrelli AM, Scarcella A, Cascioli C, Cirillo C, Coppola P, Isabella E, Parisi G. Hepatitis B immunisation with a reduced number of doses in newborn babies and children. Lancet 1985;1:949-951.
32. Poovorawan Y, Sanpavat S, Pongpunlert W, Chumdermpadetsuk S, Sentralkul P, Vandepapelière P, Safary A. Long term efficacy of hepatitis B vaccine in infants born to hepatitis B e antigen-positive mothers. Ped Infect Dis J 1992;11:816-821.
33. Canho del R, Grosheide PM, Voogd M, Huisman WM, Heijtink RA, Schalm SW. Immunogenicity of 20 µg of recombinant DNA hepatitis B vaccine in healthy neonates: a comparison of three different vaccination schemes. J Med Virol 1993;41:30-34.
34. Mazel JA, Schalm SW, de Gast GC, Nuijten ASM, Heijtink RA, Botman MJ, Bänffer JRJ, Gerards LJ, Zwijnenberg J, Mettau J, Wladimiroff JW, Fetter WPF. Passive-active immunization of neonates of HBsAg positive carrier mothers: preliminary observations. Br Med J 1984;288:513-515.

35. Schalm SW, Mazel JA, Gast GC de, Heijtink RA, Botman MJ, Bänffer JRJ, Gerards LJ, Zwijnenberg J, Fetter WPF, Nuijten ASM, Wladimiroff JW, Christiaens GCML. Prevention of hepatitis B infection in newborns through mass screening and delayed vaccination of all infants of mothers with hepatitis B surface antigen. Pediatrics 1989;83:1041-1047.
36. Stevens CE, Toy PT, Taylor PE, Lee Th, Yip HY. Prospects for control of hepatitis B virus infection: implications of childhood vaccination and long-term protection. Pediatrics 1992;90:170-173.
37. Chiron JP, Coursaget P, Yvonnet B, Auger F, Quan TL, Barin F, Denis F, Diop-Mar I. Simultaneous administration of hepatitis B and diphtheria/tetanus/polio vaccines. Lancet 1984;1:623-624.
38. Coursaget P, Yvonnet B, Relyveld EH, Barres JL, Diop-Mar I, Chiron JP. Simultaneous administration of diphtheria-tetanus-pertussis-polio and hepatitis B vaccines in a simplified immunization program: immune response to diphtheria toxoid, tetanus toxoid, pertussis, and hepatitis B surface antigen.
Infect Immun 1986;51:784-787.
39. Giammanco G, Volti SL, Mauro L, Bilancai GG, Salemi I, Barone P, Musumeci S. Immune response to simultaneous administration of a recombinant DNA hepatitis B vaccine and multiple compulsory vaccines in infancy. Vaccine 1991;9:747-750.
40. Poovorawan Y, Theamboonlers A, Sanpavat S, Pongpunlert W, Chumdermpadetsuk S, Vandepapelière P, Safary A. Reactogenicity and immunogenicity of combined tetravalent DTP and hepatitis B vaccine in infants. 8th International Symposium on Viral Hepatitis and Liver Disease 1993;Abstract 257.
41. Canho R del, Grosheide PM, Gerards LJ, Heijtink RA, Schalm SW. Hepatitis B vaccination and preterm infants. Ped Inf Dis J 1993;12:407-408.
42. Callis LM, Clanxet J, Fortuny G, Caballeria J, Carrasco JL, Lardinois R. Hepatitis B infection and vaccination in children undergoing haemodialysis. Acta Paediatr Scand 1985;74:213-218.
43. Entacher U, Jürgenssen O, Thun-Hohenstein L, Simbruner G, Khoss A, Wank H, Neuwirth G, Gadner H, Frisch-Niggemeyer W. Hepatitis B vaccination and immune response in children with malignant diseases. Eur J Pediatr 1985;144:160-163.
44. Zuin G, Principi N, Tornaghi R, Paccagnini S, Re M, Massironi E, Ragni MC. Impaired response to hepatitis B vaccine in HIV infected children. Vaccine 1992;10:857-860.
45. Alper CA, Kruskall MS, Marcus-Bagley D, Craven DE, Katz AJ, Brink SJ, Dienstag JL, Awdeh Z, Yunis EJ. Genetic prediction of nonresponse to hepatitis B vaccine. N Engl J Med 1989;321:708-712.
46. Canho del R, Schalm SW, Heijtink RA. Hepatitis B revaccination of neonates with inadequate response after primovaccination. Vaccine 1992;10:69.
47. Whittle HC, Inskip H, Hall AJ, Mendy M, Downes R, Hoare S. Vaccination against hepatitis B and protection against chronic viral carriage in The Gambia. Lancet 1991;1:747-750.
48. Moyes CD, Milne A, Waldon J. Very low dose hepatitis B vaccination in the newborn: anamnestic response to a booster at four years. J Med Virol 1990;30:216-218.
49. Coursaget P, Yvonnet B, Chotard J, Sarr M, Vincelot P, N'doye R, Diop-Mar I, Chiron JP. Seven-year study of hepatitis B vaccine efficacy in infants from an endemic area (Senegal). Lancet 1986;2:1143-1145.
50. Hadler SC. Are booster doses of hepatitis B vaccine necessary? Ann Intern Med 1988;108:457-458.
51. Carman WF, Zanetti AR, Karayannis P, Waters J, Manzillo G, Tanzi E, Zuckerman AJ, Thomas HC. Vaccine-induced escape mutant of hepatitis B virus. Lancet 1990;336:325-329.

CHAPTER 3.2

PASSIVE-ACTIVE IMMUNIZATION IN INFANTS OF HEPATITIS B e ANTIGEN-POSITIVE MOTHERS: COMPARISON OF THE EFFICACY OF EARLY AND DELAYED ACTIVE IMMUNIZATION

P.M. Grosheide[1], R. del Canho[1], R.A. Heijtink[2], A.S.M. Nuijten[3], J. Zwijnenberg[4], J.R.J. Bänffer[5], J.W. Wladimiroff[6], M.J. Botman[7], J.A. Mazel[8], G.C. de Gast[9], G.C.M.L. Christiaens[10], L.J. Gerards[11], W.P.F. Fetter[12], W. Baerts[12] and S.W. Schalm[1]

Department of Internal Medicine II[1], Academic Hospital Dijkzigt, Department of Virology[2], Erasmus University, State School of Training for Midwives[3], Department of Paediatrics[4], Ruwaard van Putten Hospital, Regional Public Health Laboratory[5], Department of Obstetrics and Gynaecology[6], Academic Hospital Dijkzigt, Rotterdam; Regional Laboratory of Pathology and Microbiology[7], Enschede, General Practitioner[8], Borne; Blood Transfusion Laboratory[9], Department of Obstetrics and Gynaecology[10], Academic Hospital Utrecht, Wilhelmina Childrens Hospital[11], Utrecht; Department of Paediatrics, St Sophia Hospital[12], Zwolle, the Netherlands.

American Journal of Diseases of Children, in press.

SUMMARY

To assess the efficacy of late active immunization against hepatitis B concomitant with diphtheria, pertussis, tetanus and polio vaccine, a randomized study was conducted among infants born to hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg)-positive mothers in three large city hospitals and one rural area providing prenatal care and obstetric services.

Eighty infants of HBsAg- and HBeAg-positive carrier mothers received 0.5 ml/kg of body weight hepatitis B immune globulin within two hours of birth and hepatitis B vaccine (10 μg) at 0, 1, 2, and 11 months of age (group A) or at 3, 4, 5, and 11 months of age concomitant with diphtheria, pertussis, tetanus and polio immunization (group B). A second dose of hepatitis B immune globulin was given to infants on schedule B at 3 months. Blood samples were collected at 0, 3, 6, 11, and 12 months of age and tested for antibodies to hepatitis B core antigen (antiHBc) and HBsAg. Follow-up visits were scheduled annually up to 5 years of age.

Eight infants were excluded from analysis. During the study period six children became HBsAg carriers, three in each group, which corresponds to a five-year incidence of infection of 9 and 8% for groups A (three of 35) and B (three of 37), respectively. Subclinical infections (persistent antiHBc positivity beyond month 12 or appearance of antiHBc) were encountered in another eight infants (four in each group).

Late active immunization starting at 3 months of age appears to provide similar protective efficacy as active immunization starting at birth when combined with hepatitis B immune globulin at 0 and 3 months of age.

INTRODUCTION

The current goal of immunoprophylaxis for newborns of mothers positive for hepatitis B surface antigen (HBsAg) is prevention of the chronic hepatitis B carrier state. In June 1982 plasma-derived hepatitis B vaccine was licensed in the Netherlands and in July 1982 a program was initiated to determine a practical and effective immunization schedule for the prevention of hepatitis B in neonates of HBsAg-positive mothers. Because more than 95% of the relevant population participates in the national immunization program in the Netherlands, incorporation of hepatitis B vaccine into the diphtheria, tetanus, pertussis (DTP) and polio vaccination program is presumed to yield the highest compliance and the lowest costs. Therefore we investigated two schedules of passive-active immunization to find out whether the simultaneous injection of hepatitis B vaccine and DTP-polio vaccine (delayed active immunization) provided the same protection against perinatal infections from HBsAg- and hepatitis B e antigen (HBeAg)-positive carrier mothers as early active immunization starting directly after birth.

Preliminary results of this study were reported earlier (1-2). Then, 34 infants of HBsAg-

and HBeAg-positive mothers had entered the study. The final results on the protective efficacy of passive-active immunization in 80 high-risk infants after a follow-up of five years are reported herein.

PATIENTS AND METHODS

Participating centers

The study was performed in four centers in the Netherlands: three large city hospitals in Utrecht (n=1) and Rotterdam (n=2) and the rural area Twente-Gelderse Achterhoek where the number of home deliveries is high.

Ethics

Early in 1982 permission for the study was obtained from the local medical ethics committees.

Hepatitis B screening

In July 1982 HBsAg screening of pregnant women was started in the four centers. Blood samples obtained from all pregnant women during their first visit to the prenatal clinic of the participating centers were tested for the presence of HBsAg. Pregnant women with a positive test during the initial visit underwent a repeated test for HBsAg at week 28 of pregnancy. A woman was considered an HBsAg-positive carrier if the repeated test was positive for HBsAg. At the prenatal visit following the diagnosis of HBsAg carriership, the mother was informed about the immunization study program. Informed consent was obtained from the mother and information on country of birth and parity was noted in the woman's file (baseline characteristics). Randomization for either early or delayed active immunization took place at the local center.

At delivery an additional blood sample was obtained from the mother to verify the eligibility of her infant for the study. When a pregnant woman came to the two participating hospitals in Rotterdam for delivery and no HBsAg test result from prenatal visits was available, a rapid HBsAg test was performed. Results were available the next day before hospital discharge of the mother. If the rapid HBsAg test was positive, the mother was asked for informed consent and her baby was randomized.

All pregnant women who were positive for HBsAg were also tested for the presence of HBeAg. The enrollment of infants born to HBsAg- and HBeAg-positive mothers closed December 31, 1987.

Immunization schedules

Infants received an intramuscular injection of 0.5 ml/kg of body weight of hepatitis B immune globulin (HBIg) within 2 hours of birth except for infants of perinatally detected HBsAg carrier mothers who received HBIg as soon as possible. HBIg prepared by the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, contained 100 to 150 international units of antibodies to the hepatitis B surface antigen (antiHBs) per milliliter and was stored at 2°C to 8°C. The HBIg was given intramuscularly in the anterolateral region of the thigh by the individual assisting in the delivery.

For active immunization, infants were referred to a pediatrician. Infants on schedule A received 0.5 ml (10 μg) of plasma hepatitis B vaccine (HBvax) intramuscularly in the anterolateral thigh within two days of birth and 1, 2, and 11 months later, whereas those on schedule B received an equal dose of vaccine at 3, 4, 5, and 11 months of age. The vaccine (Merck Sharp & Dohme, West Point, Pa, USA) contained 20 μg/ml of surface antigen and was stored at 2°C to 8°C. A second dose of HBIg (100 to 150 IU of antiHBs/ml) was given at 3 months to infants receiving delayed active immunization according to schedule B.

Blood sampling

Immediately after delivery, cord blood was obtained from all infants. Follow-up blood samples were obtained at 3, 6, 11, and 12 months of age and then annually for at least five years.

Laboratory

Serum samples obtained from mothers during the study in Utrecht and Rotterdam (the Netherlands) were assayed for HBsAg by radioimmunoassay (Ausria II, Abbott Laboratories, Chicago, Ill, USA). The reversed passive hemagglutination assay (Auscell, Abbott) was used for initial screening in the Twente-Gelderse Achterhoek area and for rapid HBsAg testing in Rotterdam. All positive samples detected by the reversed passive hemagglutination assay were confirmed by radioimmunoassay.

When a mother was HBsAg-positive, the HBeAg status was assessed (Abbott-HBe). Serum samples from infants were assayed for antiHBs by radioimmunoassay (Ausab, Abbott). The results were expressed in international units per liter (IU/l) with the aid of the World Health Organization reference serum. Samples obtained at birth and 12 months of age, as well as the annual samples, were also tested for antibodies to hepatitis B core antigen (Corab, Abbott). The presence of HBsAg was assayed in all samples with antiHBs values below 100 IU/l.

Hepatitis B infections

Among infants, two types of hepatitis B infection were registered after birth. A hepatitis B virus (HBV) infection was considered to have occurred if an infant became positive for HBsAg (cord blood excluded) or antiHBc without HBsAg was observed after month 12 (3-4). AntiHBc positivity had to be present on two or more consecutive occasions to rule out false low-level antiHBc reactivity. AntiHBc positivity without HBsAg at month 12 was considered to be due to maternal transmission of antiHBc.
AntiHBcIgM was not considered to be a representative marker of acute hepatitis B infection since infants of HBsAg carrier mothers lacked this marker when infected perinatally (5). An increase in the antiHBs level alone was not considered an HBV infection. An infant who was HBsAg-positive for more than six months was considered to be an HBsAg carrier (3). Infants who had no HBsAg-positive serum samples but were antiHBc-positive after month 12 were considered to have had a subclinical infection with HBsAg.

Randomization

From July 1, 1982 until December 31, 1987 all HBsAg-positive mothers admitted to the study were randomly assigned to one of two treatment groups. Randomization according to Peto et al. occurred in each local center separately using numbered sealed opaque envelopes (6). Each block of six envelopes contained three cards with treatment A and three cards with treatment B. Stratification according to prenatal and perinatal detection of HBsAg was applied in the hospitals in Rotterdam since strict adherence to immunization schedules could not be guaranteed for the infants of perinatally detected HBsAg-positive mothers.

Statistics

The number of infants born to HBeAg-positive HBsAg carrier mothers available during the enrollment period determined the sample size.

The degree of similarity between the two treatment groups was demonstrated by comparing baseline characteristics of the HBsAg- and HBeAg-positive mothers.
The baseline characteristics were compared for all mothers who agreed to treatment of their infants (n=80) and for mothers whose infants received proper treatment according to schedule A or B (n=72). Differences in proportions related to country of birth of the mothers, parity, and rapid screening between treatment groups were compared using the Chi-square test and Fisher's exact test in the case of small numbers. Median ages of mothers were compared using the Wilcoxon test. Differences in number of HBV events between groups were calculated with the Fisher's exact test and 95% confidence intervals (CI).
The exact values for 95% CI are given in Geigy Scientific Tables (7).

RESULTS

A total number of 527 pregnant women was found to be repeatedly HBsAg-positive by screening; 92 (17%) of these women were also HBeAg-positive. Eighty infants of HBsAg- and HBeAg-positive mothers were born in the study period and eligible for passive-active immunization (38 according to schedule A; 42 according to schedule B).

Eight infants (schedule A, three infants; schedule B, five infants) were excluded from further analysis: three infants never received vaccine (schedule A, one infant; schedule B, two infants), three infants received two doses of vaccine but no serum sample was available after month 0 (schedule A, two infants; schedule B, one infant), and one infant died of congenital abnormalities 10 days after birth (schedule B, one infant). One infant received the wrong immunization schedule (schedule B, one infant), but the last available blood sample tested was negative for HBsAg.

Table 1

Baseline characteristics of HBsAg- and HBeAg-positive mothers of infants immunized according to schedule A or B.

Mothers	Schedule A n=35	Schedule B n=37	P-value
Median age, y	23 (19;33)*	24 (17;38)	0.06†
Country of birth, No (%)			0.92‡
Netherlands + other	3 (8.6)	2 (5.4)	
Mediterranean	15 (42.9)	16 (43.2)	
Surinam	5 (14.3)	7 (18.9)	
Asia	12 (34.3)	12 (32.4)	
Rapid screening, No (%)	2 (5.7)	2 (5.4)	1.00§
Primigravidae, No (%)	16 (45.7)	10 (27.0)	0.10‡

* Numbers in parentheses indicate fifth to 95th percentiles
† Wilcoxon test
‡ χ^2 test
§ Fisher's exact test

In Table 1 baseline characteristics are presented for the 72 mothers whose infants received proper treatment. The baseline characteristics of these 72 mothers did not differ from those for the total group of 80 mothers originally randomized.

HBsAg positivity was found in three (9%) of 35 infants in group A (95% CI: 1.8-23.1) and three (8%) of 37 infants in group B (95% CI: 1.7-21.9). All HBsAg-positive infants

Table 2
Time at which infants who became HBsAg carriers despite passive-active immunization were first positive for HBsAg.

		HBsAg-positive at month					antiHBs in IU/l at month				
Case	Schedule	0†	3	6	11	12	0†	3	6	11	12
1	A	+	-	+	+	+	0	17	2	0	0
2	A	+	-	-	+	+	0	45	39	0	0
3	A	+	+	+	+	+	0	0	0	NT	0
4	B	+	+	+	+	+	0	0	0	NT	0
5	B	+	-	+	+	+	0	29	0	0	6
6	B	-	-	-	+	+	0	38	17	0	0

† Cord blood
NT indicates no test result available; plus sign indicates HBsAg-positive and minus sign indicates HBsAg-negative.

became carriers. The onset of HBsAg positivity in those infants who could not be protected occurred within the first 11 months of life (Table 2). Two infants were already HBsAg-positive in cord blood and thereafter. The other four carrier infants were first HBsAg-positive at 6 or 11 months of age; their antiHBs antibody levels varied from 17 IU/l to 45 IU/l at month 3 and were absent from month 6 onwards in all but one case. Infant 5 had concurrent HBsAg and antiHBs at month 12.

Four (13%) of 32 infants in group A (95% CI: 3.5-29.0) and four (12%) of 34 infants in group B (95% CI: 3.3-27.5) exhibited antiHBc beyond month 12 on at least two consecutive occasions; in all cases the development of antiHBs was normal during the first 12 months of age (Table 3). Four infants (patients 1 through 4), two in each group, were antiHBc-positive from month 12 onwards. The other four infants became antiHBc-positive after month 12. AntiHBs titers varied considerably in these antiHBc-positive infants and either increased or (slowly) decreased. Hepatitis B surface antigen was never observed in these infants.

The frequency of HBV events per group, seven (20%) of 35 infants in group A (95% CI: 8.4-36.9) and seven (19%) of 37 infants in group B (95% CI: 8.0-35.2), was not statistically different. The 95% CI for the difference between the two population proportions ranges from -17% to 19%.

The HBsAg- and HBeAg-positive mothers who infected their infants were all detected during pregnancy; they had a normal pregnancy and spontaneous delivery. The six infants who became HBsAg carriers received HBIg directly after birth and, except for patient 5 who received the second vaccination 3 weeks late, all infants were immunized according to schedule.

Table 3

Appearance/ persistence of antiHBc and antiHBs in follow-up sera from infants with subclinical infection despite passive-active immunization according to schedule A and B.

		antiHBc at month					antiHBs in IU/L at month				
Case	Schedule	12	24	36	48	60	12	24	36	48	60
1	A	+	+	+	+	NT	1299	2371	7643	4040	NT
2	A	+	+	NT	NT	+	1183	134	90	NT	34
3	B	+	+	+	+	+	559	3903	2962	1185	1037
4	B	+	+	+	+	+	25660	21555	10195	10989	NT
5	A	-	-	+	+	+	2280	332	309	301	144
6	A	-	+	+	+	+	4989	349	209	175	182
7	B	-	-	NT	+	+	2191	725	NT	1043	795
8	B	-	-	+	+	NT	898	78	110	32	NT

NT indicates no test result available; plus sign indicates antiHBc-positive, minus sign indicates antiHBc-negative.

Table 4

HBV infections (HBsAg carriers and antiHBc positivity after month 12) by study group and ethnic distribution.

	Number of infants			Number of HBV infections		
	Schedule			Schedule		
Country of birth	A	B	Total	A	B	Total
Mediterranean	15	16	31	5 (2)	6 (3)	11 (5)
Asia	12	12	24	2 (1)	1 (-)	3 (1)
Total	27	28	55	7 (3)	7 (3)	14 (6)

Numbers in parentheses indicate number of HBsAg carrier infants.

To determine whether mothers from Asia with suspected high levels of the virus (8-9) were more likely to infect their offspring, we evaluated the ethnic distribution of the HBsAg- and HBeAg-positive carrier mothers in relation to the perinatal transmission of HBV. The ethnic distribution of mothers with HBV-infected infants was similar in groups A and B (Table 4). When HBV-infected infants from the two groups were combined and analyzed according to maternal origin, differences became apparent. In contrast to our expectations, the rate of transmission of HBV for mothers of Asian origin (three [13%] of 24) tended to be lower (95% CI: 2.7-32.4) than that for mothers of Mediterranean origin (11 [36%] of 31; 95% CI: 19.2-54.6). This trend in frequency of HBV infections was just above statistical significance ($p = 0.05$; Chi-square test).

DISCUSSION

This study demonstrates that the frequency of hepatitis B antigenemia for infants of HBsAg- and HBeAg-positive mothers after delayed active immunization, starting at 3 months, is similar to that obtained with early active immunization starting directly after birth: 8% and 9%, respectively. Only six (8%) of 72 infants (95% CI: 3.1-17.3) became HBsAg carriers, a rate comparable with that found in other passive-active immunization studies with either plasma vaccine or recombinant vaccine (8,10-14). Our results are in agreement with the findings of Beasley et al. who demonstrated HBsAg carrier rates of 2% for the delayed vaccination also starting at 3 months vs 6% for the early vaccination given 4 to 7 days after birth (10).

Since the number of HBsAg-positive infants in both studies was relatively small and antiHBc is the most sensitive marker of HBV infection, we also compared the number of antiHBc-positive infants without HBsAg in the two groups. AntiHBc positivity was found for eight infants, four in each group. All subclinical infections were observed in infants with an active immune response to vaccine; none of these infants had development of the carrier state or became HBsAg-positive. This indicates that infants with an adequate initial antiHBs response remained protected against HBV carrier state for at least five years. After the follow-up period of five years, the total number of hepatitis B infections was similar for both groups; seven (20%) in group A and seven (19%) in group B. The 95% CI for the difference between the two groups ranges from -17% to 19%, showing the imprecision due to the limited sample size. Further studies with larger numbers of neonates will be needed to confirm these results, although the low number of HBV infections suggests no major clinical relevance.

It is unlikely that the HBV infections were generated by vaccine-induced escape mutants (seen in Mediterranean countries) since these mutants are characterized by HBsAg and concomitant adequate levels of nonneutralizing antiHBs (15).

In the Netherlands, where prenatal screening of pregnant women is current policy, immunoprophylaxis with vaccine alone is not considered and HBIg is always given at birth.

The cardinal question then becomes what vaccination schedule is feasible with high compliance and what schedule is most effective? We tested one schedule we thought would be the most effective (0, 1, 2, and 11 months) and one schedule we thought would be associated with highest compliance (3, 4, 5, and 11 months). The results showed no difference in efficacy, confirming the original observations by Beasley et al. (10), and higher immunogenicity (2). In this setting, the compliance with both schedules was similar but early active immunization does not correspond to the routinely scheduled immunization visits in practice.

In many countries hepatitis B immunization starting immediately after birth is advocated. Although this approach is scientifically well founded, both the compliance related to multiple injections and the costs related to additional physician visits might interfere with high compliance to the vaccination programs. In our opinion, both our study and the study of Beasley et al. demonstrate that the timing of the start of active immunization within the period of 0 to 3 months after birth is of minor importance for neonates receiving HBIg directly after birth (10). This conclusion is of considerable importance when designing strategies for HBV immunization programs with a high degree of compliance.

Current experience suggests that high compliance is difficult to achieve with many injections on separate months (16-17). In Italy, where mass vaccination against hepatitis B was recently introduced, the highest compliance rate was observed in the babies who received hepatitis B vaccination at the same time as the mandatory childhood vaccinations (98%), whereas it was 80% in babies who received the vaccines separately (16).

An extra dose of vaccine at month 0 with no proven benefit would mean additional effort and cost. Conversely, data on the immunogenicity and efficacy of a vaccination schedule starting at birth with a second dose at month 3 are unavailable. Increasing the interval between the first and the second dose of vaccine beyond the recommended interval of four weeks has been reported to decrease the antibody response (18).

If the hepatitis B vaccine could be given at the same time as DTP or DTP-polio immunization, the number of physician visits could be reduced; in addition, the number of injections could be reduced if hepatitis B vaccine could be incorporated into the DTP vaccine.

Furthermore, the influence of the vaccination schedule is demonstrated by significantly higher antiHBs response in infants receiving late active immunization (1-2). Age at the first injection of vaccine is a matter of controversy because, on the one hand it is important to protect the neonates as early as possible, delivery being the most crucial time to get HBV infection; on the other hand, the physiological immunosuppression of newborns may produce a less effective response to vaccination.

A potential drawback of delayed active immunization may be the need for an additional HBIg injection, as was given to infants in our study as well as in the study of Beasley et al. (10). The need for a second dose of HBIg with delayed vaccination, however, has not been established. In fact, an efficacy study showed no evidence of enhanced protection when an

additional dose of HBIg is used (Grosheide et al.: unpublished data). It seems reasonable to expect that active immunity will develop rapidly enough to eliminate the need for subsequent doses of HBIg (19).

In the Netherlands, a low prevalence area with a high percentage of home deliveries, vaccination starting directly after birth does not fit easily into the child care program; DTP-polio injections are given at the ages of 3, 4, 5, and 11 months. Passive immunization of infants of HBsAg-positive mothers immediately after birth by the midwife or obstetrician, followed by active immunization by the physician responsible for routine infant immunization, appears feasible. Because compliance to DTP-polio vaccination in the Netherlands reaches 95%, simultaneous administration of the hepatitis B vaccine with DTP-polio vaccination is likely to be associated with a similar high compliance for hepatitis B. Evaluation of infant immunization would occur within the existing health care system. At virtually no cost and without extra effort, infants can be immunized against hepatitis B. The results of this study open the way for general application of hepatitis B vaccine, incorporated into a DTP vaccine, in countries that provide hepatitis B immune globulin prophylaxis at birth to high-risk neonates.

Acknowledgments

We thank Marja Bakker-Bendik, Thea van der Laar-Kokx, Harriet Ribbers-Smeenk, Hetty van Dijk, Anneke Deurloo-Volaard and Marja Voogd-Schotanus who made this study their fulltime professional effort for several years. Special thanks go to J Boot, WCJ Hop (B Sc) and JR Klokman-Houweling for their technical assistance. This study was supported by a grant from the Praeventiefonds, the Hague, the Netherlands.

References

1. Mazel JA, Schalm SW, Gast de GC, Nuijten ASM, Heijtink RA, Botman MJ, Bänffer JRJ, Gerards LJ, Zwijnenberg J, Mettau J, Wladimiroff JW, Fetter WPF. Passive-active immunization of neonates of HBsAg-positive carrier mothers: Preliminary observations. Br Med J 1984;288:513-515.

2. Schalm SW, Mazel JA, Gast de GC, Heijtink RA, Botman MJ, Bänffer JRJ, Gerards LJ, Zwijnenberg J, Fetter WPF, Nuijten ASM, Wladimiroff JW, Christiaens GCML. Prevention of hepatitis B infection in newborns through mass screening and delayed vaccination of all infants of mothers with hepatitis B surface antigen. Pediatrics 1989;83:1041-1047.

3. Beasley RP, Hwang LY, Lin CC, Stevens CE, Wang KY, Sun TS, Hsieh FJ, Szmuness W. Hepatitis B immune globulin (HBIG) efficacy in the interruption of perinatal transmission of hepatitis B virus carrier state. Lancet 1981;2:388-393.

4. Szmuness W, Stevens CE, Harley EJ, Zang EA, Alter HJ, Taylor PE, DeVera A, Chen GTS, Kellner A, the dialysis vaccine trial study group. Hepatitis B vaccine in medical staff of hemodialysis units. Efficacy and subtype cross-protection. N Engl J Med 1982;307:1481-1486.

5. Goudeau A, Yvonnet B, Lesage G, Barin F, Denis F, Coursaget P, Chiron JP, Diop Mar I. Lack of anti-HBc IgM in neonates with HBsAg carrier mothers argues against transplacental transmission of hepatitis B virus infection. Lancet 1983;2:1103-1104.

6. Peto R, Pike MC, Armitage P, Breslow NE, Cox DR, Howard SV, Mantel N, McPherson K, Peto J, Smith PG. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. Br J Cancer 1976;34:585-612.

7. Lenter C, (ed). Geigy Scientific Tables. 8th ed. Basel, Switzerland: Geigy;1982:89-102.

8. Wong VCW, Ip HMH, Reesink HW, Lelie PN, Reerink-Brongers EE, Yeung CY, Ma HK. Prevention of the HBsAg carrier status in newborn infants in mothers who are chronic carriers of HBsAg and HBeAg by administration of hepatitis B vaccine and hepatitis B immune globulin: Double-blind randomized placebo-controlled study. Lancet 1984;1:921-926.
9. Beasley RP, Hwang LY, Stevens CE, Lin CC, Hsieh FJ, Wang KY, Sun TS, Szmuness W. Efficacy of hepatitis B immune globulin for prevention of perinatal transmission of the hepatitis B virus carrier state: final report of a randomized double-blind, placebo-controlled trial. Hepatology 1983;3:135-141.
10. Beasley RP, Hwang LY, Lee GCY, Lan CC, Roan CH, Huang FY, Chen CL. Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. Lancet 1983;2:1099-1102.
11. Stevens CE, Taylor PE, Tong MJ, Toy PT, Vyas GN, Nair PV, Weissman JY, Krugman S. Yeast-recombinant hepatitis B vaccine. Efficacy with hepatitis B immune globulin in prevention of perinatal hepatitis B virus transmission. JAMA 1987;257:2612-2616.
12. Poovorawan Y, Sanpavat S, Pongpuniert W, Chumdermpadetsuk S, Sentrakul P, Safary A. Protective efficacy of a recombinant DNA hepatitis B vaccine in neonates of HBe antigen-positive mothers. JAMA 1989;261:3278-3281.
13. Lee CY, Huang LM, Chang MH, Hsu EY, Wu SY, Sung JL, Safary A. The protective efficacy of recombinant hepatitis B vaccine in newborn infants of hepatitis B e antigen-positive-hepatitis B surface antigen carrier mothers. Pediatr Infect Dis J 1991;10:299-303.
14. Ip HMH, Lelie PN, Wong VCW, Mimms L, Reesink HW. Prevention of the HBV-carrier state in infants of HBsAg- and HBeAg-positive mothers with high and low serum levels of HBV-DNA. A 3 year placebo-controlled study comparing the efficacy of hepatitis B vaccine with and without hepatitis B immunoglobulin. Lancet 1989;1:406-410.
15. Carman WF, Zanetti AR, Karayannis P, Waters J, Manzillo G, Tanzi E, Zuckerman AJ, Thomas HC. Vaccine-induced escape mutant of hepatitis B virus. Lancet 1990;336:325-329.
16. Da Villa G, Piazza RI, Picciotto L, Peluso P, Luca de G, Basile B. A pilot model of vaccination against hepatitis B virus suitable for mass vaccination campaigns in hyperendemic areas. J Med Vir 1992;36:274-278.
17. Stroffolini T, Pasquini P and collaborating group. Five years of vaccination campaign against hepatitis B in Italy in infants of hepatitis B surface antigen carrier mothers. Ital J Gastroenterol 1990;22:195-197.
18. Inskip HM, Hall AJ, Chotard J, Loik F, Whittle H. Hepatitis B vaccine in the Gambian expanded programme on immunization: factors influencing antibody response. Int J Epidemiol 1991;20:764-769.
19. Prevention of perinatally transmitted hepatitis B infection. Lancet 1984;1:939-941. Editorial.

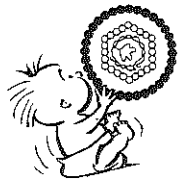
CHAPTER 3.3

ANTIHBs LEVELS IN INFANTS OF HEPATITIS B CARRIER MOTHERS AFTER DELAYED ACTIVE IMMUNIZATION WITH RECOMBINANT VACCINE CONCOMITANT WITH DTP-polio VACCINE: IS THERE NEED FOR A SECOND DOSE OF HBIg?

P.M. Grosheide[1], R. del Canho[1], M. Voogd[1], R.A. Heijtink[2], S.W. Schalm[1] and the Dutch Study Group Prevention Neonatal Hepatitis B

Department of Internal Medicine II[1], University Hospital Dijkzigt and Department of Virology[2], Erasmus University, Rotterdam, the Netherlands.

Accepted for publication in Vaccine.



SUMMARY

The need for the additional dose of HBIg was studied by comparing infants receiving 1 ml HBIg at birth followed by hepatitis B immunization, concomitant with DTP-polio vaccine, at 3, 4, 5, and 11 months (schedule E) to infants receiving the same schedule with additional HBIg at 3 months (schedule F). The immune response to recombinant hepatitis B vaccine (20 μg) was evaluated in 195 infants born to HBsAg-positive mothers allocated to groups E and F and compared to historic controls who received plasma vaccine (10 μg) according to schedule F. Blood samples were drawn at 0, 3, 4, 6, 11, 12, and 24 months of age.

No difference in the efficacy between both schedules was observed; 8% and 6% of infants born to HBeAg-positive HBsAg carrier mothers in groups E and F became HBsAg carriers, respectively. Passively acquired antibodies at birth remained present for about 5 months in most infants. The seroprotection rates (antiHBs $\geq$ 10 IU/l) were over 90% at all instants and similar for groups E and F. The titers of antiHBs attained during the first 6 months were statistically lower ($p \leq 0.02$) for group E than for group F but similar thereafter. AntiHBs titers in infants receiving the recombinant vaccine were significantly lower than in infants receiving the plasma vaccine ($p << 0.001$).

Supplemental doses of HBIg in infants receiving a high dose of HBIg (1 ml) at birth and the first dose of vaccine at the age of 3 months is not advised.

INTRODUCTION

In a large Dutch study a schedule of passive immunization starting within 2 hours after birth and active immunization with plasma-derived vaccine at 3, 4, 5, and 11 months prevented significant hepatitis B virus infection in infants of hepatitis B surface antigen (HBsAg) carrier mothers (1-3). The delayed active hepatitis B immunization, starting at 3 months of age concomitant with DTP-polio vaccine, had a similar protective efficacy as the generally recommended schedule of immunization starting immediately after birth (0, 1, 2, 11 months) but gave rise to higher antiHBs titers (2-3). Infants on the delayed immunization schedule received a second dose of antiHBs immune globulin (HBIg) at 3 months of age, at the same time active immunization with the plasma-derived vaccine was started.

For practical and economic reasons, the simultaneous administration of DTP-polio vaccine and the hepatitis B vaccine was accepted in the Netherlands as the regimen of choice for the prevention of perinatal hepatitis B. However, in view of the levels of antiHBs observed at 3 months of age the need for the second dose of HBIg is uncertain (2-4). Practical and economic reasons strongly favour the elimination of the HBIg injection at 3 months of age. The present study examines the antiHBs levels in infants of HBsAg-positive mothers receiving a high dose of HBIg at birth and recombinant hepatitis B vaccine at 3, 4, 5, and 11 months, either with or without a second dose of HBIg at 3 months of age.

To confirm evidence that the immune response to the yeast-derived recombinant hepatitis B vaccine is similar to the response to the plasma-derived vaccine, we also compared the antiHBs levels of infants receiving recombinant vaccine to the results found previously in infants using the same immunization regimens with the Merck plasma vaccine (2,5).

PATIENTS AND METHODS

Procedure

The study population consisted of healthy infants born to HBsAg-positive carrier mothers in three large city hospitals in Utrecht (n=1), in Rotterdam (n=2), and one rural area providing prenatal and obstetric services. Entry to the study which was approved by the medical ethics committees at each of the four participating centers started January 1, 1988 and ended on October 1, 1989.

All pregnant women who attended the prenatal clinic at one of the participating centers were screened for the presence of HBsAg during their first visit. Pregnant women with a positive test result from the initial visit underwent a repeated test for HBsAg at delivery to verify the eligibility of infants for the study. Pregnant women who were positive for HBsAg were also tested for the presence of HBeAg. At the prenatal visit following the diagnosis of HBsAg positivity, the mother was informed about the immunization study program. Informed consent was obtained from the mother for the participation of her infant. Each infant received 1 ml injection of HBIg intramuscularly within two hours of birth. After referral to the pediatrician, infants in Rotterdam were given 1 ml of recombinant vaccine at 3, 4, 5, and 11 months of age (group E). Infants born in Utrecht and the rural area were assigned to group F and were given the same schedule as infants in group E, but received an

	↓	↓	↓		↓	↓	↓	↓	
group E	I	V	V	V		V			
group F	I	I+V	V	V		V			
	0	3	4	5	6	11	12	24	months

Figure 1
Passive-active immunization schedule: I=HBIg administered (200-250 IU antiHBs/ml); V=vaccine administered (20 µg Engerix-B/ml); ↓=blood sample taken.
(Historic controls received 10 µg plasma vaccine (HBvax) according to schedule F).

additional dose of 1 ml HBIg at 3 months of age. The parent or guardian was asked to record any local or systemic reaction for 5 days after each vaccine injection.

Immediately after delivery cord blood was obtained after cleansing of the umbilical cord. Follow-up blood samples were taken at 3, 4, 6, and 11 months and at the age of one and two years (Figure 1).

Infants of HBsAg-positive mothers on the same schedule of passive-active immunization as the infants in group F, but who received 10 µg of the plasma-derived vaccine (HBvax; Merck Sharp & Dohme 20 µg/ml) served as historic controls. Results of the study on the protective efficacy and immunogenicity of the plasma-derived hepatitis B vaccine in infants of HBsAg-positive mothers have been reported previously (1-3).

Laboratory

All serum samples obtained from mothers during the course of the study were tested for HBsAg and HBeAg by radioimmunoassay (Abbott Laboratories, Chicago, Ill, USA). Serum samples from infants were assayed for antiHBs by radioimmunoassay (Abbott). The results were expressed in international units per liter (IU/l). Blood samples obtained at 12 and 24 months of age were also assayed for antiHBc and in cases where antiHBs titers had dropped below 100 IU/l for HBsAg.

Hepatitis B immune globulin

Hepatitis B immune globulin was prepared by the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam. The HBIg was supplied in vials of 1 ml and stored at 2°C to 8°C. The full dose of HBIg, 1 ml containing 200 to 250 IU antiHBs, was given intramuscularly in the anterolateral region of the thigh.

Vaccine

The recombinant hepatitis B vaccine, alum adsorbed, prepared by SmithKline Biologicals, Rixenart, Belgium, was used. The vaccine was stored at 2°C to 8°C. The vaccine dose of 1 ml (20 µg) was given intramuscularly in the anterolateral part of the other thigh in cases of concomitant injection of HBIg.

Statistics

The degree of similarity between the two treatment groups was demonstrated by comparing baseline characteristics (e.g. parity, age) of the HBsAg-positive mothers. Differences in proportions were compared using the Chi-square test and Fisher's exact test. Median ages of mothers were compared using the Wilcoxon test. Differences in antiHBs seroconversion rates between treatment groups were calculated with the Fisher's exact test

and 95% confidence intervals (95% CI). The exact values for 95% CI are given in Geigy Scientific Tables (6). The geometric mean titers (GMTs) were calculated only for those infants who had antiHBs ≥ 10 IU/l. AntiHBs levels were compared using the Wilcoxon Rank sum test. The Wilcoxon Mann Whitney test was used to compare the median immunization intervals between groups.

RESULTS

Participants

During the study period 210 infants were born to the mothers who had agreed to participate in the study. Nine infants were withdrawn from the trial by their parents before the treatment had started. 201 infants entered the study of whom 6 infants, three in each group, were excluded from the final analysis because they received a wrong vaccination schedule. In all 6 infants the last available blood sample tested was negative for HBsAg.

Results for the 195 infants who were studied for at least 6 months are presented; 102 were on schedule E and 93 on schedule F. In Table 1 comparisons between treatment groups are presented.

In both groups 97% of infants received HBIg at birth and all injections: 99 infants in group E and 90 infants in group F. Blood samples at 12 and 24 months of age were taken

Table 1

Comparison of the characteristics between study groups.

Mothers	Schedule E n=102	Schedule F n=93	P-value
Median age, y	26 (18;38)*	27 (18;40)	0.86†
Country of birth, No (%)			0.002‡
Netherlands + other	27 (26)	12 (13)	
Mediterranean	48 (47)	64 (69)	
Surinam	14 (14)	3 (3)	
Asia	13 (13)	14 (15)	
HBeAg-positive, No (%)	13 (13)	17 (18)	0.28‡
Primigravidae, No (%)	36 (35)	26 (28)	0.27‡

* Numbers in parentheses indicate fifth to 95th percentiles.
† Wilcoxon test
‡ χ^2 test

from 88%, or 86%, of infants in group E and 74%, or 73%, of infants in group F.

AntiHBs response

The distribution of the antiHBs titers of infants during the two years of follow-up is shown in Table 2. The seroconversion rates (antiHBs ≥ 10 IU/l) were similar for both groups at all months studied; more than 92% at all times and above 97% from month 6 onwards. The geometric mean antibody titers of vaccinees with antiHBs ≥ 10 IU/l were significantly higher for treatment group F at months 3, 4, and 6. These differences were no longer significant at 11, 12, and 24 months of age.

Recombinant vaccine compared with plasma vaccine

A comparison of infants on schedule F and the historic controls receiving the same vaccination regimen with the plasma vaccine is presented in Table 3. The percentage of infants seroconverting for antiHBs was similar for both vaccines. Significantly higher GMTs were obtained with the plasma vaccine than with the recombinant vaccine, both after the initial series of vaccination at month 6 and during follow-up.

HBV infections

Despite passive-active immunization 1% (2/195) of infants, one in each group, became HBsAg-positive and developed the HBV carrier state. Both infants had HBeAg-positive HBsAg carrier mothers with high levels of HBV DNA (223 pg/ml and 193 pg/ml by Abbott HBV DNA assay) and had detectable HBsAg before the age of 4 months. The HBsAg carrier rates among infants born to HBsAg- and HBeAg-positive mothers were 8% (95% CI: 0.19-36.03) and 6% (95% CI: 0.15-28.69) in groups E and F, respectively. At 12 months of age, 18% of infants (18/98) on schedule E compared with 20% (14/70) of infants on schedule F were antiHBc-positive. At the age of 24 months 7 infants, the two infants who were HBsAg-positive included, tested antiHBc-positive. The total number of HBV infections was similar for both groups; three (3%) of 102 infants in group E (95%CI: 0.62-8.77) and four (4%) of 93 infants in group F (95%CI: 1.18-10.65). The inapparent HBV infections at 24 months of age (antiHBc-positive only) were observed in both infants born to HBsAg- and HBeAg-positive mothers and infants born to HBeAg-negative mothers.

Table 2

AntiHBs levels in infants of HBsAg-positive mothers who responded to passive-active immunization according to schedule E or F.

	AntiHBs seroconversion (≥ 10 IU/l)					AntiHBs levels in IU/l				
	Schedule E		Schedule F			Schedule E		Schedule F		
Month	n	(%)	n	(%)	P-value*	GMT	(± 2 SEM)	GMT	(± 2 SEM)	P-value†
3	90/94	(96)	67/73	(92)	NS	40	(36-44)	58	(48-71)	<< 0.001
4	82/89	(92)	74/75	(99)	NS	25	(21-29)	148	(124-176)	<< 0.001
6	89/91	(98)	74/76	(97)	NS	374	(264-530)	733	(507-1060)	= 0.02
11	90/91	(99)	64/65	(98)	NS	746	(567-981)	1126	(776-1636)	NS
12	89/90	(99)	68/69	(99)	NS	9317	(6558-13237)	9699	(6475-14528)	NS
24	85/88	(97)	67/68	(99)	NS	1727	(1216-2452)	1125	(767-1649)	NS

* Fisher's exact test

† Wilcoxon Rank sum test

NS indicates not significant.

Table 3

AntiHBs levels in infants of HBsAg-positive mothers after administration of plasma vaccine (historic controls) or recombinant hepatitis B vaccine (group F).

	AntiHBs seroconversion					AntiHBs levels in IU/l				
	Plasma		Recombinant			Plasma		Recombinant		
Month	n	(%)	n	(%)	P-value*	GMT	(± 2 SEM)	GMT	(± 2 SEM)	P-value†
3	94/99	(95)	67/73	(92)	NS	32	(29-37)	58	(48-71)	<< 0.001
6	105/107	(98)	74/76	(97)	NS	1120	(812-1545)	733	(507-1060)	<< 0.001
11	95/98	(97)	64/65	(98)	NS	2360	(1832-3041)	1126	(776-1636)	<< 0.001
12	95/99	(96)	68/69	(99)	NS	15739	(11738-21104)	9699	(6475-14528)	<< 0.001
24	87/91	(96)	67/68	(99)	NS	1728	(1284-2325)	1125	(767-1649)	<< 0.001

* Fisher's exact test

† Wilcoxon Rank sum test

NS indicates not significant.

Table 4
Length of time (in days) between birth and the administration of the first dose of vaccine to infants on schedule E or F.

1st dose of vaccine	Schedule E	Schedule F	P-value
Infants (no)	102	93	
Target age (days)	91	91	
Median age (days)	96	94	0.12*
Mean age (SD)	100 (16)	99 (17)	
5th;95th percentile	84;125	83;148	

* Wilcoxon Mann Whitney test

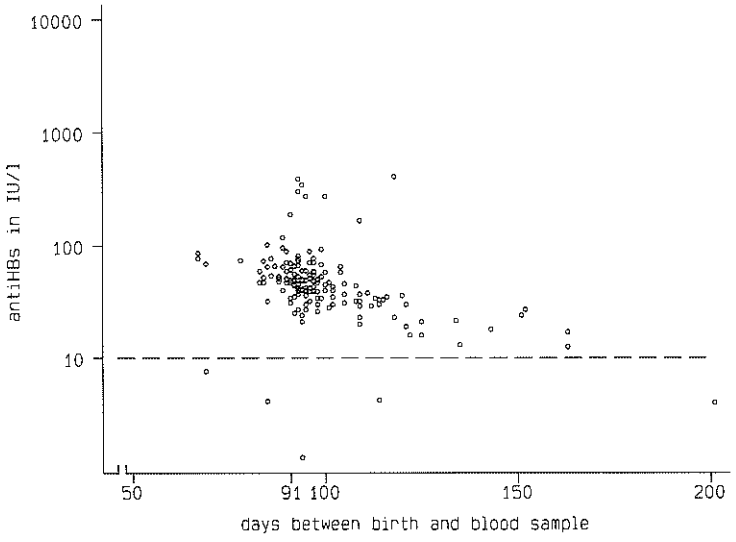


Figure 2
The effect of time on the passively acquired antiHBs levels between birth and the administration of the first dose of vaccine (target age 91 days). AntiHBs titers ≥ 1 IU/l are presented.

Vaccination interval

The median ages in days at which infants were given the 1st dose of vaccine are given in Table 4. For some infants the 0 to 3 month interval was extremely long. For instance, 21% of infants on schedule E (21/99) and 20% of infants (18/90) on schedule F received their injection at 3 months of age more than two weeks later than the target age (91 days).

Figure 2 illustrates the relationship between the moment at which the blood sample of month 3 was taken (target age 91 days) and the level of antiHBs acquired passively at birth. As the 0 to 3 month interval increased the antibody level decreased significantly ($p < 0.001$). From Figure 2 it can also be deduced that the passively acquired antibodies last approximately 5 months in most infants before falling below the critical level of 10 IU/l. Ten infants, however, had no protective levels of antiHBs at 3 months of age. Five infants had less than 10 IU/l antiHBs and the other five infants had no detectable antiHBs. Only two of these infants, one in each group, received their first dose beyond the mean age of vaccine administration plus the standard deviation. Except for the one infant who became an HBsAg carrier all infants with antiHBs < 10 IU/l at 3 months of age were born to HBeAg-negative mothers and developed a protective immune response from month 6 onwards.

DISCUSSION

We examined the need for an additional HBIg dose at 3 months of age in infants receiving a high dose of HBIg at birth and simultaneous injections of hepatitis B vaccine and DTP-polio vaccine at 3, 4, 5, and 11 months of age. The rates of seroprotection (antiHBs ≥ 10 IU/l) in group E, without the additional dose of HBIg, and group F were similar in all cases. Especially during the first six months of life when the effect of HBIg given at birth is waning (7) a significant benefit from the additional HBIg dose at 3 months was not observed.

More importantly, we found no differences in the number of infants who became HBsAg-positive in group E (8%) and group F (6%), whereas the number of infants born to HBsAg- and HBeAg-positive mothers was similar in both groups (Table 1).
Although the calculation of the 95% CI for the difference between the two population proportions ranges from -16% to 20%, showing the relative imprecision due to the limited sample size, these precentages are comparable to the number of HBsAg-positive infections (8%; 95% CI: 1.7-21.9) observed in the larger group of historic controls who received two doses of HBIg at 0 and 3 months and the plasma-derived vaccine at 3, 4, 5, and 11 months of age (3). The percentage of infants on schedule E or F with inapparent HBV infection (antiHBc-positive only) was also similar for both groups.

After a high dose of HBIg given at birth, antibodies tend to remain above the critical level of 10 IU/l antiHBs for approximately five months (Figure 2).

The immune response provided by vaccination initiated at 3 months of age is rapid and strong (2,8-10), resulting in similar protective efficacy (98% versus 90-93%) as compared to

vaccination starting at birth (2,8). Our study results with excellent protective efficacy after delayed active immunization are confirmatory to the findings by Beasley et al. (8).

Ten infants had no protective levels of antiHBs at 3 months of age. All but one infant were born to HBeAg-negative mothers and reasons for the absence of antiHBs levels ≥ 10 IU/l are not clear. The low levels of antiHBs in these infants may have been caused either by failures in the administration of HBIg or by consumption of the passively administered antiHBs antibodies. For programs with delayed active immunization, monitoring of HBIg administration is indicated. In addition, we advocate the use of 1 ml of HBIg instead of the usually recommended dose of 0.5 ml.

The practical consequences of our findings are that a schedule with high efficacy and compliance at relatively low cost can be implemented in the Dutch child care system to prevent perinatal infections in infants of HBsAg-positive mothers, provided the timely administration of hepatitis B vaccine in clinical practice.

The data from this study further demonstrate that the recombinant hepatitis B vaccine is efficacious in inducing high antibody levels in infants of HBsAg-positive mothers. Although we, like others, found that the recombinant vaccine and the plasma vaccine are equally immunogenic in inducing levels of antiHBs ≥ 10 IU/l (5), the GMTs of antiHBs antibodies in infants given the recombinant vaccine were significantly lower than those observed with plasma vaccine. Others also demonstrated that the recombinant vaccine (10 µg or 20 µg Engerix-B) produces a relatively lower antibody titer than the plasma vaccine (10 µg or 20 µg HBvax, MSD) (11-12).

Differences in GMTs between the current recombinant vaccine study and our previous study using the plasma vaccine need careful interpretation. Even though the vaccination regimen and methods of testing (radioimmunoassay, Ausab, Abbott) were similar, the vaccine doses were different and the study populations might have changed over time. It should also be noted that antibodies were assayed with kits containing plasma-derived HBsAg as the antigen. Conceivably, antibodies induced by recombinant-derived HBsAg may be only partially homologous to the plasma-derived HBsAg (13).

The higher geometric mean titers in group F during the first six months are likely the result of the passively acquired antibodies since simultaneous administration of HBIg and vaccine was not found to either reduce or stimulate the immune response (14).

The possibility of giving reduced doses of vaccine to lower the cost was evaluated by Lee and colleagues (15). As the protective efficacy decreased with the dose of antigen given, the authors recommend not to use lower doses of vaccine for infants of carrier mothers. Since a rapid and strong immune response to the vaccine may enhance optimal early protection and long-lasting immunity, we support the recommendations of the Dutch Health Authorities to use the adult dose of the recombinant vaccine.

The recommendations on HBIg and hepatitis B vaccine apply only for developed countries where maternal screening for HBsAg and passive-active immunization in infants of HBsAg-positive women is the standard policy for the prevention of perinatal hepatitis B infection. In the Netherlands, where many home deliveries take place, HBIg should be given

at birth by the person assisting in the delivery. The dose should be adequate in order to maintain protection until the age of 3 months when hepatitis B vaccination in infants is started at the same time as DTP-polio vaccination at the Child Health Clinics.

Acknowledgments

This study is generously funded by a grant from the Praeventiefonds, the Netherlands (no. 28-760-2). The authors thank J Boot and WCJ Hop for their technical assistance and statistical advice. We also thank the members of the Virology Department for their support.

References

1. Mazel JA, Schalm SW, de Gast GC, Nuijten ASM, Heijtink RA, Botman MJ, JRJ Bänffer, Gerard LJ, Zwijnenberg J, Mettau J, Wladimiroff YW, Fetter WPF. Passive-active immunization in neonates of HBsAg-positive carrier mothers: preliminary observations. Br Med J 1984;288:513-515.
2. Schalm SW, Mazel JA, de Gast GC, Heijtink RA, Botman MJ, Bänffer JRJ, Gerards LJ, Zwijnenberg J, Fetter WPF, Nuijten ASM, Wladimiroff YW, Christiaens GCML. Prevention of hepatitis B infection in newborns through mass screening and delayed vaccination of all infants of mothers with hepatitis B surface antigen. Pediatrics 1989;83:1041-1048.
3. Grosheide PM. del Canho R, Heijtink RA, Nuijten ASM, Zwijnenberg J, Bänffer JRJ, Wladimiroff JW, Botman MJ, Mazel JA, de Gast GC, Christiaens GCML, Gerards LJ, Fetter WPF, Baerts W, Schalm SW. Passive-active immunization in infants of hepatitis B e antigen-positive mothers: comparison of the efficacy of early and delayed active immunization. AJDC 1993 (in press).
4. Beasley RP, Hwang LY, Stevens CE, Lin CC, Hsieh FJ, Wang KY, Sun TS, Szmuness W. Efficacy of hepatitis B immune globulin for prevention of perinatal transmission of the hepatitis B virus carrier state: final report of a randomized double-blind, placebo-controlled trial. Hepatology 1983;3:135-141.
5. Cadranel S, Zeghlache S, Fernandez S, Safary A, Andre FE. Vaccination of newborns of HBsAg positive carrier mothers with a recombinant DNA hepatitis B vaccine. Postgrad Med J 1987;63(Suppl 2):159-160.
6. Lenter C, (ed). Geigy Scientific Tables. 8th ed. Basel, Switzerland: Geigy 1982:89-102.
7. Beasley RP, Hwang LL. Postnatal infectivity of hepatitis B surface antigen-carrier mothers. J Infect Dis 1983;147:185-190.
8. Beasley RP, Hwang L-Y, Lee GC-H, Lan C-C, Roan C-H, Huang F-Y, Chen C-L. Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. Lancet 1983;2:1099-1102.
9. Piazza M. Picciotto L, Villari R, Guadagnino V, Orlando R, Isabella L, Macchia V, Memoli AM, Vegnente A, Borrelli AM, Scarcella A, Cascioli C, Cirillo C, Coppola P, Isabella E, Parisi G. Hepatitis B immunization with a reduced number of doses in newborn babies and children. Lancet 1985;1:949-951.
10. Piazza M, Picciotto L, Villari R, Guadagnino V, Orlando R, Macchia V, Memoli AM, Vegnente A, Guida S, Fusco C. Two-dose hepatitis B immunisation regimen for infants. Lancet 1985;2:1120-1121.
11. Panda SK, Ramesh R, Rao KVS, Gupta A, Zuckerman AJ, Nayak NC. Comparative evaluation of the immunogenicity of yeast-derived (recombinant) and plasma-derived hepatitis B vaccine in infants. J Med Virol 1991;35:297-302.
12. Payton CD, Scarisbrick DA, Sikotra S, Flower AJE. Vaccination against hepatitis B: comparison of intradermal and imtramuscular administration of plasma derived and recombinant vaccines. Epidemiol Infect 1993;110:177-180.
13. McCartney RA, Harbour J, Roome APCH, Caul EO. Comparison of enhanced chemiluminescence and microparticle enzyme immunoassay for the measurement of hepatitis B surface antibody. Vaccine 1993;11:941-945.

14. Lelie PN, Reesink HW, Grijm R, de Jong-van Manen STh, Reerink-Brongers EE. Simultaneous passive and active immunization against hepatitis B; non-interference of hepatitis B immune globulin with the anti-HBs response to reduced doses of heat-inactivated hepatitis B vaccine. Hepatology 1986;6:971-975.
15. Lee CY, Hwang LY, Beasley RP. Low-dose hepatitis B vaccine. Lancet 1989;2:860-861.

CHAPTER 3.4

TEN-YEAR NEONATAL HEPATITIS B VACCINATION PROGRAM, THE NETHERLANDS, 1982 TO 1992: PROTECTIVE EFFICACY ACCORDING TO MATERNAL SERUM LEVELS OF HBV DNA AND LONG-TERM IMMUNOGENICITY

R. del Canho[1], P.M. Grosheide[1], J.A. Mazel[2], R.A. Heijtink[3], W.C.J. Hop[4] L.J. Gerards[5], G.C. de Gast[6], W.P.F. Fetter[7] , J. Zwijnenberg[8], S.W. Schalm[1] and the Dutch Study Group Prevention Neonatal Hepatitis B

Department of Internal Medicine II[1], Academic Hospital Dijkzigt, Rotterdam; General Practitioner[2], Borne; Department of Virology[3] and Department of Epidemiology and Biostatistics[4], Erasmus University, Rotterdam; Department of Paediatrics[5], Wilhelmina Childrens Hospital, Blood Transfusion Laboratory[6], University Hospital Utrecht, Utrecht;Department of Paediatrics[7], St Sophia Hospital, Zwolle; Department of Pediatrics[8], Ruwaard van Putten Hospital, Rotterdam, the Netherlands.

Submitted for publication.

SUMMARY

From 1982-1989, 705 infants born to HBsAg-positive mothers entered the Dutch neonatal hepatitis B vaccination program and received passive-active hepatitis B immunization, according to 6 schedules, varying in time of vaccination, dose of hepatitis B immune globulin (HBIg) and type and dose of vaccine. 118 (17%) of the mothers were also HBeAg-positive. This final report describes the protective efficacy and long-term immunogenicity of passive-active hepatitis B immunization over a period of 10 years.

During a follow-up, 9 infants became HBsAg carriers: 8, all born to HBeAg-positive mothers within the first year of life and one child, born to an HBeAg-negative mother at the age of 5 years. No evidence of emergence of hepatitis B escape mutants was found. The protective efficacy rate (PER) of passive-active immunization at 12-month follow-up was 92% for the entire group with no significant differences between groups starting active immunization at birth or at 3 months of age; receiving one or two doses of HBIg; or receiving plasma-derived or recombinant hepatitis B vaccine. The PER at month 12 in the group with maternal HBV DNA levels less than 150 pg/ml was 100%, significantly higher than 68% for the group with HBV DNA levels above 150 pg/ml.

After 5 years of follow-up, the group with active immunization starting at birth had significantly more infants with antiHBs levels less than 10 IU/l (15%) than the corresponding group starting at 3 months of age (2%). Geometric mean titers of antiHBs were significantly higher in the group that started at 3 months of age with plasma vaccine than in the corresponding group that received recombinant vaccine.

This program showed that passive-active immunization can be highly effective in the prevention of neonatal hepatitis B, except for children born to women with high hepatitis B viraemia. Evaluation of vaccination schedules should take into account risk assessment according to maternal HBV DNA levels. The excellent efficacy of delayed active immunization allows incorporation of the hepatitis B vaccine into the standard infant immunization programs for countries with a passive-active immunization strategy for hepatitis B. For long-term protection, dosage of recombinant vaccine with equal immunogenicity to that of plasma vaccine should be considered.

INTRODUCTION

Passive-active immunization with hepatitis B immune globulin (HBIg) and hepatitis B vaccine has proven to be highly effective in preventing perinatal transmission of hepatitis B infection for more than a decade (1-3).

These studies were performed on infants born to HBeAg-positive mothers, who were considered at nearly 90% risk for developing chronic hepatitis B virus infection (1,4-6). Recently the risk of mother-to-infant transmission of HBV was shown to be related to the presence of HBV DNA in serum of HBeAg carrier mothers (7-9). The protective efficacy

rate of passive-active hepatitis B immunization has not been assessed according to maternal HBV DNA levels.

When hepatitis B vaccine was licensed in the Netherlands in 1982, a program was started to screen mothers for HBsAg positivity and to immunize their offspring. In addition to various passive-active immunization schedules starting at birth the vaccination program evaluated a schedule with passive immunization at birth and active immunization starting at 3 months of age, concomitant with diphtheria-tetanus-pertussis and polio (DTP-polio) vaccination. We hypothesized that the efficacy of delayed active immunization would be similar but the long-term immunogenicity would be superior to immunization starting at birth. Such a result would allow incorporation of active hepatitis B immunization into the standard infant immunization program which was considered to be advantageous with respect to logistics and costs. Preliminary results confirmed the hypothesis (10).

With the introduction of recombinant vaccine in 1987, confirmation was also needed for the recombinant vaccine (11). This study also allowed testing to determine the need for supplementary hepatitis B immune globulin (HBIg) at the start of delayed active immunization.

Our finding that within the population studied vaccination failure is primarily related to high maternal HBV DNA levels (12) made it necessary to re-evaluate the effects of components of the various schedules: time of starting active immunization, types and doses of HBIg and vaccine used, in relation to maternal HBV DNA levels.

This final report of the Dutch program for prevention of perinatal hepatitis B describes the protective efficacy and long-term immunogenicity of passive-active hepatitis B immunization over a period of 10 years.

PATIENTS AND METHODS

Hepatitis B screening

The study was started in July 1982 in three large city hospitals in Rotterdam and Utrecht and in one large rural area, Twente-Gelderse Achterhoek. Blood samples were obtained from all pregnant women during their first visit to the prenatal clinic of the participating centers and in addition to the routine determination of blood groups and screening for syphilis, were tested for the presence of HBsAg. In case of a positive finding for HBsAg, a repeated HBsAg test was performed at 28 weeks of pregnancy. If HBsAg positivity was confirmed, allocation to one of the immunization schedules took place after informed consent was obtained from the mother. In the two participating hospitals in Rotterdam the HBsAg status of expectant mothers was checked soon after arrival in the delivery room. Whenever prenatal HBsAg test results were missing, blood was obtained and tested the next morning with a rapid hemagglutination HBsAg test. If the rapid HBsAg test was positive, the mother was asked for informed consent and the baby was randomized and included in

the immunization trial. All pregnant HBsAg-positive women were also tested for the presence of HBeAg. In December 1992 maternal HBV DNA levels were quantified retrospectively in the available stored serum samples positive for HBeAg.

Subjects and immunization schedules

From July 1982 to January 1988, 495 eligible infants were randomly allocated to one of four plasma vaccine immunization schedules (Table 1: groups A-D). From January 1988 to October 1989 another 210 eligible infants were allocated to one of the two immunization schedules using recombinant vaccine (Table 1: groups E-F).

Within two hours of birth all infants received HBIg (200 to 300 IU, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam) intramuscularly by the physician or midwife assisting in the delivery. For active immunization, infants were referred to a pediatrician. Infants received plasma vaccine (10 μg and 5 μg HBvax, Merck Sharp & Dohme, West Point, Pa, USA) or recombinant vaccine (20 μg Engerix-B, SmithKline Beecham, Rixensart, Belgium). Nine infants (1 in group B, 3 in group C and 5 in group D) with an antiHBs level ≤ 10 IU/l at 12 months of age and a negative test for HBsAg received an additional series of plasma or recombinant vaccine in their second year of life (13).

Serological assays and laboratory methods

Blood samples were obtained from infants at birth (cord blood) and in groups A and B at months 3, 6, 11, 12, and then yearly until 9 years of age; in groups C and D at months 3, 6, 12, and then yearly until 5 years of age; in groups E and F at months 3, 4, 6, 11, 12 and at 2 years of age. All serum samples were tested for antiHBs and antiHBc; HBsAg was assayed in all samples with antiHBs below 100 IU/l. HBsAg, HBeAg, antiHBc and antiHBs were assessed using a commercial radioimmunoassay kit (Abbott Laboratories, Chicago, Ill, USA). HBV DNA was assessed quantitatively by a solution hybridization assay (HBV DNA, Abbott).

Definition of HBV infection

The HBsAg carrier state was defined as HBsAg positivity for more than 6 months. Transient HBV infection was characterized by being HBsAg-positive less than 6 months. Inapparent HBV infection was defined as antiHBc-positivity without HBsAg on two or more occasions after month 12.

Table I
Immunization schedules of study groups.

Groups	Entry period	Number of infants: Total (mother HBeAg+)	Number of infants: Evaluated for immunogenicity	Number of infants: Evaluated for efficacy§	Immunization schedule: HBIg[a] months/dose after birth	Immunization schedule: Vaccine type/dose	Immunization schedule: Administration at month
A	1982-1984*	117 (38)†	110 (35)	103 (37)	0/200	plasma[b] 10 µg	0,1,2 and 11
B	„	121 (42)	109 (37)	105 (41)	0,3/200,125‡	plasma 10 µg	3,4,5 and 11
C	1984-1987*	133 (3)	128 (2)	127 (3)	0/200	plasma 10 µg	0,1,6
D	„	124 (4)	122 (4)	115 (2)	0/200	plasma 5 µg	0,1,6
E	1988-1989	112 (14)	102 (13)	98 (14)	0/300	recomb.[c] 20 µg	3,4,5 and 11
F	„	98 (17)	93 (17)	83 (17)	0,3/300,300	recomb. 20 µg	3,4,5 and 11
Total	1982-1989	705 (118)	664 (108)	631 (114)			

* Infants of HBeAg-positive mothers entered in group A and B until December 1987.
† Number of infants born to HBeAg-positive mothers in parentheses.
§ Number of infants evaluated for the protective efficacy at 12 months of age.
‡ Infants in group B received 1 ml HBIg (100-150 IU/ml) at 3 months of age.
[a] Hepatitis B immune globulin, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, the Netherlands.
[b] HBvax, Merck Sharp & Dohme, Westpoint, Pa, USA.
[c] Engerix-B, SmithKline Beecham Biologicals, Rixensart, Belgium.

Statistical analysis

Available data were analyzed according to the 'intention to treat' principle. Separate per-protocol analysis, i.e. analysis of data of children who received vaccinations according to protocol, was also performed. The results of these analyses did not significantly differ. Therefore we report on the protective efficacy using results of the 'intention to treat' analysis (including infants who violated the protocol). The results on the immunogenicity are reported using the outcome of the per-protocol analysis.

Protection by the different immunization schedules was expressed as the protective efficacy rate (PER):

$$\frac{\text{expected number of HBV infections without immunoprophylaxis minus measured number of HBV infections with immunoprophylaxis}}{\text{the expected number of HBV infections without immunoprophylaxis}} \times 100$$

The expected number of HBV infections without immunoprophylaxis was estimated to be 90% of infants born to HBeAg-positive mothers per immunization group (3-5). The PER was also calculated for a percentage of 67 for the expected number of HBV infections without immunoprophylaxis, in view of the finding that one third of the HBeAg-positive mothers had an HBV DNA level less than 5 pg/ml and therefore were unlikely to transmit hepatitis B to their infants (8).

To investigate whether patients with incomplete data affected the outcome of the long-term immunogenicity, the percentages of infants with antiHBs levels < 10 IU/l were also calculated for those infants who had no missing data (excluding occasional missing antiHBs titers, that could be obtained by linear interpolation of 2 adjacent points). The percentages thus obtained differed at all time-points at most 4% from those given in Figure 3, indicating that loss to follow-up did not significantly affect the outcomes presented.

Differences in percentages were analyzed by Chi-square test or Fisher's exact test in case of small numbers. Continuous variables were analyzed by the two-sample Wilcoxon rank-sum test. The limit for significance was set to 0.05 (two-sided). In case of evaluations at various timepoints, the limit for significance was set according to Bonferoni's principle to allow for the multiplicity of statistical tests. AntiHBs levels were expressed in IU/l. Geometric mean titers (GMT) were calculated only for those infants who had antiHBs ≥ 10 IU/l. Exact confidence limits for odds ratios were calculated using the statistical software package 'STATXACT'.

Ethics

The study was approved by the local medical ethics committees of the participating centers.

RESULTS

Follow-up

From July 1982 until October 1989, 705 infants of HBsAg-positive mothers were assigned to one of six treatment groups (Table 1). Sixteen infants were withdrawn after informed-consent but before vaccination, 12 infants received at least 1 vaccination, but no serum sample was available after month 0. 677 infants, received passive-active immunization. Thirteen infants received vaccinations but not according to the protocol (1 in group A, 4 in group B, 1 in group C, 1 in group D, 3 in group E and 3 in group F).
A number of 664 infants received passive-active immunization according to the protocol; 650 of 664 infants (98%) received all planned vaccinations. Serum samples were available from 590 children after 1 year follow-up, 546 after 2 years, 262 after 5 years and 126 after 8 years (Table 5). Incomplete data from these children were primarily due to secondary refusal of the parents, related to the frequency of blood sampling (up to 10 samples) during the study, and to migration.

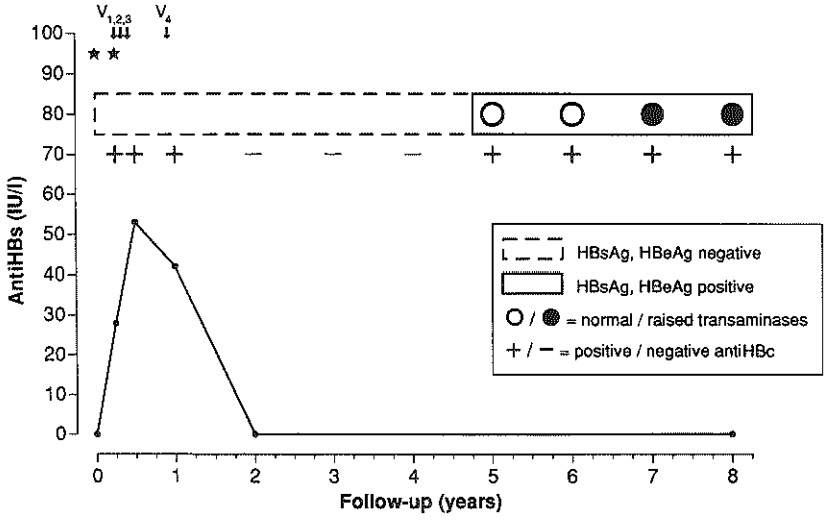


Figure 1
Chronic HBV infection after a follow-up of 5 years in one infant in group B, born to an HBeAg-negative mother despite a response (albeit weak) to passive-active immunization.

Analysis of serum samples of family members showed that this patient has an HBeAg-positive brother. This might explain the horizontally transmitted HBV infection in this patient at the age of 5 years. There was no coexistence of HBsAg and antiHBs positivity in the patient; full in vitro neutralization of HBsAg by HBIg was observed, indicating that the HBV infection was not caused by an escape mutant.

Protective efficacy of perinatal HBV immunization

At month 12, HBsAg positivity was found in 8 of 590 infants. No serum sample was available from 33 infants at month 12 but they were HBsAg-negative thereafter, so assumption is made that there was no HBsAg positivity at month 12. Serum samples were also available from 8 out of the 13 infants, who received a vaccination schedule not according to protocol, and then were found HBsAg-negative at month 12 or thereafter. From the 631 (i.e. 590+33+8) infants analyzed, 8 (1.3%) were found to be HBsAg-positive during the first year of life; all HBsAg-positive children were born to HBeAg-positive mothers. One infant of an HBeAg-negative mother became HBsAg-positive at the age of 5 years (Figure 1). This child and all infants found to be HBsAg-positive in the first year became hepatitis B carriers.

Table 2

Protective efficacy of passive-active hepatitis B immunization at month 12 in infants of HBeAg-positive mothers, according to different immunization components.

Immunization components	Number of infants: Total	Number of infants: HBsAg-positive (%)	Difference in HBsAg positivity (95% CI)	Protective efficacy† %: 90%§	Protective efficacy† %: 67%‡
HBIg month 0	56	4 (7.1)	0.2 (-9.2,+9.2)	92	89
HBIg months 0,3	58	4 (6.9)		92	90
Vaccine starting month 0	42	3 (7.1)	0.2 (-9.5,+9.9)	92	89
Vaccine starting month 3	72	5 (6.9)		92	90
Plasma vaccine	83	6 (7,2)	0.7 (-9.6,+11.0)	92	89
Recombinant vaccine	31	2 (6.5)		93	90
Total	114	8 (7.0)		92	90

† $\frac{\text{expected number of HBV infections without immunoprophylaxis minus measured number of HBV infections with immunoprophylaxis}}{\text{the expected number of HBV infections without immunoprophylaxis}} \times 100$

§ The expected number of HBV infections without immunoprophylaxis for infants of HBeAg-positive mothers is estimated 90% (3-5).

‡ The protective efficacy based on a chronic carier rate, without prophylaxis, of 67% is assumed in view of the finding that one third of infants of HBeAg-positive mothers had HBV DNA less than 5 pg/ml and therefore were unlikely to transmit hepatitis B to their infants (8).

Inapparent HBV infection (antiHBc positivity with negative HBsAg tests on two or more occasions after month 12), was observed in 8 infants, all born to HBeAg-positive mothers. A total of 614 (i.e. 631-9-8) infants developed antiHBs after vaccination, without signs of HBV infection; in 9 children antiHBs levels of more than 10 IU/l developed only after revaccination.

At month 12, the protective efficacy rate of passive-active immunization for the 114 infants (all immunization groups) of HBsAg- and HBeAg- positive mothers was 92% (Table 2). No significant differences were found between the PER for the groups starting active vaccination at birth and groups starting at 3 months, between groups receiving either one or two doses of HBIg, and between groups receiving the plasma vaccine or the recombinant vaccine (Table 2).

In 72 of the 114 HBeAg-positive mothers, residual serum was available for quantitative HBV DNA assessment in 1992. Table 3 shows the relation between the maternal HBV DNA levels (divided in 3 groups of equal size) and the number of infants who became HBV carriers. The PERs at month 12 for the 2 groups with maternal HBV DNA levels < 150 pg/ml were 100% and significantly higher than the PER (68%) for the group with maternal HBV DNA levels ≥ 150 pg/ml (p = 0.009).

Table 3

Protective efficacy rates (PER) in infants at 12 months of age receiving passive-active immunization according to the maternal levels of HBV DNA.

	Number of infants		
HBV DNA (pg/ml)	Total	HBsAg-positive (%)	PER† %
< 6	24	0 (0)	n.a.§
7-150	24	0 (0)	100
> 150*	24	7 (29)	68

† The PER is based on a chronic carrier rate, without prophylaxis, of 90% at 12 months.

* p = 0.009 (Fisher's exact test) between number of HBsAg-positive infants in the group with maternal HBV DNA > 150 pg/ml and the groups with maternal HBV DNA between 7-150 pg/ml and < 6 pg/ml, respectively. Due to small numbers of infants, we calculated the exact 95% CI of the ratio of the odds of HBsAg positivity in the group of infants with maternal HBV DNA < 150 pg/ml (odds = 0/48) versus the corresponding odds (7/17) in the group with HBV DNA > 150 pg/ml. This 95% CI ranged from 0 to 0.22.

§ Not applicable in view of the finding that one third of infants of HBeAg-positive mothers had HBV DNA less than 5 pg/ml and were therefore unlikely to transmit hepatitis B to their infants (8).

Figure 2 shows the maternal HBV DNA levels for HBV-infected infants and non-infected infants. The median maternal HBV DNA levels of the HBV carrier infants and of the inapparently infected infants were ten times higher (approximately 350 pg/ml) than the median maternal HBV DNA level (31 pg/ml) of the infants without HBV infection ($p = 0.001$ and $p = 0.03$, respectively).

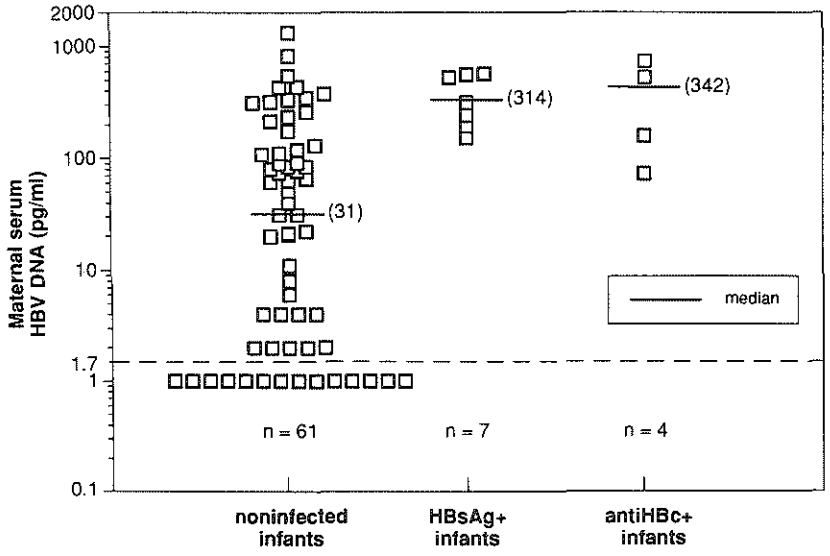


Figure 2
Maternal HBV DNA levels of 7 infants with persistent HBV infection, 4 transiently HBV-infected infants and 61 noninfected infants. The cutt-off level of the HBV DNA assay (HBV DNA, Abbott) is 1.7 pg/ml.

Long-term immunogenicity

Assuming a minimal risk for hepatitis B infection in vaccinees with antiHBs levels above 10 IU/l, and a potential risk with antiHBs levels less than 10 IU/l (14), we calculated the percentages of infants with antiHBs < 10 IU/l in the different immunization groups (Figure 3). At the age of 5 years, the group that started at 3 months of age with plasma vaccine (group B) had a significantly lower percentage (2%, 95% CI: 0-6%) of children with antiHBs < 10 IU/l than group A (14.5%, 95% CI: 6-23%) that started immunization at birth (Fisher's exact test; $p = 0.02$). The percentage of infants with antiHBs < 10 IU/l in group B never exceeded 5% during a 5-year follow-up, whereas the corresponding percentage in the other immunization groups increased during follow-up to more than 15%.

Follow-up total of infants with antiHBs levels of less than 10 IU/l amounted to 186 person-years: 38 years from nineteen of 441 infants with antiHBs titers ≥ 1000 IU/l at

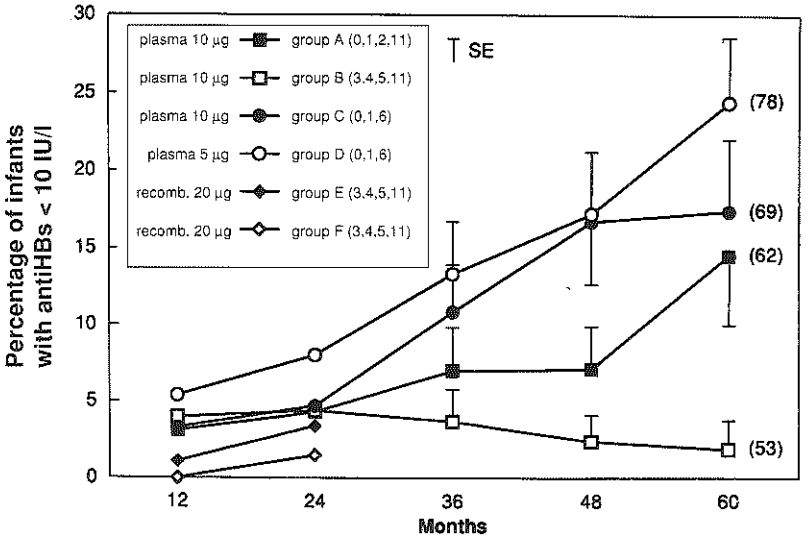


Figure 3

Percentages of infants with antiHBs < 10 IU/l in the six immunization groups. Bars indicate standard errors. Results at 2 years are similar for groups starting immunization at birth or at 3 months of age. Long-term follow-up suggests excellent persistence of protective immunity in infants who started immunization at 3 months age.

month 12; 78 years from thirty of 97 infants with antiHBs titers between 100 IU/l and 1000 IU/l; 69 years from twenty-one of 35 infants with antiHBs titers between 10 IU/l and 100 IU/l and 1 year from 1 of 9 infants with less than 10 IU/l antiHBs (the latter all developed more than 10 IU/l after revaccination).

Six of the 8 infants with inapparent HBV infection had antiHBs titers ≥ 1000 IU/l at month 12 and the other two infants had antiHBs titers between 10 IU/l and 100 IU/l.

Table 4 shows the GMTs (antiHBs ≥ 10 IU/l) for the different immunization groups during follow-up. The GMTs of antiHBs in group B (10 µg plasma vaccine administration from 3 months of age onwards) were approximately twice higher than the GMTs in group A (starting at birth with 10 µg plasma vaccine). In the corresponding groups with different dosages of vaccine, the GMT at month 12 in group C with 3 doses of 10 µg plasma vaccine administered from birth onwards was approximately twice higher than in group D, receiving the same schedule with only 5 µg of plasma vaccine. However, this difference was not significant after 36 months of follow-up. A comparison of the GMTs in groups given plasma or recombinant vaccine from 3 months onwards showed, that the group receiving the plasma vaccine (group B) had approximately one and a half times higher GMTs than the corresponding group receiving the recombinant vaccine (group F) ($p < 0.001$ at all times measured).

Side-effects

No clinically important side effects of vaccination were reported by the parents or observed by the pediatricians.

Table 4

Geometric mean titers (antiHBs ≥ 10 IU/l) in the 6 immunization groups during follow-up.

	GMT with (95% CI) in IU/l at month				
Groups	12	24	36	48	60
A 0,1,2,11; 10 µg pl.	8730 (6238-12217)	737 (533-1019)	353 (248-502)	207 (145-296)	137 (92-203)
B 3,4,5,11; 10 µg pl.	15739 (11738-21104	1728 (1284-2325)	820 (596-1129)	484 (356-231)	331 (231-473)
P-value§	0.023	0.001	0.0006	0.0004	0.0002
C 0,1,6; 10 µg pl.	1142 (849-1537)	331 (245-447)	202 (151-271)	138 (100-90)	100 (70-143)
D 0,1,6; 5 µg pl.	608 (438-846)	203 (146-283)	163 (116-228)	114 (78-166)	75 (52-109)
P-value†	0.0012	0.0066	0.19	0.31	0.11
E 3,4,5,11; 20 µg rec.	9317 (6558-13237)	1727 (1216-2452)	-	-	-
F 3,4,5,11; 20 µg rec.	9699 (6475-14528)	1125 (767-1649)	-	-	-
P-value‡	0.13	0.84			

§ P-value for group A vs B
† P-value for group C vs D
‡ P-value for group E vs F

Table 5
Number of serum samples available during follow-up of infants immunized according to six passive-active immunization scedules.

	Number of serum samples at month									
Groups	3	6	11	12	24	36	48	60	72	84
A	102	106	99	98	92	86	85	62	73	67
B	100	107	98	99	91	82	84	53	66	59
C	115	119	-	122	107	102	84	69	-	-
D	114	113	-	112	100	98	87	78	-	-
E	94	91	91	90	88	-	-	-	-	-
F	73	6	65	69	68	-	-	-	-	-
Total	598	542	353	590	546	368	340	262	139	126

DISCUSSION

In this study the protective efficacy against hepatitis B infection for infants of HBeAg-positive HBsAg carrier mothers was 92% at 12 months of age; for infants of HBeAg-negative mothers it was 100%. These rates are comparable to those found in other passive-active immunization studies, with either plasma-derived or recombinant vaccine (1-3,15-16). During follow-up beyond 1 year, extending to 9 years, one vaccinated infant of an HBeAg-negative mother became positive for HBsAg.

The timing of the initial vaccine injection, the number of doses of HBIg and the type of vaccine had no effect on the PERs. Although confidence in the results is not absolute, these results are confirming earlier findings. Beasley et al. already reported that, with HBIg coverage from birth, the timing of the start of active vaccination appeared of no importance (1). Stevens et al. published results indicating that yeast-recombinant vaccine was as effective as plasma-derived vaccine in preventing hepatitis B virus infection (3). We found no evidence that there is a need for a second dose of HBIg in combination with delayed active immunization. Evidence from other studies for the necessity of such action has not been forthcoming. Although some uncertainty on this point may persist, we found a major clinically relevant factor that did influence the PER.

The PERs in the groups of infants with maternal HBV DNA levels less than 150 pg/ml was 100%, significantly more than 68% in the group with maternal HBV DNA levels of more than 150 pg/ml.

To verify the finding that the PER is directly influenced by maternal HBV DNA levels, we analyzed the protective efficacy rate at 12 months of age according to the quantified maternal HBV DNA levels from the neonatal hepatitis B vaccination program in Hong Kong (PN Lelie; written communication). In the Hong Kong study no persistent HBsAg positivity at 12 months was detected in infants with maternal HBV DNA below 5 pg/ml, irrespective of immunization (8). Infants with maternal HBV DNA levels of more than 150 pg/ml were at high risk for hepatitis B infection; 25-50% of infants became persistent HBsAg-positive despite immunization. These results strongly support the concept that the level of maternal HBV DNA is the major factor influencing the PER of hepatitis B immunization.

In the Hong Kong study, infants with maternal HBV DNA levels between 7-150 pg/ml were also at risk for hepatitis B infection. In comparison to the 15% to 28% HBsAg carrier rate in the Hong Kong study the absence of HBsAg carriers in the group with moderately high maternal HBV DNA in the Dutch study needs additional discussion.

Since the rate of intrauterine infection is estimated to be only 1-2% (15), the difference observed is likely due to differences in intervention (HBIg, vaccine) or to differences in maternal-fetal transfusion during labour. The dose of hepatitis B immune globulin used in the Hong Kong study (50 IU) was lower than that used in the Dutch study (200-300 IU) and the dose of vaccine in the Hong Kong study (3 μg) was also lower than the vaccine dose used in the Dutch study (10 μg). Although the dose of vaccine may not reflect immunogenicity, the GMTs in the Dutch study were almost 10 times higher than in the Hong Kong study, indicating higher immunogenicity of the vaccine used in the study in the Netherlands. These results also suggest that efficacy of dose of HBIg and vaccine can now be assessed more precisely in cohorts with maternal quantified HBV DNA levels.

Recently, hepatitis B "escape mutants", lacking the "a" epitope on the viral envelope were found in vaccinated infants (17). We did not observe coexistence of HBsAg and antiHBs in 7 of the infants with persistent HBsAg. Additional laboratory investigations including in vitro neutralization of HBsAg by polyclonal antiHBs and molecular sequencing of the "a" domain of HBsAg provided no evidence of the presence of surface antigen variants in our HBsAg-positive infants. Also, a clinical relevance of precore mutants was not found in our study since the PER at month 12 for infants of HBeAg-negative mothers was 100%.

The long-term immunogenicity was significantly higher in the group receiving late active immunization than in the group starting immunization directly after birth. At the age of 5 years, the group with delayed active immunization had a significantly lower percentage (2%) of children with antiHBs less than 10 IU/l than the groups starting immunization at birth (15-25%). This finding is in agreement with others who also found an enhancement of the immune response if the infant is older at the time of the initial injection, probably related to a more mature immune system (18).

The implications of these findings are at present unclear. If protection against hepatitis B infection depends in a major way on the degree of immunologic priming, reflected in the persistence of antibody, then a strong argument could be made for adoption of schedules

that maximize the antiHBs levels. In the present study, there was a total follow-up of 186 person-years of 71 infants with antiHBs titers less than 10 IU/l. One infant, born to an HBeAg-negative mother, with an initial antiHBs response between 10 and 100 IU/l, became an HBsAg carrier after 4 years of follow-up without detectable antiHBs. In other studies, no HBsAg positivity after 5 years of follow-up was found in infants with initial antiHBs ≥ 10 IU/l, whether the infants lost their antiHBs or not (18,19). In long-term follow-up studies of immunized adults, no persistent HBsAg positivity was detected in persons with an initial antiHBs response > 10 SRU but transient HBsAg positivity and/or antiHBc positivity was detected in this group (14). For the time being, until more long-term follow-up studies are available, it seems advisable to aim for an initial antiHBs response of more than 100 IU/l for the prevention of clinically important forms of hepatitis B (14,20).

Implications of the finding that there was no effect on the PER of timing of active immunization are straightforward: it allows incorporation of the hepatitis B vaccine into the Expanded Program on Immunization (EPI). The number of physician visits can then be reduced as well as the number of injections, if multivalent vaccine becomes available. The finding that maternal HBV DNA is the most relevant clinical factor influencing the efficacy of standard passive-active hepatitis B immunization is important for improving results of intervention, particularly in countries which can afford high level individual care.
In practice, we advocate that HBV DNA levels be determined quantitatively in all HBeAg-positive HBsAg carrier mothers. On the basis of the current study we advise that additional preventive measures be offered for infants at high risk for hepatitis B infection, i.e. administration of 300 IU of HBIg (1 ml instead of 0.5 ml) and the adult dose of vaccine instead of the pediatric dose to infants of HBeAg-positive mothers with maternal HBV DNA level above 5 pg/ml. Further research is warranted to prevent hepatitis B infections in infants born to mothers with HBV DNA levels above 150 pg/ml, like the evaluation of delivery by elective Caesarean section as proposed by Lee (21) or additional HBIg injections as suggested by the Hong Kong study (15).

Acknoweledgments

We thank M Bakker-Bendik, Th van der Laar-Kokx, H Ribbers-Smeenk, HC van Dijk and M Voogd-Schotanus for their outstanding help in this study for several years. Special thanks go to YAM Weber, C Kerkhof and J Kruining for performing the laboratory tests, J Boot for his technical assistance and PN Lelie for making available the maternal HBV DNA assays of the Hong Kong neonatal hepatitis B vaccination program. This study was supported by a grant from the Praeventiefonds, the Netherlands (no. 28-760-2).
The following participants of the Dutch Study Group Prevention Neonatal Hepatitis B are acknowledged for their contribution:
Wladimiroff JW, Dept. of Obstetrics and Gynaecolgy, Academic Hospital Dijkzigt, Rotterdam; Nuijten ASM, State School of Training for Obstetricians, Rotterdam; Zwijnenberg J, Dept. of Pediatrics, Ruwaard van Putten Hospital, Rotterdam; Bänffer JRJ, Regional Public Health Laboratory, Rotterdam; Botman MJ, Regional Laboratory of Pathology and Microbiology, Enschede; Christiaens GCML, Dept. of Obstetrics and

Gynaecology, Academic Hospital, Utrecht; Fetter WPF and Baerts W, Dept. of Pediatrics, St Sophia Hospital, Zwolle.
The study group is grateful to the Steering Committee formed by: Professor dr J Huisman, Professor dr JW Stoop, dr H Bijkerk and Professor S Krugman.

References

1. Beasley RP, Hwang LY, Lee GC, Lan C, Roan CH, Huang FY, Chen CL. Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. Lancet 1983;2:1099-1102.
2. Coursaget P, Yvonnet B, Chotard J, Sarr M, Vincelot P, N'doye R, Diop-Mar I, Chiron JP. Seven-year study of hepatitis B vaccine efficacy in infants from an endemic area (Senegal). Lancet 1986;2:1143-1145.
3. Stevens CE, Taylor PE, Tong MJ, Toy PT, Vyas GN, Nair PV, Weissman JY, Krugman S. Yeast-recombinant hepatitis B vaccine: efficacy with hepatitis B immune globulin in prevention of perinatal hepatitis B virus transmission. Jama 1987;257:2612-2616.
4. Okada K, Kamiyama I, Inomata M, Imai M, Miyakawa Y, Mayumi M. E antigen and anti-e in the serum of asymptomatic carrier mothers as indicators of positive and negative transmission of hepatitis B virus to their infants. N Engl J Med 1976;294:746-749.
5. Reesink HW, Reerink-Brongers EE, Lafeber-Schut BJTh, Kalkhoven-Benschop J, Brummelhuis HGJ. Prevention of chronic HBsAg-carrier state in infants of HBsAg-positive mothers by hepatitis B immunoglobulin. Lancet 1979;2:436-438.
6. Beasley RP, Hwang LY. Postnatal infectivity of hepatitis B surface antigen-carrier mothers. J Inf Dis 1983;147:185-190.
7. Lee SD, Lo KJ, Wu JC, Tsai YT, Wang JY, Ting LP, Tong MJ. Prevention of maternal-infant hepatitis B virus transmission by immunization: the role of serum hepatitis B virus DNA. Hepatol 1986;6:369-373.
8. Ip HMH, Lelie PN, Wong VCW, Kuhns MC, Reesink HW. Prevention of hepatitis B virus carrier state in infants according to maternal serum levels of HBV DNA. Lancet 1989;1:406-410.
9. Lin HH, Chang MH, Chen DS, Sung JL, Hong KH, Young YC, Yang KH, Lee TY. Early predictor of the efficacy of immunoprophylaxis against perinatal hepatitis B transmission: analysis of prophylaxis failure. Vaccine 1991;9:457-460.
10. Schalm SW, Mazel JA, Gast de GC, Heijtink RA, Botman MJ, Bänffer JRJ, Gerards LJ, Zwijnenberg J, Fetter WPF, Nuijten ASM, Wladimiroff JW, Christiaens GCML. Prevention of hepatitis B infection in newborns through mass screening and delayed vaccination of all infants of mothers with hepatitis B surface antigen. Pediatrics 1989;83:1041-1048.
11. Grosheide PM, Canho del R, Voogd M, Heijtink RA, Schalm SW, Dutch Study Group Prevention Neonatal Hepatitis B. AntiHBs levels in infants of hepatitis B carrier mothers after delayed active immunization with recombinant vaccine concomittant with DTP-polio vaccine: is there need for a second dose of HBIg? Vaccine (accepted for publication).
12. Canho del R, Grosheide PM, Schalm SW, Vries de RRP, Heijtink RA. Failure of neonatal hepatitis B vaccination: the role of HBV-DNA levels in hepatitis B carrier mothers and HLA antigens in neonates. J Hepatol (in press).
13. Canho del R, Schalm SW, Heijtink RA. Hepatitis B revaccination of neonates with inadequate response after primovaccination. Vaccine 1992;10:69.
14. Hadler SC, Francis DP, James DS, Maynard JE, Thompson SE, Judson FN, Echenberg DF, Ostrow DG, O'Malley PM, Penley KA, Altman NL, Braff E, Shipman GF, Coleman PJ, Mandel EJ. Long-term immunogenicity and efficacy of hepatitis B vaccine in homosexual men. N Engl J Med 1986;315:209-214.
15. Wong VCW, Ip HMH, Reesink HW, Lelie PN, Reerink-Brongers EE, Yeung CY, Ma HK. Prevention of the HBsAg carrier state in newborn infants of mothers who are chronic carriers of HBsAg and HBeAg by administration of hepatitis B vaccine and hepatitis B immunoglobulin. Lancet 1984;1:921-926.

16. Poovorawan Y, Sanpavat S, Pongpuniert W, Chumdermpadetsuk S, Sentrakul P, Safary A. Protective efficacy of a recombinant DNA hepatitis B vaccine in neonates of HBe antigen-positive mothers. JAMA 1989;261:3278-3281.
17. Carman WF, Zanetti AR, Karayiannis P, Waters J, Manzillo G, Tanzi E, Zuckerman AJ, Thomas HC. Vaccine-induced escape mutant of hepatitis B virus. Lancet 1990;336:325-329.
18. Stevens CE, Toy PT, Taylor PE, Lee T, Yip HY. Prospects for control of hepatitis B virus infection: implications of childhood vaccination and long-term protection. Pediatr 1992;90:170-173.
19. Lo KJ, Lee SD, Tsai YT, Wu TC, Chan CY, Chen GH, Yeh CL. Long-term immunogenicity and efficacy of hepatitis B vaccine in infants born to HBeAg-positive HBsAg-carrier mothers. Hepatology 1988;8:1647-1650.
20. Jilg W, Schmidt M, Deinhardt F. Decline of antiHBs after hepatitis B vaccination and timing of revaccination. Lancet 1990;1:173-174.
21. Lee SD, Lo KJ, Tsai YT, Wu JC, Wu TC, Yang ZL, Ng HT. Role of Caesarean section in prevention of mother-infant transmission of hepatitis B virus. Lancet 1988;2:833-834.

CHAPTER 4.1

INTRODUCTION OF THE NATIONAL PROGRAM

Background information

The total population of the Netherlands is 15 million with approximately 200,000 deliveries per year. Based on the estimated prevalence of the HBsAg carrier state in the general population (0.1% to 0.5%), the country is considered an area of low endemicity. The strategy for the prevention of hepatitis B has been to immunize certain groups at high risk of hepatitis B virus exposure.

In 1983 the National Health Council (Gezondheidsraad) recommended hepatitis B vaccination for high risk groups including infants of HBsAg-positive mothers (1).
Since then, several policy decisions are worth noting. The Sickness Funds Council (Ziekenfondsraad) placed hepatitis B vaccine at disposal free of charge for those infants born to HBsAg- and HBeAg-positive mothers in 1984 only after authorization of the individual health insurances (2). At that time Mazel and colleagues presented the promising results of a multicenter trial for the prevention of perinatal HBV infection.
It was determined that screening of all pregnant women for HBsAg could effectively be introduced in prenatal care. Delayed active immunization starting at 3 months of age, concomitant with DTP-polio vaccination, in infants born to HBsAg-positive mothers was found an effective and, for reasons of compliance and low cost, attractive alternative to early active immunization starting at birth (3-4).
Based on these study results a National Hepatitis Advisory Committee was installed in 1985 by the Chief Medical Officer of Health (Geneeskundige Hoofdinspectie van de Volksgezondheid). This Committee, composed of government officials and medical experts, was organized to provide policy guidance and allocation of resources for a national program on the prevention of perinatal hepatitis B infection.
In January 1986, recommendations were given to the Minister of Health that included routine screening of pregnant women for HBsAg and passive-active immunization of infants of HBsAg-positive mothers (5). A cost-benefit analysis indicated annual savings of 167,400 Dutch florins (about 83.700 US dollars at that time).
Thereupon, the Minister asked the technical advice of the Sickness Funds Council in 1987. This led to the provision of routine screening for HBsAg paid for by the Exceptional Medical Expenses Act (AWBZ) and provision of hepatitis B vaccine without previous authorization in 1988 by the individual health insurances for all infants regardless the HBeAg-status of the mother (6).
Thereafter, the National Hepatitis B Steering Committee, consisting of experts and health officers of all parties involved, was formed to implement and publicize the national program guidelines.
In September 1989, one month before the commencement of the national program for the prevention of perinatal HBV infection, an instruction manual outlining the exact procedures to be used was distributed to health care professionals (7). The screening of pregnant women for HBsAg, preferably in their first trimester of pregnancy, was added to the recommended prenatal laboratory tests (i.e. rhesus blood groups and syphilis). The infants of HBsAg-positive mothers should receive hepatitis B immune globulin directly after birth and

hepatitis B vaccine is to be administered concomitantly with the DTP-polio vaccine at 3, 4, 5, and 11 months of age.

The National Institute of Public Health and Environmental Protection (RIVM) is responsible for the evaluation of the coverage of the program as well as technical advice and research support to the Ministry of Health. The National Hepatitis B Steering Committee evaluates the ongoing program at annual meetings.

References

1. Gezondheidsraad. Advies inzake hepatitis B. Rijswijk, 1983;22 (in Dutch).
2. Ziekenfondsraad. Advies inzake wijziging bijlage 3 behorende bij het Besluit Farmaceutische Hulp Ziekenfondsverzekering. Amstelveen, 1984 (in Dutch).
3. Mazel JA, Schalm SW, Gast GC de, Nuijten ASM, Heijtink RA, Botman MJ, Bänffer JRJ, Gerards LJ, Zwijnenberg J, Mettau J, Wladimiroff JW, Fetter WPF. Passive-active immunization of neonates of HBsAg-positive carrier mothers: preliminary observations. Br Med J 1984;288:513-515.
4. Mazel JA, Heijtink RA, Schalm SW, Gerards LJ, Botman MJ, Bänffer JRJ, Gast de GC, Nuijten ASM, Wladimiroff JW, Zwijnenberg J, Mettau J, Fetter WPF. Gecombineerde passieve en actieve immunisatie van zuigelingen van HBsAg-positieve moeders. Ned Tijdschr Geneeskd 1985;129:590-594 (in Dutch).
5. Advies werkgroep van deskundigen ingesteld door de Geneeskundige Hoodinspectie van de Volksgezondheid. Preventie van hepatitis B bij pasgeborenen d.m.v. screening van zwangeren op hepatitis B antigenen en inenting van de pasgeborenen van positief bevonden moeders. Leidschendam, Januari 1986 (in Dutch).
6. Ziekenfondsraad. Advies inzake screening op hepatitis-B bij zwangeren. Amstelveen, Februari 1988 (in Dutch).
7. Geneeskundige Hoofdinspectie van de Volksgezondheid. Preventie hepatitis B bij pasgeborenen. Organisatie en werkwijze van de HBsAg-screening van zwangeren en de passieve en actieve immunisatie van de pasgeborenen van HBsAg-positieve moeders. GHI-bulletin, Rijswijk, September 1989 (in Dutch).

CHAPTER 4.2

PROGRAM FOR THE PREVENTION OF PERINATAL HEPATITIS B INFECTION THROUGH SCREENING OF PREGNANT WOMEN AND IMMUNIZATION OF INFANTS OF HEPATITIS B CARRIER MOTHERS IN THE NETHERLANDS, 1989 TO 1992

P.M. Grosheide[1], J.M. Klokman-Houweling[1] and the National Hepatitis B Steering Committee

Center of Infectious Disease Epidemiology[1], National Institute of Public Health and Environmental Protection (RIVM), Bilthoven, the Netherlands.

Submitted for publication.

SUMMARY

A program for the prevention of perinatal hepatitis B infection was launched in the Netherlands on October 1, 1989. Infants of hepatitis B positive carrier mothers detected, by routine screening, receive hepatitis B immune globulin at birth and four doses of hepatitis B vaccine in conjunction with routine immunization at 3, 4, 5, and 11 months of age. Results of screening and immunization from 1989 to 1992 indicate that the coverage of screening increased from 46% in 1989 to 84% in 1992. Hepatitis B surface antigen was detected in 2,145 women (0.44%). The coverage of postnatal immunoprophylaxis in 1,645 neonates born to hepatitis B carrier mothers was 85%; in 3% there was a delay in immune globulin administration beyond 24 hours. In 1991, 96%, 95%, 94% and 87% of the infants received the first, second, third and fourth dose of vaccine, respectively. Vaccination was refused by the parents in 5 infants. There was considerable variation in vaccine administration: 17% of the infants received their first dose more than two weeks late. Of the 59% of infants who received the fourth dose more than 2 weeks beyond target age, 14% also received their first dose too late. It is concluded that a perinatal hepatitis B prevention program in a low prevalence area - when incorporated into existing health care - is feasible and achieves satisfactory coverage rates. Intensive follow-up is needed to improve adherence to the immunization schedule.

INTRODUCTION

In low endemic countries like the Netherlands, the prevention of hepatitis B infection focuses on individuals at risk. Immunization with hepatitis B vaccine is therefore recommended for certain well-defined groups (1). Since the vertical transmission of the hepatitis B virus (HBV) plays an essential role in the maintenance of the reservoir of chronic carriers of HBV, neonates born to carriers of the hepatitis B surface antigen (HBsAg) constitute an important target group. A multicenter study in the Netherlands showed an HBsAg prevalence of 0.7% among the pregnant population screened (2). Of these HBsAg-positive women, 17% was hepatitis B e antigen (HBeAg) positive and therefore highly infectious for their offspring (3-4). With an annual number of births of approximately 200,000, about 1,400 infants will be born to HBsAg-positive women each year. Infections acquired soon after birth are almost always asymptomatic but are most likely to become persistent (5).

Administration of hepatitis B immune globulin (HBIg) and hepatitis B vaccine to infants of HBsAg-positive mothers has been shown to be safe and highly efficacious in preventing perinatal HBV infection (6-8). By identifying HBsAg-positive women through antenatal testing, HBIg can be offered to their infants soon after birth. HBIg given directly after birth followed by hepatitis B vaccination at the ages of 3, 4, 5, and 11 months, concomitant with the diphtheria-tetanus-pertussis-polio (DTP-polio) injections, can prevent more than 90% of

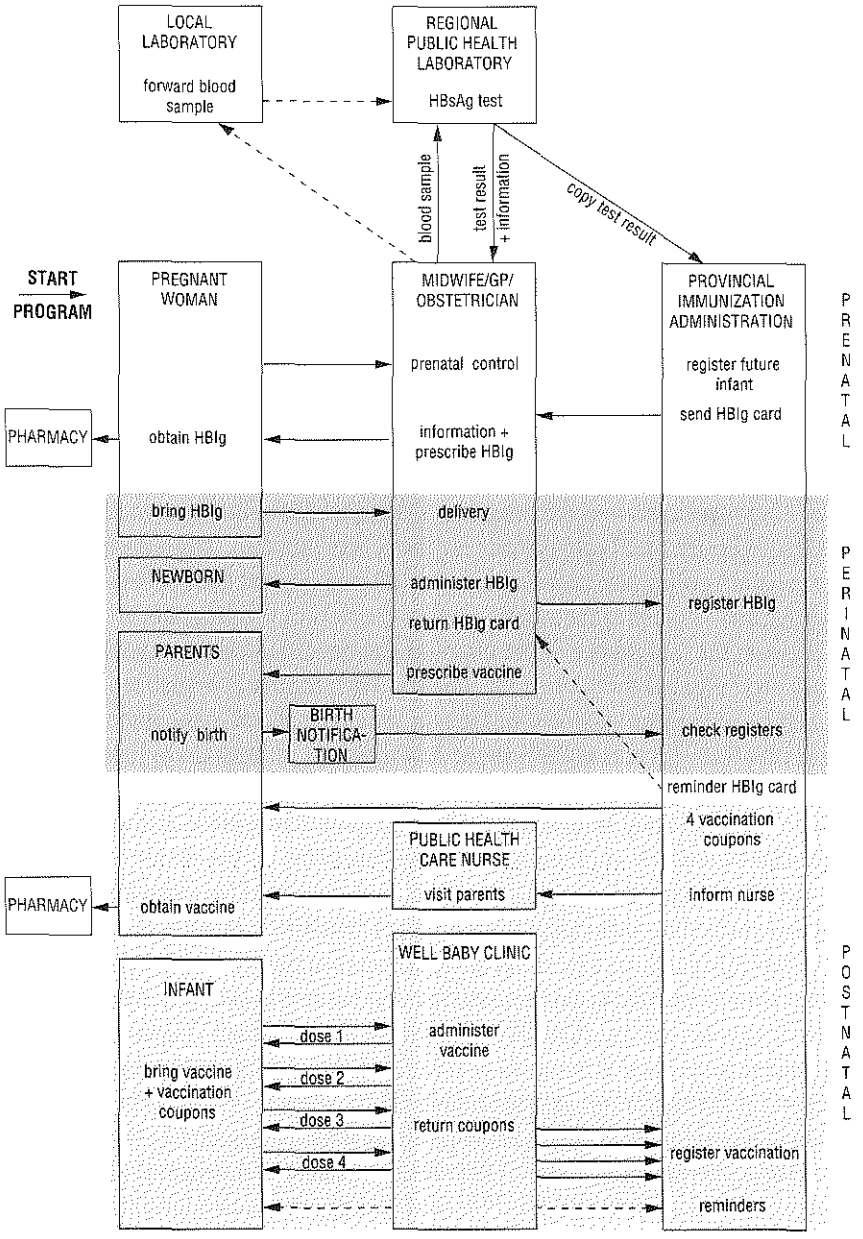


Figure I

Flow chart of the hepatitis B prevention program in the Netherlands through screening of pregnant women for HBsAg and passive-active immunization of infants of mothers found HBsAg-positive.

hepatitis B infections and is associated with high antibody responses (8-10).
With a high dose of HBIg at birth, the timing of the start of vaccination appears less important, so for reasons of compliance and low cost, delayed active immunization in conjunction with visits for routine immunization was considered an attractive alternative to early active immunization as recommended by the manufacturers of the vaccine (8-10).

On October 1, 1989 a nationwide program was launched to detect HBsAg-positive women by routine antenatal screening in the first trimester of pregnancy. Passive-active immunization against hepatitis B is advised for infants of HBsAg-positive women.
This paper describes the program logistics and coverage rates between October 1, 1989 and December 31, 1992.

PATIENTS AND METHODS

National guidelines

Two years before initiation of the program, a National Steering Committee with representatives of health care professionals involved, was installed. This Committee was to provide the program policy, the coordination, the information and the implementation in clinical practice and to monitor the results. Before the start of the program, an instruction booklet explaining the importance of the program and the procedures was widely distributed to health care professionals throughout the country. The program is visualized in Figure 1.

The screening of pregnant women for HBsAg should be added to the routine testing for ABO, rhesus blood groups and syphilis in the first trimester of pregnancy. Laboratory facilities for this "prenatal panel" were enhanced in the existing 16 Regional Public Health Laboratories (RPHL) already responsible for blood typing and syphilis testing. The HBsAg test is performed by enzyme or radioimmunoassay depending on the choice of the laboratory. All HBsAg-positive samples are confirmed and tested for other HBV markers, i.e. HBeAg and antibodies to hepatitis B core antigen (antiHBc). Positive test results are forwarded to the person taking care of the pregnant woman together with a protocol defining how an infant born to an HBsAg-positive mother is to receive hepatitis B immune globulin and hepatitis B vaccine. An information leaflet for the future mother is issued. Screening at delivery should be performed if no result of HBsAg testing during pregnancy is available.

The Provincial Immunization Administration (PIA) which maintains data on births and childhood vaccination for every infant in the province until the age of 13 years receives a copy of the HBsAg-positive test result of the women residing in the region. This copy also states the expected date of delivery. The PIA sends a registration card for the future administration of HBIg to the professional who takes care of the HBsAg-positive woman.
In the Netherlands, where the proportion of home deliveries is high, the professional prescribes HBIg. Infants receive 1 ml HBIg containing 300 IU antiHBs/ml (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service) intramuscularly as soon as possible after delivery, preferably within 2 hours after birth. After

the administration of HBIg, the registration card is returned to the PIA and the mother is given a prescription for hepatitis B vaccine by the attending professional.

The PIA is notified of all births by the local Registry of Births and thereupon checks the files on infants of HBsAg-positive mothers. If no HBIg card is returned two weeks after the expected delivery date indicated on the laboratory form, the PIA contacts the caretaker of the woman on the outcome of pregnancy and HBIg administration.

Public health care nurses visit mothers around the tenth day after delivery. Information about the need for immunization of her infant and how to obtain hepatitis B vaccine is given to the HBsAg-positive mothers. Access to primary health care is excellent: almost all infants visit the Child Health Clinics (CHC) for routine check ups and childhood vaccinations which are free of charge. A vaccination booklet containing four coupons for DTP-polio vaccine plus four coupons for hepatitis B vaccine is mailed by the PIA to the mother. Infants receive 1 ml of recombinant hepatitis B vaccine intramuscularly in the anterolateral part of the thigh (or arm). The DTP-polio injection is given in the contralateral thigh at the same visit. The vaccination coupons containing basic information about the vaccinee are returned to the PIA.

If the appropriate coupon is not received within 2 weeks after the target age, the PIA sends a reminder for vaccination to the parents.

Evaluation of coverage and compliance

Quarterly results of the screening, laboratory procedures and information on parity and country of origin of the expectant HBsAg-positive women are sent to the National Institute of Public Health and Environmental Protection (RIVM). The coverage of screening was estimated by the number of screening tests of pregnant women reported by the RPHL divided by the number of notified births obtained from the PIA in the same period. The actual number of doses of HBIg and hepatitis B vaccine administered are obtained from the PIA on registration forms also stating vaccination failures and the reasons. Data on infants born in Amsterdam are not included since follow-up of infants of HBsAg-positive mothers is in the hands of the Municipal Health Service that uses the 0, 1, 6 months vaccination schedule.

The target ages for the general vaccination schedule in the Netherlands are 13, 17, 22, and 48 weeks, respectively. The data on the coverage of vaccination presented include the birth cohort 1991, whose youngest member had reached the target age for the fourth dose of hepatitis B vaccine at 11 months of age. The infants studied for adherence to the vaccination schedule were those born between October 1, 1989 and December 31, 1992.

Table 1

Coverage of screening and prevalence for HBsAg in pregnant women in the Netherlands per year and coverage rates of passive-active immunization in infants born to the HBsAg-positive mothers between 1989 and 1991 are given.

	1989* N (%)	1990 N (%)	1991 N (%)	1992 N (%)
Live births	46,351	197,198	199,057	195,730
Samples tested	21,275§ (46)	142,878 (73)	158,255 (80)	165,076 (84)
Samples HBsAg+	97 (0.46)	639 (0.45)	732 (0.46)	677 (0.41)
Born to HBsAg+↕	56	457	560	572
HBIg	39 (70)	385 (84)	483 (86)	484 (85)
Vaccine 1	49 (88)	423 (93)	537 (96)	n.g.
Vaccine 2	48 (86)	414 (91)	532 (95)	
Vaccine 3	45 (80)	402 (88)	525 (94)	
Vaccine 4	38 (68)	327 (72)	489 (87)	

* Last quarter only.
\+ Plus sign indicates HBsAg-positive mother.
§ Data of 3 Regional Public Health Laboratories are missing.
↕ Data Amsterdam excluded.
n.g. Data not given.

RESULTS

During the 1989-1992 period 487,484 blood samples of pregnant women were tested for HBsAg in one of the RPHL. The coverage of screening rose from 46% in 1989 to 84% in 1992 (Table 1). The overall prevalence for HBsAg was 0.44% (n=2145). The marked variation between the prevalence found in the urban centers Amsterdam and Rotterdam (0.79%) and the other mixed urban-rural regions (0.33%) is shown in Figure 2. Screening for HBsAg at the time of delivery was performed in 174 cases; of these samples 3 (1.7%) were HBsAg-positive. Of the HBsAg-positive women identified 15% was HBeAg-positive. The country of origin was stated in only 42% of the HBsAg-positive women: most women originated from Mediterranean countries such as Turkey (36%) and Morocco (10%) or Asia (23%). Only 11% was of Dutch-Caucasian origin (Figure 3). Of the HBsAg-positive women 10% was already known as HBsAg-positive through previous testing. A percentage of 34 of the HBsAg-positive women was nulliparous.

1,645 infants were born to these HBsAg-positive mothers. HBIg was given to 85% of the

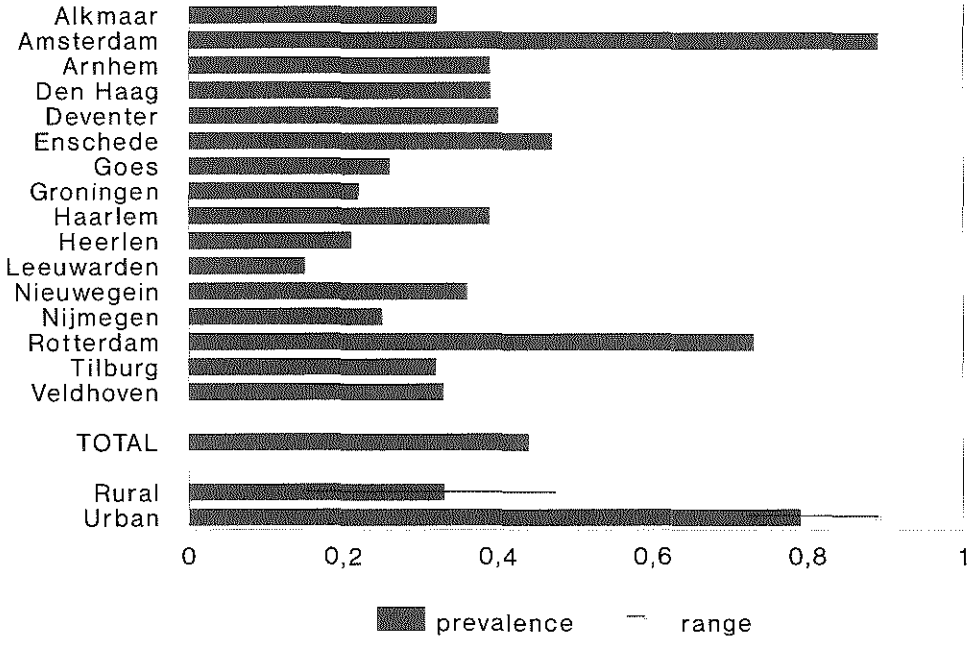


Figure 2
Prevalence for HBsAg among pregnant women per Regional Public Health Laboratory and for the large cities Amsterdam and Rotterdam (urban) versus the other areas (rural), 1989-1992.

infants (n=1391); 97% received HBIg within 24 hours after birth and 99% within the first week of life. In the remaining 15% of infants, the HBIg injection was either not registered or not administered. In 259 cases (16%) no HBIg card was sent to the person taking care of the parturient because the PIA had not been notified of the HBsAg-positive woman. Throughout the study, the coverage rates of vaccination showed a rising trend and in 1991 a coverage of 96%, 95%, 94% and 87% were registered for the four doses of vaccine, respectively (Table 1). However, the coverage for the PIAs ranged from 45-100% for HBIg and 77-100%, 69-100%, 62-98% and 46-96% for the first, second, third and fourth dose of vaccine, respectively. The lowest coverage rates involved two PIAs.
Of the infants who were not immunized, it was noted that 18 left the country, 7 infants died of reasons not associated with the HBV vaccination and 5 parents refused vaccination on religious grounds. Information on the number of vaccinees and moment of administration of the vaccine is given in Tables 2 and 3. 31 infants started vaccination within their first week of life. 17% of the infants received the first dose of vaccine more than two weeks later than planned. This percentage increased to 59% for the fourth dose of vaccine of which 14% of

infants already received the first dose of vaccine beyond the designated time (Table 3). No side effects of HBV vaccination were reported.

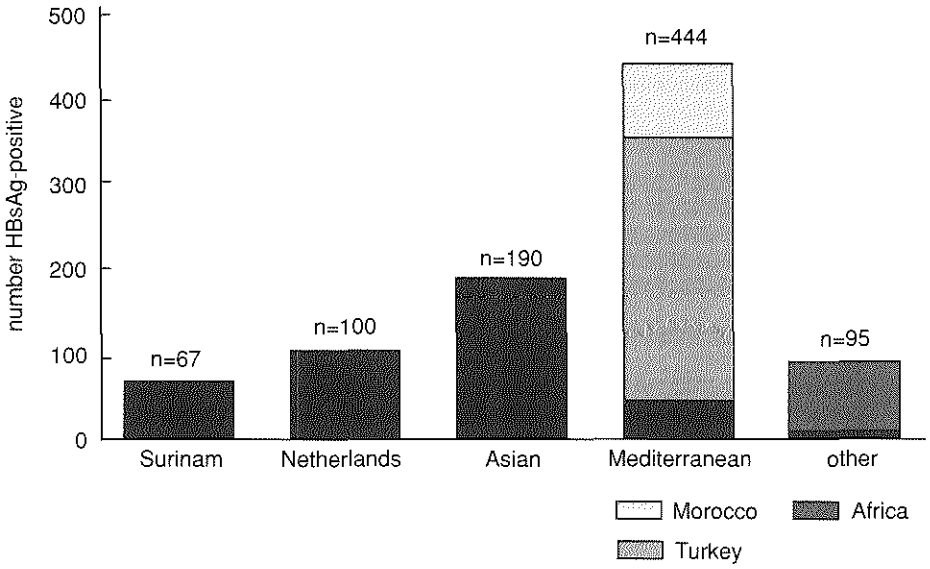


Figure 3
Country of origin of the HBsAg-positive women detected in the Regional Public Health Laboratories, 1989-1992.

DISCUSSION

The results show the success of prenatal screening of pregnant women for HBsAg to identify infants at risk for HBV infection in a country with low endemicity. The integration of screening into routine antenatal care early in pregnancy and the integration of hepatitis B vaccine into the existing childhood immunization program are effective because of excellent access to primary health care and contribute to encouraging coverage rates.

The coverage of screening was more complete than reported in Italy after 3 years of screening (51%) but similar to that reached in Taiwan after fifteen months (78%) (11-12). In both countries, national screening programs were introduced in 1984 but these countries differ from the Netherlands with respect to endemicity for HBV and organization of health care. In Taiwan, the program was launched after an intensive information campaign utilizing mass media. In Italy and the Netherlands, only general guidelines for the program were

Table 2
Time in weeks at which infants of HBsAg-positive mothers received active immunization in relation to the target age, 1989-1992.

Vaccine	Number	Weeks		
		Target age	Median	5th;95th percentile
Dose 1	1509	13	14	9;20
Dose 2	1461	17	18	15;26
Dose 3	1378	22	24	21;36
Dose 4	982	48	51	47;67

Table 3
Compliance to the active immunization schedule projected as the number of infants immunized more than 2 weeks after the target age, 1989-1992.

Vaccine	Total immunized	> 2 weeks after target age		Dose 1 also > 2 weeks late*	
	n	n	(%)	n	(%)
Dose 1	1509	258	(17)	-	-
Dose 2	1461	371	(25)	219	(15)
Dose 3	1378	520	(38)	205	(15)
Dose 4	982	583	(59)	141	(14)

* When an infant receives the first dose of vaccine > 2 weeks later than planned it is likely that the additional doses are also delayed.

forwarded to the professionals concerned. In Italy the coverage rose to 71% after 5 years. Recently screening of pregnant women in Italy has been made compulsory by law (13).

Migration as well as termination of an unknown number of pregnancies preclude accurate calculation of the proportion of pregnant women screened for HBsAg who actually gave birth in the study period. It is not known how many pregnant women were not screened or were possibly tested in non-participating laboratories since 16% of infants were reported to the PIA in the postnatal period. It is encouraging that the coverage of screening in the RPHL increased over the years. However, the compliance to screening varied from region to region depending on the presence of other laboratories involved in antenatal screening.

The overall prevalence for HBsAg among the pregnant population was 0.4%. This percentage is influenced by the high prevalence found in Amsterdam and Rotterdam (0.8%). The 0.3% prevalence for HBsAg found for the other RPHL appears a better estimate for the pregnant population in the Netherlands. The 0.4% prevalence is lower than described in previous studies where 0.7% of the pregnant women tested were HBsAg-positive (2). This may have several causes.

Firstly, the ignorance of professionals regarding the screening program and failure to re-screen women known as hepatitis B carriers in subsequent pregnancies are noted. Secondly, the pregnant population from which screening data are missing could belong to a socially underprivileged group not seeking routine antenatal care. For instance, screening at delivery in Rotterdam, in those cases in which no antenatal HBsAg test results were available, showed that the prevalence of HBsAg for this group of women was more than double than observed for routinely screened women (2,8-9). Screening at delivery, usually outside office hours, was performed in a limited number of cases for financial and logistical reasons.

Of the 174 tests performed at delivery, HBsAg positivity rate was found to be four times higher (1.7% versus 0.4%). Thirdly, selection of populations previously studied may also play a role since the prevalence of HBsAg in Rotterdam was 0.7% and a 1.5% prevalence was described earlier (2).

85% of the infants born to HBsAg-positive mothers received HBIg. Non registration by the PIA in the remaining 15% may not necessarily mean that HBIg was not administered. Some candidates for immunoprophylaxis were apparently detected in laboratories other than the RPHL and administration of HBIg was not always retrievable in those cases. In large hospitals where the number of attending professionals is high, the registration appeared to be incomplete. The suboptimal coverage of HBIg needs full attention since active immunization is delayed until the age of 3 months.

Active immunization was completed in 87% of the infants in 1991. Again, the coverage rates presented are probably an underestimate since registration of immunizations appears to be incomplete.

Unnecessary reminders took place because parents lost the vaccination coupons or the attending professionals failed to return the relevant coupons. Inquiries are under way to analyze reasons for incomplete records in certain regions. Incompleteness of the records was not influenced by the number of infants in need for vaccination in these regions.

Nevertheless, the completion rate is much higher than reported for the United States where 33%, 59% and 76% of infants received HBIg and three doses of vaccine according to the 0, 1, and 6 months schedule (14-16). Since most infants in the United States are born in hospital, the first dose of vaccine is preferably administered before discharge from the hospital; this was achieved in 84% of New York infants (14).
It is likely that the administration of hepatitis B vaccine in combination with the national childhood vaccination program results in a high compliance (8-9,17). Still, the 87% of infants that completed the series of hepatitis B vaccine is less than the 93 percent of infants that completed the series of DTP-polio vaccine. Unfortunately, data on the coverage of DTP-polio vaccination in our infants are not available. However, lower immunization rates for DTP-polio vaccine are found in certain groups and regions of the country, including a substantial proportion of children of foreign workers (18). A recent study performed among over 6,500 infants in Amsterdam confirmed that four doses of DTP-polio vaccine were administered in 95% of the Dutch infants, 90% of infants from Surinam, 87% from Morocco and 88% from Turkey (H Pouw; personal communication). Since most of the infants requiring hepatitis B vaccine have parents originating from Mediterranean or other immigrant countries, demographic variables and language barriers could therefore be important reasons for incomplete immunization. It is unknown how many of these infants returned to their native countries. A decline in the administration of the booster dose is also reported by others (11,19).

Although the coverage rates may not be fully reliable, the results over the years are encouraging. It should be stressed, however, that in 17% of the infants, vaccination was not started according to the schedule. Mostly vaccinations were delayed although 31 infants started immunization within the first week of life as recommended by the manufacturers of the vaccine. If an infant receives the first dose of vaccine later than the target age, it is most likely that the 2nd and 3rd dose of vaccine will also be delayed since the recommended time interval between doses is four weeks. Therefore, we looked in more detail to the vaccination data of 258 infants who received their first dose of vaccine more than two weeks later than planned. Of these infants 15% also received their subsequent doses later than recommended (Table 3). An additional 45% of infants received their booster dose later than the target age of 11 months. Off schedule immunization remains a matter of concern although some delay in the administration of vaccine does not appear to have an effect on the protective efficacy in Gambian infants (19).

In the Netherlands the antibody levels after vaccination are not tested since a health care system responsible for blood sampling of infants does not exist. The need for follow-up serologic testing of infants is questionable; several controlled studies have shown that antiHBs seroconversion occurs in over 90% of the infants (6-8). Protective antibody levels develop using a wide range of schedules (20). In order to establish the efficacy of the present program in clinical practice, however, serological follow-up of infants is strongly recommended.

In conclusion, the national program for the prevention of perinatal hepatitis B infection

was successfully implemented in the existing health care. However, improvements have to be made. Routine antenatal screening for HBsAg should be enhanced, preferably in the selected laboratories. Vaccination should be improved through motivation of the parents involved and education of health care workers. The registration procedures also need improvement.

Acknowledgments

The authors wish to thank all health care professionals responsible for the implementation of the prevention program. Members of the National Hepatitis B Steering Committee are: HP Verbrugge and R Latuperisa (Chief Inspectorate for Public Health), JA Mazel (Society of General Practitioners), AA Lems (Organization of Midwives in the Netherlands), TjD Ypma (Dutch Society of Obstetrics and Gynaecology), LJ Gerards (Dutch Paediatric Association), GJ Aarts (Municipal Public Health Service), DJA Bolscher (Youth Health Care Service), GJJ Duffels (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service), A Koppenaal, B Engel and PM Grosheide (National Institute of Public Health and Environmental Protection), JG van der Voorn (Provincial Immunization Administration), EH Koster (Association of Dutch Health Insurance), JB van der Steur (Sickness Funds Council).

References

1. Gezondheidsraad. Advies inzake hepatitis B vaccinatie. 's-Gravenhage 1983; rapport nr 22 (in Dutch).
2. Grosheide PM, Wladimiroff JW, Heijtink RA, Mazel JA, Christiaens GCML, Nuijten ASM, Schalm SW. Antenatal screening for hepatitis B surface antigen: policy proposal for routine screening at 14 weeks. Submitted for publication.
3. Okada K, Kamiyama I, Inomata M, Imai M, Miyakawa Y, Mayumi M. E antigen and anti-e in the serum of asymptomatic carrier mothers as indicators of positive and negative transmission of hepatitis B virus to their infants. N Engl J Med 1979;294:746-749.
4. Stevens CE, Neurath RA, Beasley RP, Szmuness W. HBeAg and antiHBe detection by radioimmunoassay: correlation with vertical transmission of hepatitis B virus in Taiwan. J Med Virol 1979;3:237-241.
5. Beasley RP, Hwang LY, Lee GC, Lan CC, Roan CH, Huang FY, Chen CL. Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. Lancet 1983;2:1099-1102.
6. Beasley RP, Hwang LY, Stevens CE, Lin CC, Hsieh FJ, Wang TS, Szmuness W. Efficacy of hepatitis B immune globulin for prevention of perinatal transmission of the hepatitis B virus carrier state: final report of a randomized double-blind, placebo-controlled trial. Hepatology 1983;3:135-141.
7. Stevens CE, Toy PT, Tong MJ, Taylor PE, Vyas GN, Nair PV, Gudavalli M, Krugman S. Perinatal hepatitis B virus transmission in the United States: Prevention by passive-active immunization. JAMA 1985;253:1740-1745.
8. Schalm SW, Mazel JA, de Gast GC, Heijtink RA, Botman MJ, Bänffer JRJ, Gerards LJ, Zwijnenberg J, Fetter WPF, Nuijten ASM, Wladimiroff JW, Christiaens GCML. Prevention of hepatitis B infection in newborns through mass screening and delayed vaccination of all infants of mothers with hepatitis B surface antigen. Pediatrics 1989;83:1041-1047.
9. Mazel JA, Schalm SW, de Gast GC, Nuijten ASM, Heijtink RA, Botman MJ, Bänffer JRJ, Gerards LJ, Zwijnenberg J, Mettau J, Wladimiroff JW, Fetter WPF. Passive-active immunization of neonates of HBsAg-positive carrier mothers: preliminary observations. Br Med J 1984;288:513-515.
10. Grosheide PM, del Canho R, Heijtink RA, Nuijten ASM, Zwijnenberg J, Bänffer RJR, Wladimiroff JW, Botman MJ, Mazel JA, de Gast GC, Christaens GCML, Gerards LJ, Fetter WPF, Baerts W, Schalm SW. Comparison of the efficacy of early and delayed active immunization in infants of HBsAg and HBeAg-positive mother receiving hepatitis B immune globulin at birth. AJDC 1993;147 (in press).

11. Chen DS, Hsu NHM, Sung JL, Hsu TC, Hsu ST, Kuo YT, Lo KJ, Shih YT. The Hepatitis Steering Committee and The Hepatitis Control Committee. A mass vaccination program in Taiwan against hepatitis B virus infection in infants of hepatitis B surface antigen carrier mothers. JAMA 1987;257:2597-2603.
12. Stroffolini T, Pasquini P, Mele A and Collaborating Group for Vaccination against Hepatitis B in Italy. A nationwide vaccination program in Italy against hepatitis B virus infection in infants of hepatitis B suface antigen carrier mothers. Vaccine 1989;7:152-154.
13. Stroffolini T, Pasquini P and Collaborating Group. Five years of vaccination campaign against hepatitis B in Italy in infants of hepatitis B suface antigen carrier mothers. Ital J Gastroenterol 1990;22:195-197.
14. Henning KJ, Pollack DM, Friedman SM. A neonatal hepatitis B surveillance and vaccination program: New York City, 1987 to 1988. Am J Public Health 1992;82:885-888.
15. Jonas MM, Reddy RK, Madina de M. Hepatitis B infection in a large municipal obstetrical population: characterization and prevention of perinatal transmission. Gastroenterol 1990;85:277-280.
16. Niu MT, Targonski PV, Stoll BJ, Albert GP, Margolis HS. Prevention of perinatal transmission of the hepatitis B virus. Outcome of infants in a community prevention program. AJDC 1992;146:793-796.
17. Villa da G, Piazza M, Iorio R, Picciotto L, Peluso P, de Luca G, Basile B. A pilot model of vaccination against hepatitis B virus suitable for mass vaccination campaigns in hyperendemic areas.
J Med Virol 1992;36:274-278.
18. Verbrugge HP. The national immunization program of the Netherlands.
Pediatrics 1990;86(suppl):1060-1063.
19. Inskip HM, Hall AJ, Chotard J, Loik F, Whittle H. Hepatitis B vaccine in the Gambian Expanded Programme on Immunization: factors influencing antibody response. Int J Epidemiol 1991;20764-769.
20. West DJ, Calandra GB, Hesley TM, Ioli V, Miller WJ. Control of hepatitis B through routine immunization of infants: the need for flexible schedules and new combination vaccine formulations. Vaccine 1993;11:S21-27.

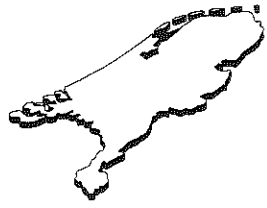
CHAPTER 4.3

IDENTIFICATION OF FACTORS ASSOCIATED WITH NON-COMPLIANCE TO THE NATIONAL PROGRAM FOR THE PREVENTION OF PERINATAL HEPATITIS B INFECTION IN THE NETHERLANDS

P.M. Grosheide[1], A.L. den Ouden[2], G.H. Verrips[2], J.G. Loeber[3], M.A.E. Conyn-van Spaendonck[1], S.P. Verloove-Vanhorick[2]

Center for Infectious Disease Control[1] and Unit Teratology, Endocrinology and Perinatal Screening[3], National Institute of Public Health and Environmental Protection (RIVM), Bilthoven;Department of Child Health[2], TNO Institute of Preventive Health Care (NIPG-TNO), Leiden, the Netherlands.

An adapted version of this paper has been accepted for publication in Nederlands Tijdschrift voor Geneeskunde.



SUMMARY

A program for the prevention of perinatal hepatitis B infection in the Netherlands was introduced in October 1989. The program included the following elements: HBsAg screening for all pregnant women was added to routine antenatal testing in the Regional Public Health Laboratories and hepatitis B immunization of infants of HBsAg-positive mothers was added to the routine program on immunization. The effectiveness of the program in 1990 was reviewed and factors associated with incomplete coverage of screening and deficient immunization were identified. Semi-structured interviews were carried out among the participating health care professionals to evaluate their experience and future proposals.

In about 70% of the pregnant women, prenatal HBsAg screening was performed according to protocol. For logistical and financial reasons, screening at delivery where prenatal screening for HBsAg failed, seldom occurred. An additional 10% of women were tested in other laboratories than selected; in case of an HBsAg-positive test result, the passive immunization at birth was not ensured.
Further shortcomings were lack of awareness and lack of coordination between professionals. Missed opportunities to immunize include difficult accessibility of hepatitis B vaccine and follow-up problems since most of the risk population involves certain ethnic groups unaware of the benefits of immunization. Poor functioning of the registration system leads to underestimation of vaccine coverage.

The acceptance and implementation of the program were encouraging one year after initiation. More attention should be targeted on education of health care professionals and risk groups involved. Screening should be enhanced in the appointed laboratories. Identification of coordinators who are responsible for the program logistics in every region should result in increased coverage. Centralization of the purchase of vaccine ensures the availability at the appointed time. The efficacy of the program must be assessed continuously.

INTRODUCTION

The hepatitis B virus (HBV) is efficiently transmitted perinatally to infants born to mothers who test positive for the hepatitis B surface antigen (HBsAg) (1-2). Infections acquired soon after birth are almost always asymptomatic and are most likely to become persistent. Immunization of infants of HBsAg-positive carrier mothers can effectively prevent perinatal infection (3-4). By identifying HBsAg-positive women through prenatal testing, immunoprophylaxis can be delivered to their infants soon after birth to prevent HBV infection.

In 1982 a study was initiated in the Netherlands to determine on the one hand whether screening for HBsAg could be introduced into prenatal care successfully (i.e. high

compliance) and on the other hand, to determine whether hepatitis B vaccination given concomitantly with the diphtheria-tetanus-pertussis-poliomyelitis (DTP-polio) vaccination was superior to hepatitis B vaccination initiated immediately after birth as far as compliance, immunogenicity and protective efficacy were concerned (5-6).
It was concluded that prenatal screening of pregnant women for HBsAg could be introduced effectively at reasonable cost in a country with a low prevalence of HBsAg and a high proportion of home deliveries. Delayed active immunization starting at 3 months of age, concurrent with the DTP-polio vaccine, has been shown to prevent more than 90% of infections in infants and resulted in high antibody titers and high compliance to the vaccination schedule (5-6).

These study results provided the basis for the introduction of a national program to prevent perinatal HBV infection using the existing networks of health care services. Starting October 1989, screening for HBsAg was incorporated in the routine laboratory testing during the first antenatal visit. In case of a HBsAg-positive test result, a chain of action should follow to offer passive-active immunization to those infants born to HBsAg-positive mothers. The implementation of the national guidelines during the first 15 months of the program was not complete; about 30% of pregnant women were not tested for HBsAg and the coverage of immunization of infants at risk for HBV infection was also incomplete (7). These results provided a reason for the Sickness Funds Council, financially supporting the screening program, to assess the short-term effectiveness of the program and to identify potential non-economic barriers to adoption of the guidelines. Hereto, the TNO Institute of Preventive Health Care (NIPG-TNO) and the National Institute of Public Health and Environmental Protection (RIVM) were asked to investigate the coverage and shortcomings of the program. We report the reasons for the incomplete execution of the program and suggest means for future improvement.

METHODS

In September 1989, one month before the official start of the national program, an instruction manual which outlined the exact procedures of the program was distributed to the health care professionals involved. In short, a screening test for HBsAg is performed in venous blood from pregnant women that should already be sent to one of the 16 Regional Public Health Laboratories (RPHL) in the Netherlands for rhesus blood groups and syphilis testing. Blood samples drawn from women outside the RPHL, should be sent to the RPHL for prenatal testing. In case of an HBsAg-positive test result, the RPHL notifies the professional taking care of the pregnant woman and the Provincial Immunization Administration (PIA) of the oncoming birth of an infant in need for immunoprophylaxis. The PIA, which maintains immunization records for each infant in the province, provides the attending professional with a card for the registration of the hepatitis B immune globulin (HBIg) injection directly after birth. Analogous to the DTP-polio program, the parents

receive four vaccination coupons for the future administration of hepatitis B vaccine from the PIA. Infants receive their hepatitis B vaccinations, concomitantly with DTP-polio vaccine, at the Child Health Clinic. After each injection the relevant coupon is returned to the PIA which sends reminders to the parents in case of incomplete immunization registers.

In September 1992 all 16 RPHLs were mailed a questionnaire to determine the outcome of HBsAg screening in 1990 and to assess possible drawbacks of the program. A list of other laboratory facilities in the region of each RPHL was included and the RPHL were asked to indicate those laboratories where antenatal testing was supposed to be performed. Data on screening of pregnant women and the number of deliveries in these local laboratories (mainly large hospitals) were then requested by telephone. The coverage of antenatal screening per RPHL region was thereafter estimated from the number of blood samples tested and the number of births in each region in the same period, derived from the Central Bureau of Statistics.

Based upon the data provided by the RPHL, five RPHL regions weighted for urbanisation, screening coverage and prevalence of HBsAg positivity, were selected for further investigation. The practical experience of health care professionals involved in the execution of the program (i.e. midwives, obstetricians, general practitioners, provincial pediatricians, health care nurses) was gathered from three representatives of each profession per region and three local laboratories in each selected region through personal visits. In addition, the seven PIAs maintaining the registers of all infant immunizations in these regions were visited to obtain data on the coverage of hepatitis B vaccination and registration procedures. During the interviews semi-structured questionnaires were used dealing with factors related to non-compliance to the program (i.e. workload, cost) and suggestions for improvement.

RESULTS

All RPHLs were well informed about the hepatitis B prevention program. Some RPHLs have tried to optimize HBsAg screening procedures in their region by sending personalized letters to professionals and laboratories providing obstetric services. In general, the professionals in charge of the pregnant population and immunizations of their offspring were less aware than the RPHL of the program guidelines to identify HBsAg-positive women prenatally and to administer appropriate prophylaxis to their newborn infants.

Screening

In Table 1 the coverage of HBsAg screening in the RPHL and in other than the selected laboratories are presented. Similar coverage rates were achieved for rhesus blood groups and syphilis testing. The coverage ranged from less than 50% to over 100%; the lowest coverage rates were found for those regions where the RPHL had indicated that no screening was

Table 1

Coverage of HBsAg screening of pregnant women in the Regional Public Health Laboratories (RPHL) and in other laboratories in the same region in 1990.

Region	Births	Coverage in RPHL		Coverage in other laboratory		Total coverage	
	n	n	%	n	%	n	%
Alkmaar	7,300	5,920	81	0		5,920	81
Amsterdam	14,273	13,034	91	0		13,034	91
Arnhem	11,033	7,484	68	0		7,484	68
Den Haag	18,490	12,485	68	3,600	19	16,085	87
Deventer	12,163	7,957	65	2,300	19	10,257	84
Enschede	9,019	9,583	106	837	9	10,420	116
Goes	4,504	3,255	72	0		3,255	72
Groningen	12,599	10,977	87	750	6	11,727	93
Haarlem	7,470	6,319	85	626	8	6,945	93
Heerlen	9,620	4,882	51	4,332	45	9,214	96
Leeuwarden	7,499	5,757	77	0		5,757	77
Nieuwegein	20,358	9,201	45	431	2	9,632	47
Nijmegen	10,261	5,632	55	4,975	48	10,607	103
Rotterdam	23,890	21,423	90	1,100	5	22,523	94
Tilburg	18,833	11,183	59	0		11,183	59
Veldhoven	9,199	7,786	85	0		7,786	85
Total	196,511	142,878	73	18,951	10	161,829	82

performed outside the RPHL. On average, about 10% of prenatal testing took place in other laboratories. These local laboratories failed to send blood samples for prenatal testing to the RPHL in the region, for the following reasons: costs and lack of organization to send selected blood samples to the RPHL and impracticability if the same blood sample should be used for additional testing (i.e. haemoglobin) in the laboratory where the blood sample was drawn. Prenatal screening is funded through the Exceptional Medical Expenses Act (AWBZ) if performed in the RPHL. Local laboratories, however, receive a higher fee for the same tests, entered as diagnostic expenses, from the individual insurance companies of pregnant women.

Several other reasons for incomplete coverage of screening in the RPHL exist. Even though blood is drawn from women prenatally, it appeared that the HBsAg assay is omitted in some instances; some professionals do not routinely determine the HBsAg-status and repeated testing in subsequent pregnancies does not always occur. In most of the instances where prenatal screening failed to occur, the women involved were not screened for HBsAg at the time of delivery either, as recommended by the protocol. In addition, for

financial and logistical reasons, few RPHL are willing to perform screening on command outside office hours. In one region, midwives with private practices have to pay postage costs when sending blood samples to the RPHL.
An underestimate of the screening coverage may be observed in regions where professionals do not or cannot indicate on the laboratory forms that the blood samples drawn for HBsAg testing originate from pregnant women.

HBsAg-positive test results

When a woman is identified as HBsAg-positive outside the RPHL, the results of the positive test do not become known to the PIA. When a PIA is not notified of the oncoming birth of an infant in need for immunoprophylaxis, no record is kept for that infant and no card for the registration of the HBIg injection is sent to the professional in charge of the delivery of the HBsAg-positive woman. The providers of obstetric health care neither receive the instructions for the management of the delivery and immunoprophylaxis of the newborn infant, nor do they receive an information leaflet for the future mother from the laboratory.
Many of the positive test results received by the PIA are incomplete and do not state the expected date of delivery of the HBsAg-positive women. This information, necessary for controlling the timely administration of HBIg to the infant, is often difficult to obtain from the attending professionals.

Passive immunization

Several reasons for the incomplete coverage of passive immunization were identified. In some regions, up to 50% of the infants born to HBsAg-positive mothers were reported to the PIA after birth by the public health nurse, the Child Health Clinic (CHC) or the mother. In those cases, screening for HBsAg generally took place outside the RPHL (Table 1) or the mother was previously known as HBsAg-positive and was not offered re-screening.
Much effort of the PIA is needed to ensure whether these infants indeed received their immunoprophylaxis. In hospital settings it is not always clear whether the obstetric or the paediatric services are responsible for the administration and registration of the HBIg. The HBIg card is often not returned to the PIA and information on the actual administration of HBIg to these infants after birth cannot always be obtained.
The person in charge of the delivery of an HBsAg-positive woman has to prescribe the vial of HBIg; the future mother should obtain the HBIg from the pharmacy and store it at home. Consequently, the HBIg is not always available at the time of delivery.

Active immunization

Important factors associated with incomplete immunization start with the inaccurate registration of the vaccine administration. Frequently, hepatitis B vaccination coupons were not returned to the PIA or only after considerable delay. Follow-up problems also occur because of departure of immigrants to their native country or language barriers within certain ethnic groups in need for immunization. The PIAs do not always have the personal support to trace infants that are lost to follow-up.

In addition, other schedules than the schedule nationally recommended, were in use since the manufacturers of the two registered hepatitis B vaccines in the Netherlands advise to start immunization directly after birth and monthly thereafter. Such recommendations may lead to vaccination failure as confusion may arise with regard to whether infants fell into the planned early or delayed national vaccination schedule when the first dose of vaccine had been given at birth.

Moreover, hepatitis B vaccine was not always available when infants did attend the CHC. Parents, dependent on a prescription from the attending professional at delivery, have to obtain and bring the vaccine with the appropriate vaccination coupons to the CHC at the appointed time.

DISCUSSION

This study evaluated the response of professionals to the national recommendations for the prevention of perinatal hepatitis B infection in the Netherlands. To identify factors related to non-compliance, the experience and opinions of representatives of health care services involved in the execution of the program were analyzed. In addition to the fact that the program was considered to be of little importance by some professionals, lack of information may have hindered the implementation of the program. No other governmental action than the distribution of the instruction manual one month before the start of the program was initiated to promulgate the recommendations.

The program for the prevention of perinatal hepatitis B infection in the Netherlands focused on the identification of pregnant women who are HBsAg-positive. A centralized program of routine HBsAg screening for all pregnant women was introduced in October 1989. HBsAg testing was added to the protocol for routine antenatal testing in the 16 RPHLs, therefore, no extra blood samples or requisitions from the obstetric services were needed to carry out the HBsAg screening.

Our study demonstrates the relative success of the introduction of HBsAg screening making use of existing health care services; 73% of the pregnant population was tested for HBsAg in the RPHLs in 1990. It was disappointing that similar coverage rates for rhesus blood groups and syphilis testing were achieved in these RPHL since these tests were considered to be widely accepted.

Several factors, however, make it difficult to determine accurate rates of screening.

Many of the pregnant women screened for HBsAg in 1990 actually gave birth in 1991 so the division of the number of pregnant women screened by the number of births in the same period represents a rough estimate of the coverage of screening. Migration of women out of the region as well as termination of an unknown number of pregnancies also preclude the accurate calculation of the proportion of women screened per RPHL.
There was great variety in the coverage of screening in the various regions (Table 1). In Enschede, where HBsAg screening was initiated in 1982 as part of a multicenter vaccination trial (5-6), a coverage rate of 116% was reached. This suggests that more attention focused on obstetric services to convince them of the importance to screen all pregnant women for HBsAg, may enhance coverage. In those regions where screening was presumed to occur in the RPHL exclusively, the coverage rates were surprisingly low. Communication between RPHL and local laboratories is a critical component and where screening services overlap it should be defined which service has the responsibility for the HBsAg testing.
The local laboratories interviewed were all well aware of the national guidelines for screening but some failed to send prenatal blood samples to the RPHL for practical and financial reasons. More importantly, when an HBsAg-positive test result is found in one of the local laboratories, these laboratories failed to send a copy of the test result to the PIA. In some regions up to 50% of infants born to HBsAg-positive mothers were reported to the PIA only after birth and passive immunization directly after birth could not be guaranteed in many of these infants. It would be preferable for prenatal testing to be centralized in the RPHL to be able to ascertain the completeness of screening and guarantee the correct management of the HBsAg-positive mothers and their offspring at reasonable cost.
To enhance screening in the RPHL, individual insurance companies should refrain from the reimbursement of costs to local laboratories. In cases where testing does occur in other than the RPHL, the laboratories involved should strictly adhere to the program guidelines.
With an overall prevalence of 0.4% HBsAg positivity among the pregnant population the local laboratories will find relatively few HBsAg-positive test results and chances that the program guidelines will be ensured are small.
In general, the appointment of coordinators in every region, responsible for screening logistics and, once an HBsAg-positive woman is identified, for the communication between the health care providers involved, may result in improved program coverage. These individuals could also develop practical guidelines for screening on admission in those cases where prenatal screening for HBsAg did not take place.

Of great concern is the uncertain coverage of the HBIg injection directly after birth. The problem is not posed by the administration itself but by the registration of the HBIg injection. In order to stimulate better use of the HBIg and/or HBIg cards, reimbursement or suppletion of vials could, for instance, be linked to filing out special registration forms after the administration of a vial. A similar system is operative for rhesus-negative mothers in need for anti-D-immunoglobulin.

Access to child health care in the Netherlands is good and up to 93% coverage rates for DTP-polio immunization are achieved during the first year of life (8). Using the

organizations already in place for the administration of infant vaccines and registration of these injections by the CHC and the PIA, respectively, saves additional physician visits and is likely associated with high compliance to hepatitis B immunization in those infants at risk of HBV infection. Factors related to incomplete hepatitis B immunization, however, are found among attending professionals and parents of the infants identified at risk.
Since most infants in need for hepatitis B immunization belong to ethnic minorities, a number of social-cultural variables are directly related to incomplete immunization. Many parents are unaware of the benefits of immunization and immigrant people frequently move or leave for a longer period to their native country. Thus, the coverage of DTP-polio vaccine may also be less than 93% for this group. Better information, adapted to the cultural level of the population involved, is needed. Demonstration of the responsibility the parents share with professionals for securing their children's health may prove helpful.

Opportunities for immunization were missed if the parents did not bring the hepatitis B vaccine to the CHC. Centralization of the purchase of hepatitis B vaccine should be the responsibility of the PIAs with great experience already in the management of vaccines for the national infant vaccination program. An additional advantage is that the vaccine needs no longer to be kept at home where the required storage conditions are not always guaranteed.

Improvement of acceptance and awareness of program guidelines by those professionals involved is a further aspect which may lead to better immunization coverage, as would better instructions on how to deal with infants who are off schedule. Flexible immunization schedules and administration of vaccine whenever there is contact with the CHC should lead to positive results. However, to prevent confusion, the instructions by the manufacturers of the vaccine should be in agreement with the vaccination schedule recommended in the instruction manual.

Validity of the immunization registers kept by the PIA constitute serious limitations and in fact may lead to estimations of immunization coverage below the real levels. Some of the manual systems of resident children and their hepatitis B immunizations were not up-to-date, particularly in areas of high mobility. Hepatitis B injections were not always registered or the vaccination coupons were not returned to the PIA. Clear instructions and appointments between health care providers should be made about improvement of the registration system, not in the least to prevent unnecessary reminders for vaccination to the parents. Again, the identification of coordinators could help to deal with program logistics in every region. The active management of the program by designated individuals will contribute substantially to improvement of coverage and coordination between institutions.

Computerized data management by the PIA may show higher coverage rates and warrant a more adequate system for vaccination reminders within a specified time. They also create opportunities to analyze vaccination coverage in certain groups. For instance, it is important to identify whether incomplete coverage of DTP-polio immunization in infants of HBsAg-positive mothers occurs as well in order to conduct intensified immunization activities in those underserved infants.

Discrepancies in the coverage rates of DTP-polio and hepatitis B vaccination in infants at risk for HBV infection or discrepancies in the moment of administration of both vaccines can be studied in more detail since some parents may well refuse the simultaneous administration of several doses of vaccine to their infants.
Fear for adverse effects or refusal of two injections (hepatitis B and DTP-polio) at one visit were not noticed at present. However, since July 1993 *Haemophilus influenzae* type b injections are added to the primary immunization series already in place and this may result in an infant receiving three injections at each visit. In the near future this could prove to be a new factor limiting the acceptance and administration of the hepatitis B vaccine.

In conclusion, the results of this study suggest several areas where the coverage of the prevention program can be enhanced. In general the organization of more extensive programs that focus on public education and better information for the pregnant population, adapted to the cultural levels, can be recommended. The effectiveness of the program should be increased at both the level of screening and immunization. Professionals should improve their performance and show a more active attitude to raise the responsibility of the parents for their children's health. Appointment of coordinators in every region who are responsible for the development of practical guidelines and program logistics in their district is advised. Continuous assessment of coverage, disease incidence and program efficacy should be made on a national base; the aim of surveillance is to identify potential problems which can then be investigated further.

Although it is important to stress that the interpretation of the results is based on personal experience of some professionals interviewed, we believe that similar concerns exist throughout the country. The issues raised are relevant and therefore deserve full attention.

Acknowledgments

The authors thank all professionals who willingly participated in this study. Special thanks go to professor SW Schalm and professor J Huisman for their critical comments and helpful advise. The study was financed by the Sickness Funds Council, Amstelveen (Ziekenfondraad).

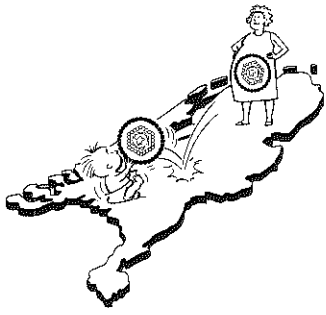
References

1. Beasley RP, Trepo C, Stevens CE, Szmuness W. The e antigen and vertical transmission of hepatitis B surface antigen. Am J Epidemiol 1977;105:94-98.
2. Delaplane D, Yogev R, Crussi F, Shulman ST. Fatal hepatitis B in early infancy: the importance of identifying HBsAg-positive pregnant women and providing immunoprophylaxis to their infants. Pediatrics 1983;72:176-180.
3. Beasley RP, Huang LY, Lee GCY, Lan CC, Roan CH, Huang FY, Chen CL. Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. Lancet 1983;2:1099-1102.

4. Wong VCW, Ip HMH, Reesink HW, Lelie PN, Reerink-Brongers EE, Yeung CY, Ma HK. Prevention of the HBsAg carrier state in newborn infants of mothers who are chronic carriers of HBsAg and HBeAg by administration of hepatitis B vaccine and hepatitis B immunoglobulin. Double-blind placebo-controlled study. Lancet 1984;1:921-926.
5. Mazel JA, Schalm SW, de Gast GC, Nuijten ASM, Heijtink RA, Botman MJ, Bänffer JRJ, Gerards LJ, Zwijnenberg J, Mettau J, Wladimiroff JW, Fetter WPF. Passive-active immunization of neonates of HBsAg positive carrier mothers: preliminary observations. Br Med J 1984;288:513-515.
6. Schalm SW, Mazel JA, de Gast GC, Heijtink RA, Botman MJ, Bänffer JRJ, Gerards LJ, Zwijnenberg J, Getter WPF, Nuijten ASM, Wladimiroff JW, Christiaens GCML. Prevention of hepatitis B infection in newborns through mass screening and delayed vaccination of all infants of mothers with hepatitis B surface antigen. Pediatrics 1989;83:1041-1047.
7. Grosheide PM, Loeber JG. Hepatitis B screening bij zwangeren: een overzicht van 1989 en 1990. Bilthoven, 1991, RIVM rapportnr 199003006 (in Dutch).
8. Verbrugge HP. The national immunization program of the Netherlands. Pediatrics 1990;86(suppl): 1060-1063.

CHAPTER 5

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS



Determination of the vaccination strategy

A successful vaccination program may be defined as one which achieves its aim in terms of disease prevention in the vaccinated group. Two essential ingredients are: first, a safe and effective vaccine and second, an appropriate vaccination strategy with adequate vaccine coverage. In addition to defining the target population an implementation strategy which will ensure good coverage is essential.

In the 1980s, initial controlled clinical trials showed the safety and efficacy of the hepatitis B vaccine (1-3). A decade ago, when the hepatitis B vaccine was first entering the market, a program for the delivery of immunoprophylaxis to neonates born to HBsAg-positive mothers did not exist in the Netherlands. In 1982, a multicenter study for the prevention of perinatal hepatitis B was initiated taking into account the fact that the degree of protection provided by a prevention program is dependent on the efficiency of detection of HBsAg-positive mothers, the efficacy of immunoprophylaxis and the degree of compliance during the immunization program (4). During the subsequent years, it was shown that for a country with low prevalence of HBsAg and well-developed primary health care, incorporation of HBsAg screening into the routine "prenatal panel" for pregnant women was more effective than restriction of screening to certain high-risk groups (4-5, chapter 2.2). Passive immunization of infants, immediately after birth, followed by delayed active immunization concomitant with routine infant immunizations at 3, 4, 5, and 11 months of age provided similar protective efficacy but also higher levels of antibody than active immunization starting at birth as is generally recommended (5-6). Thus, in 1989 theoretical as well as practical considerations supported routine HBsAg screening of pregnant women and the integration of hepatitis B vaccine into the routine infant immunization program for infants at risk for HBV infection. At present, more than ten years after the introduction of the hepatitis B vaccine and three years after the introduction of the national program, this strategy has been proven feasible.

For countries of intermediate and high endemicity, however, such a strategy cannot be recommended. The cost of screening may be prohibitive and the organization of health care cannot support prenatal screening. The cost and limited availability of HBIg also make passive immunization at birth often not possible. Schedules that use the vaccine alone have been shown to be efficacious in preventing HBV infections as well (7-8). Because of the importance of perinatal HBV transmission in these areas, universal hepatitis B immunization of infants as part of their routine immunization schedule, without prior screening of pregnant mothers and HBIg injection, is considered the ideal prevention strategy (9). To maximize protection and minimize cost hepatitis B vaccination should be initiated at confinement.

In contrast, a program featuring universal HBsAg screening of pregnant women and immunoprophylaxis of infants born to HBsAg-positive mothers is the strategy par excellence to control perinatal hepatitis B infection in countries of low endemicity. Our experience may be of practical strategic help to other countries where the epidemiology of hepatitis B is similar to that in the Netherlands. The logistics of whether to initiate

vaccinations in hospital or at the time of the first pediatric out-patient visit will depend on the organization of health care in every country.

In this chapter the following aspects concerning the prevention program are discussed: screening of pregnant women, treatment of infants born to HBsAg-positive women, costs of administering the program, recommendations for increased program effectiveness and future issues.

Practical considerations of screening for HBsAg

Prevention of perinatal hepatitis B infection depends on identifying pregnant women who are HBsAg-positive. Ideally, the identification of HBsAg carriers should occur late in the third trimester or at the time of delivery. In the Netherlands, detection of women at risk for HBV infection at the time of delivery will not be an easy task because of the large number of home deliveries. Instead, HBsAg screening was incorporated into routine antenatal care around 14 weeks of gestation in combination with the screening tests for rhesus blood groups and syphilis. Repeated testing in the third trimester if they were HBsAg-negative can be done in women with ongoing risk for future infection. The number of new HBV infections in pregnant women, however, is low in the Netherlands; between 1982 and 1989, repeated serological testing of 705 pregnant women, initially found HBsAg-positive, showed that only one woman probably had signs of an acute HBV infection (chapter 2.2).

Over 90% of pregnant women found HBsAg-positive by screening originate from countries endemic for hepatitis B where infections are mostly acquired at an early age. Nevertheless, selective screening of women considered at high risk for hepatitis B appeared not to be effective since the criteria failed to identify about half of the Dutch women who were HBsAg carriers (chapter 2.2). Other investigators also reported the failure of risk-factor assessment to detect all women seropositive for HBsAg (10-11).

In 1992, over 84% of pregnant women were offered HBsAg screening in one of the Regional Public Health Laboratories (chapter 4.2). The screening program has one disappointing aspect which is that so far not all professionals involved have collaborated. In 1990 an additional 10% of pregnant women were screened for HBsAg in other than the appointed laboratories (chapter 4.3). At present, screening in other laboratories is not justified because a positive HBsAg result was no incentive to notify institutions involved in the arrangements for treatment of infants of HBsAg-positive mothers and to provide health care information.

Women admitted for delivery who have not had prenatal HBsAg testing should have blood drawn for testing. Women without prior prenatal testing appeared to have a more than two fold higher risk for hepatitis B infection than women who received routine prenatal care (chapter 2.2). The results of serological tests for HBsAg can be available within 2 hours but logistical and financial problems hinder the performance of "rapid" testing in the Regional Public Health Laboratories that have been appointed for screening. In cases where the

laboratory results are pending for more than 12 hours after delivery the administration of HBIg to the newborn infant should be considered.

For the Netherlands, an HBsAg carrier rate of 0.4% among pregnant women is anticipated with a significantly higher rate in inner-city populations associated with a relatively large number of immigrants and parenteral use of drugs (chapter 4.2). If we consider the total number of births at 200,000 per year, 1 in 250 infants is at risk for HBV infection.

Among the women found HBsAg-positive about 15% were also positive for HBeAg. Initially it was demonstrated that the presence of HBeAg indicated the infectiousness of the mother (12-13). More recently the risk of mother-to-infant transmission of HBV appeared primarily related to the presence of HBV DNA in the sera of the HBeAg-positive HBsAg carrier mothers (14-15) and additional testing of maternal HBV DNA is desirable in HBsAg- and HBeAg-positive women (chapter 3.4).

Pregnancy in women found HBsAg-positive at prenatal screening should be managed just as it is managed at other times, that is, conservatively. Neither acute nor chronic hepatitis B infection appears to have adverse effects on the pregnant mother or her offspring, although the risk of prematurity was reported to be increased if the woman was infected in the last trimester (16-18). It seems safe to refrain from invasive procedures in HBsAg-positive pregnancies as they might provide a porte d'entrée for spread of infection to the fetus. In this respect, special attention was focused on HBsAg-positive women with an indication for amniocentesis or chorion villus sampling early in pregnancy. No signs of intrauterine transmission of HBV were found in 15 HBsAg-positive women after invasive antenatal diagnosis had been performed (chapter 2.3). Numbers were small and all but one woman were HBV DNA negative so that firm conclusions on the safety of these procedures early in pregnancy cannot be drawn. Although the risk of HBV transmission early in pregnancy is considered small, further studies are needed to investigate the potential drawbacks of these procedures in HBsAg- and HBeAg-positive mothers (19-20).

The finding that HBV is mainly transmitted at birth and not transplacentally makes infants suitable for post-exposure prophylaxis directly after birth (21). The combination of HBIg and vaccine will protect all but about 1% to 10% of infants born to HBsAg- and HBeAg-positive mothers (5-6,22-23). Failure of passive-active immunization in those infants may be due to in utero infection or to inadequate treatment. The mechanism of in utero infection was explained by placental leakage of maternal blood through premature labour or threatened abortion (24). As prolonged labour and assisted deliveries (i.e. forceps) increase the risk of maternal-fetal transfusion (25-26), care should be taken to prevent long-lasting uterine contractions in those women that have high levels of HBV DNA. The role of elective Caesarian section to reduce the risk of HBV transmission should be established; conflicting results on Caesarian section have been given (27-28). High(er) doses of HBIg directly after birth may also prove effective in preventing hepatitis B infection in the offspring of HBsAg-positive mothers (29).

In addition to identifying infants at risk for HBV infection, screening of pregnant women

for HBsAg has other advantages. Screening tests apparently sort out well persons who probably have a disease. HBsAg screening may, therefore, detect unrecognized asymptomatic disease in pregnant women but the tests are not intended to be diagnostic. If a woman tests positive for HBsAg during pregnancy a referral to a physician for further diagnosis and investigation of liver enzymes to ascertain the hepatic function is recommended after delivery. Similarly, household members who may benefit from hepatitis B vaccination are also identified by prenatal screening at the same time as the HBsAg-positive women are identified. The sex partner(s) and other family members of the HBsAg-positive women should be guaranteed hepatitis B vaccine if their susceptibility to HBV infection is determined by serological testing.

Treatment of infants of HBsAg-positive mothers

Passive immunization of newborns who are identified by HBsAg screening of their mothers can effectively reduce the risk of transmission of HBV infection (30-31). Because there is convincing evidence that hepatitis B immune globulin should be given as soon as possible to babies of HBsAg- and HBeAg-positive carrier mothers we set a time allowance of no later than 24 hours after birth to give this dose (31). It may be debatable scientifically whether 24 hours is actually the outside limit for effectiveness, but, in practical terms, this appeared to have a positive impact on the prompt delivery of HBIg. Dose-response studies with HBIg have never been published.

We studied several active immunization regimens in infants at risk for HBV infection varying in number of doses, dose and type of vaccine and moment of administration. The aim was to determine whether hepatitis B vaccination given concomitantly with the national regimen of DTP-polio vaccination was superior to hepatitis B vaccination initiated immediately after birth as is generally recommended, as far as protective efficacy, immunogenicity, and compliance are concerned (4-5). As the main purpose of hepatitis B immunization in infants is the prevention of the HBsAg carrier state and its consequences later in life, protective efficacy rates (PER) of more than 90% were aimed for. Differences in vaccination schedules appeared to have little impact on the PER (4-6, chapters 3.2 and 3.4). In our study the PER at 12 months of age for infants of HBeAg-positive mothers was 92% and for infants of HBeAg-negative mothers 100% (chapter 3.4). Like Beasley et al., we also demonstrated that with HBIg coverage from birth, the timing of the start of active vaccination was not important (3). A major clinically relevant factor that did influence the PER were the maternal levels of HBV DNA (14-15). When the efficacy of immunization was analyzed according to the quantitative levels of maternal HBV DNA the PER decreased to 68% in infants of mothers who had more than 150 pg/ml HBV DNA whereas immunization in infants of mothers who had levels below 150 pg/ml reached 100% protective efficacy. In comparison, Ip et al. found no HBV infections in infants when maternal levels of HBV DNA were below 5 pg/ml but up to 16% of infants became HBsAg-positive despite passive-active immunization if their mothers had levels above 5 pg/ml (15).

Since those infants received both lower doses of HBIg (100 IU/ml) and vaccine (3 μg), the protective efficacy may also be influenced by the immunization regimen in use. At present, Dutch infants receive 300 IU/ml HBIg at birth but the effect of higher or additional doses of HBIg to enhance the capture of HBV within the first days after birth must be evaluated for infants of mothers with HBV DNA levels > 150 pg/ml.

Enhanced immunogenicity is obtained by the use of higher doses of vaccine (32-33), start of vaccination at an older age (34-35) and by the use of plasma instead of recombinant vaccine (36). In our studies, antiHBs levels measured at the age of 12 months were approximately two times higher in infants receiving 3 doses of 10 μg plasma vaccine than in infants receiving the same schedule with 5 μg vaccine (chapter 3.4). This difference was no longer significant after 36 months of follow-up. We confirmed the finding that plasma vaccine yields higher antiHBs levels than the recombinant vaccine (chapters 3.3 and 3.4). After delayed immunization concomitant with DTP-polio vaccine antiHBs levels were significantly higher than those achieved after immunization starting at birth (4-6).

The importance of high antibody levels is presently unclear. If protection against HBV infection depends on the persistence of antibodies, then a strong argument could be made for the adoption of schedules that maximize antiHBs levels. However, follow-up studies suggest that long-term immunity is conferred on infants who initially respond to the hepatitis B vaccine (33,37). Antibody levels may decline after several years but asymptomatic breakthrough infections (antiHBc seroconversion) seldom occur even if antibodies are no longer detectable (38). So far, it appears that there is no need for booster doses but further follow-up is necessary to confirm such a recommendation.

At present, smaller doses of recombinant vaccine should not be given to infants born to HBsAg-positive mothers; it is recommended to use 1 ml (adult dose) of recombinant hepatitis B vaccine in the Netherlands. Active immunity develops quickly when immunization is started at 3 months of age and the passively acquired antibodies at birth remain present for several months. Thus, the second dose of HBIg at 3 months of age in infants receiving HBIg at birth and delayed active immunization appeared unnecessary as far as protective levels of antibody are concerned (chapter 3.3). However, this finding should be confirmed for infants born to HBeAg-positive HBsAg carrier mothers with high levels of HBV DNA.

Finally, the degree of protection provided by a hepatitis B prevention program is also dependent on the compliance to the vaccination schedule. The results of the present immunization program using the existing networks of services showed promising results. HBIg was administered to about 85% of infants at birth by the professionals attending the delivery (chapter 4.2). Inaccurate registration may lead to estimates of immunization coverage below the real level. In 1991 87% of infants received all four hepatitis B immunizations. In spite of this encouraging figure, the coverage rate is lower than achieved for the fourth dose of DTP-polio vaccine (93%). Most unvaccinated infants of HBsAg-positive mothers, 90% foreign born, are presumed to have either left the country without knowledge or simply to have been lost to follow-up. The challenge at present is to develop a

strategy that would deliver the hepatitis B vaccine to the ethnic groups that do not appear to receive routine childhood immunization delivery (39).

The hepatitis B vaccine was administered at variable intervals; about 15% of infants received the first dose of vaccine more than two weeks later than planned (chapter 4.2). It is important to determine the implication of this delay in scheduled vaccinations. If a proportion of children is still infected because active immunization is delayed beyond the target age of 3 months, the present schedule will fail. As the program does not examine seroconversion rates in infants after vaccination, the precise level of protective immunity is unknown. To assess the efficacy of the program in place and the potential need for alternative schedules, serological surveys in infants born to mothers with high levels of HBV DNA should be the subject of further work.

Costs of the prevention program

Public health policy makers are increasingly interested in using economic analysis to guide their decisions. Therefore, the net costs of the prevention program are derived by calculating the number of neonatal HBV infections with and without the program in place. In Table 1 the estimated cost of the program for the prevention of perinatal hepatitis B infection per year is given for the Netherlands. The cost is calculated in US dollars (1 US dollar ≈ 1.86 Dutch florins).

Table 1

Estimated direct costs of the national program for the prevention of perinatal hepatitis B infection.

Number of pregnant women	200,000
Prevalence of HBsAg (%)	0.44
Expected number of HBsAg-positive women	880
Cost of screening (US $ 6.20/test)	$ 1,240,000
Cost of HBIg + vaccine (US $ 254.00/child)	$ 223,520
Total costs	$ 1,463,520
Cost per HBsAg-positive woman detected	$ 1,663
Number of infections prevented	175
Cost of preventing chronic infection per infant	$ 8,363

With 200,000 births per year and an HBsAg prevalence of 0.44% among pregnant women, an estimated 880 children of HBsAg carrier mothers will be born in the Netherlands (chapter 4.2). 15% of the HBsAg-positive women is HBeAg-positive. The cost of complete

coverage of screening all pregnant women for HBsAg will be 200,000 x $ 6.20 or $ 1,240,000. This figure is based on the cost of screening reimbursed to the contracting Regional Public Health Laboratories. Since screening is incorporated into the existing "prenatal panel" no extra costs for venipuncture, administration and laboratory fees are calculated. At current prices, the cost of the recommended passive-active immunization schedule reported by the pharmacies, is $ 254 per infant: $ 79 for HBIg (300 IE antiHBs/ml) and $ 175 for four doses of hepatitis B vaccine. No additional costs of medical visits, vaccine administration, follow-up and monitoring are calculated since the immunization is incorporated into the existing postnatal health care program. In comparison, the total cost for the national childhood immunization program amounts to $ 72,540,000 per year or $ 50 per injection.

We assume that the rate of perinatal transmission of HBV and risks of developing chronic hepatitis B infection is 10% for infants of HBsAg-positive mothers and 90% for infants of HBeAg-positive mothers. If screening is not performed and immunization is not given, the number of infants with hepatitis B infection would be 194. When the protective efficacy of the passive-active immunization in use is estimated at 90%, the program will prevent 175 cases of neonatal hepatitis B infection. The total cost of preventing one infant from hepatitis B infection will then be about $ 8,400. The cost of detecting a carrier woman is shown in Table 1. The costs of the national program strongly depend on the cost of the serologic test in use; screening for HBsAg accounts for about 85% of the total cost. Thus the availability of a cheap though highly sensitive serologic test should markedly decrease the cost of the program.

How does the cost of the hepatitis B prevention program compare to the costs of established screening programs in the Netherlands? The widely accepted program of screening for congenital hypothyroidism and phenylketonuria, offered to every newborn within the first week of life, costs $ 3,980,400 and $ 1,447,080 per year, respectively. With an estimated incidence of 1:3,300 births for neonatal hypothyroidism (n=60), it costs $ 66,340 to prevent one case of cretinism. Similarly, with a frequency of 1:18,000 cases of phenylketonuria (n=11), it costs $ 131,553 to prevent a serious case of mental retardation.

Recommendations for increased program effectiveness

Despite the encouraging results, there are some deficiencies at the level of screening and treatment of infants where the program effectiveness might be enhanced:

- obstetric services should be better informed about the need to routinely screen pregnant women prenatally and to re-screen in subsequent pregnancies.
- screening on command should be made available for women who have not had prenatal HBsAg testing; the development of a cheap and rapid HBsAg test 'on the spot' is advocated.
- additional testing for HBV DNA in women found positive for both HBsAg and HBeAg is advised.

- health care services should have a regulation clearly stating how an infant born to an HBsAg-positive mother is to receive HBIg and hepatitis B vaccine.
- centralization of the purchase of hepatitis B vaccines is important to guarantee availability of the vaccine at the Child Health Center at the appointed time.
- more awareness on the part of both parents and paediatric services of the importance of immunization will facilitate the (timely) administration of the hepatitis B vaccine.
- better resources to establish accurately the immunization status and reasons for loss to follow-up of infants are necessary.
- serologic testing after hepatitis B vaccination, preferably in infants born to mothers with high infectivity, is advised to establish the programs efficacy.
- the appointment of regional assistants is recommended to develop program logistics in the region, to improve registration procedures, and to check on the coverage rates of screening and vaccination.

Future issues

The World Health Organization aims to control hepatitis B on a global scale by mass vaccination of infants and persons belonging to high risk groups (40). Many countries with high endemicity for HBV have already integrated or are moving towards integration of hepatitis B vaccination into their Expanded Program on Immunization. It has also been recommended that countries with low endemicity consider the possibilities of a universal vaccination program by the year 1997 (40). Active immunization in early childhood may be more effective than in adults but the strategy of universal infant hepatitis B immunization in the Netherlands to prevent infection primarily acquired in adulthood raises a variety of questions relating to vaccination schedules, duration of protection and cost.

Vaccination in the first year of life to prevent morbidity and mortality of disease later in life is an ethical issue, especially in a country with low prevalence of HBV and where certain groups already refuse routine infant immunization on religious and anthroposophical grounds.

As of July 1993 routine infant immunization in the Netherlands consists of two immunizations at each visit; DTP-polio and *Haemophilus influenzae* type b vaccinations are given concomitantly at the ages of 3, 4, 5, and 11 months. It is a matter of concern whether the high coverage rate achieved for DTP-polio will suffer from the additional vaccine injections to be added at the same visit. In order to maintain the high coverage rates multivalent vaccines to reduce the number of injections or physician visits should be available before universal hepatitis B vaccination may be considered in the Netherlands. At present, the DTP-polio vaccine is produced in an governmental institution, the National Institute of Public Health and Environmental Protection (RIVM). All other infant vaccines are produced by private pharmaceutical companies but packaged and labelled at the RIVM. The governmental producers need to recognize that close interaction with private producers of childhood vaccines is necessary in order not to limit the technological development of

new multivalent vaccines in the Netherlands.

The present program of infant immunizations starting at 3 months of age, does not allow to leave out the HBIg injection at birth for infants of HBsAg-positive mothers and the current program of screening pregnant women for HBsAg should remain in place.
The feasibility and effectiveness of a universal approach with new schedules and vaccines in order to circumvent the maternal screening and HBIg administration, have to be further evaluated. In addition, the duration of protection after hepatitis B vaccination is unknown at this time. This is a major issue in the consideration of childhood vaccination to prevent infection in adolescence and adulthood.

Vaccine prices have been reduced marginally in recent years (usually for large-volume purchasers); they remain, however, substantially higher than in other parts of the world where universal vaccination programs are underway. The vaccine costs about $ 130 for primary adult vaccination. The cost is a major impediment to a more effective use, particularly in high-risk groups whose vaccine costs are not covered by the medical insurance.

References

1. Maupas P, Chiron JP, Barin F, Coursaget P, Goudeau A, Perrin J, Denis F, Diop Mar I. Efficacy of hepatitis B vaccine in prevention of early HBsAg carrier state in children. Controlled trial in an endemic area (Senegal). Lancet 1981;1:289-292.
2. Tada H, Yanagida M, Mishina J, Fujii T, Baba K, Ishikawa S, Aihara S, Tsuda F, Miyakawa Y, Mayumi M. Combined passive-active immunization for preventing perinatal transmission of hepatitis B virus carrier state. Pediatrics 1982;70:613-619.
3. Beasley RP, Hwang LY, Lee GC, Lan CC, Roan CH, Huang FY, Chen CL. Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. Lancet 1983;2:1099-1102.
4. Mazel JA, Schalm SW, Gast GC de, Nuijten ASM, Heijtink RA, Botman MJ, Bänffer JRJ, Gerards LJ, Zwijnenberg J, Mettau J, Wladimiroff JW, Fetter WPF. Passive-active immunization of neonates of HBsAg-positive carrier mothers: preliminary observations. Br Med J 1984;288:513-515.
5. Schalm SW, Mazel JA, Gast de GC, Heijtink RA, Botman MJ, Bänffer JRJ, Gerards LJ, Zwijnenberg J, Fetter WPF, Nuijten ASM, Wladimiroff JW, Christiaens GCML. Prevention of hepatitis B infection in newborns through mass screening and delayed vaccination of all infants of mothers with hepatitis B surface antigen. Pediatrics 1989;83:1041-1047.
6. Grosheide PM, Canho del R, Heijtink RA, Nuijten ASM, Zwijnenberg J, Bänffer JRJ, Wladimiroff JW, Botman MJ, Mazel JA, Gast de GC, Christiaens GCML, Gerards LG, Fetter WPF, Baerts W, Schalm SW. Passive-active immunization of infants of hepatitis B e antigen-positive mothers: comparison of the efficacy of early and delayed active immunization. AJDC 1993, in press.
7. Xu ZY, Liu CB, Francis DP, Purcell RH, Gun ZL, Duan SC, Chen RJ, Margolis HS, Huang CH, Maynard JE, and the United States-China Cooperative Study Group on Hepatitis B. Prevention of perinatal acquisition of hepatitis B virus carriage using vaccine; preliminary report of a randomized, double-blind placebo-controlled and comparative trial. Pediatrics 1985;76:713-718.
8. Poovorawan Y, Sanpavat S, Pongpuniert W. Protective efficacy of recombinant DNA hepatitis B vaccine in neonates of HBs antigen-positive mothers. JAMA 1989;261:3278-3281.
9. World Health Organization, Expanded Programme on Immunization, Global Advisory Group. Weekly Epidemiol Record 1992;3:11-16.

10. Jonas MM, Schiff ER, O'Sullivan MJ, Medina de M, Reddy KR, Jeffers LJ, Fayne T, Roach KC, Steel BW. Failure of Centers for Disease Control criteria to identify hepatitis B infection in a large municipal obstetrical population. Ann Intern Med 1987;107:335-337.
11. Immunization Practices Advisory Committee, Centers for Disease Control. Prevention of perinatal transmission of hepatitis B virus: prenatal screening of all pregnant women for hepatitis B surface antigen. MMWR 1988;37:341-351.
12. Okada K, Kamiyama I, Inomata M, Imai M, Miyakawa Y, Mayumi M. E-antigen and anti-e in the serum of asymptomatic carrier mothers as indicators of positive and negative transmission of hepatitis B virus to their infants. N Engl J Med 1976;294:746-749.
13. Stevens CE, Neurath RA, Beasley RP, Szmuness W. HBeAg and anti-HBe detection by radioimmunoassay: correlation with vertical transmission of hepatitis B virus in Taiwan. J Med Virol 1979;3:237-241.
14. Lee SD, Lo KJ, Wu JC, Tsai YT, Wang JY, Ting LP, Tong MJ. Prevention of maternal-infant hepatitis B virus transmission by immunization; the role of serum hepatitis B virus DNA. Hepatology 1986;6:369-373.
15. Ip HMH, Lelie PN, Wong VCW, Kuhns MC, Reesink HW. Prevention of hepatitis B virus carrier state in infants according to maternal serum levels of HBV DNA. Lancet 1989;1:406-410.
16. Pastorek JG, Miller JM, Summers PR. The effect of hepatitis B antigenemia on pregnancy outcome. Am J Obstet Gynecol 1988;158:486-489.
17. Os van HC, Drogendijk AC, Fetter WPF, Heijtink RA, Zeilmaker GH. The influence of contamination of culture medium with hepatitis B virus on the outcome of in vitro fertilization pregnancies. Am J Obstet Gynecol 1991;165:152-159.
18. Hieber JP, Dalton D, Shorey J, Combes B. Hepatitis and pregnancy. J Pediatr 1977;91:545-549.
19. Schweitzer IL, Dunn AEG, Peters RL, Spears RL. Viral hepatitis B in neonates and infants. Am J Med 1973;55:762-771.
20. Tong MJ, Thursby M, Rakela J, McPeak C, Edwards VM, Mosley JW. Studies on the maternal-infant transmission of the viruses which cause acute hepatitis. Gastroenterol 1981;80:999-1004.
21. Stevens CE, Beasley RP, Tsui J, Lee WC. Vertical transmission of hepatitis B antigen in Taiwan. N Engl J Med 1975;292:771-774.
22. Beasley RP, Hwang LY, Lin CC, Stevens CE, Wang KY, Sun TS, Hsieh FJ, Szmuness W. Hepatitis B immune globulin (HBIG) efficacy in the interruption of perinatal transmission of hepatitis B virus carrier state. Lancet 1981;2:388-393.
23. Wong VCW, Ip HMH, Reesink HW, Lelie PN, Reerink-Brongers EE, Yeung CY, Ma HK. Prevention of the HBsAg carrier state in newborn infants of mothers who are chronic carriers of HBsAg and HBeAg by administration of hepatitis B vaccine and hepatitis B immunoglobulin. Lancet 1984;1:921-926.
24. Ohto H, Lin HH, Kawana T, Etoh T, Tohyama H. Intrauterine transmission of hepatitis B virus is closely related to transplacental leakage. J Med Virol 1987;21:1-6.
25. Wong VCW, Lee AKY, Ip HMH. Transmission of hepatitis B antigens from symptom free carrier mothers to the fetus and the infant. Br J Obstet Gynaecol 1980;87:958-965.
26. Eimer H. Untersuchungen zur maternofetalen transfusion bei geburtshilflichen operationen. Geburtsh u Frauenheilk 1972;32:657-661.
27. Beasley RP, Stevens CE. Vertical transmission of HBV and interruption with globulin. In: Vyas GN, Cohen SN, Schmid R (eds). Viral Hepatitis. Philadelphia, Franklin Institute Press, 1978:333-345.
28. Lee SD, Lo KJ, Tsai YT, Wu JC, Wu TC, Yang ZL, Ng HT. Role of Caesarian section in prevention of mother-infant transmission of hepatitis B virus. Lancet 1988;2:833-834
29. Schalm SW, Pit-Grosheide PM. Prevention of hepatitis B transmission at birth. Lancet 1989;1:44.
30. Dosik H, Jhaveri R. Prevention of neonatal hepatitis B infection by high dose hepatitis B immune globulin. N Engl J Med 1978;298:602-603.
31. Beasley RP, Hwang LY, Stevens CE, Lin CC, Hsieh FJ, Wang KY, Sun TS, Szmuness W. Efficacy of hepatitis B immune globulin for prevention of perinatal transmission of the hepatitis B virus carrier state: final report of a randomized double-blind placebo-controlled trial. Hepatology 1983;3:135-141.

32. Goh KT, Tan KL, Kong KH, Oon CJ, Chan SH. Comparison of the immune response of four different dosages of a yeast-recombinant hepatitis B vaccine in Singapore children: a four-year follow up study. Bull WHO 1992;70:233-239.
33. Lo KJ, Lee SD, Tsai YT, Wu TC, Chan CY, Chen GH, Yeh CL. Long-term immunogenicity and efficacy of hepatitis B vaccine in infants born to HBeAg-positive HBsAg-carrier mothers. Hepatology 1988;8:1647-1650.
34. Piazza M, Piccioto L, Villari R, Guadagnino V, Orlando R, Isabella L, Macchia V, Memoli AM, Vegnente A, Borrelli AM, Scarcella A, Cascili C, Cirillo C, Coppola P, Isabella E, Parisi G. Hepatitis B immunisation with a reduced number of doses in newborn babies and children. Lancet 1985;1:949-951.
35. Stevens CE, Toy PT, Taylor PE, Lee Th, Yip HY. Prospects for control of hepatitis B virus infection: implication of childhood vaccination and long-term protection. Pediatrics 1992;90:170-173.
36. Panda SK, Ramesh R, Rao KVS, Zuckerman AJ, Nayak NC. Comparative evaluation of the immunogenicity of yeast-derived (recombinant) and plasma-derived hepatitis B vaccine in infants. J Med Virol 1991;35:297-302.
37. Whittle HC, Inskip H, Hall AJ, Mendy M, Downes R, Hoare S. Vaccination against hepatitis B and protection against chronic viral carriage in The Gambia. Lancet 1991;1:747-750.
38. Coursaget P, Yvonnet B, Chotard J, Sarr M, Vincelot P, N'Doye R, Diop Mar I, Chiron JP. Seven-year study of hepatitis B vaccine efficacy in infants from an endemic area (Senegal). Lancet 1986;2:1143-1144.
39. Verbrugge HP. The national immunization program of the Netherlands. Pediatrics 1990;86(suppl):1060-1063.
40. World Health Organization. Expanded Programme on Immunization, Global Advisory Group. Weekly Epidemiol Record 1992;3:11-16.

CHAPTER 6

SUMMARY

Perinatal transmission of hepatitis B virus (HBV) from mothers who are hepatitis B surface antigen (HBsAg)-positive is one of the main causes for the development of hepatitis B infections and for the maintenance of the hepatitis B virus reservoir. Combined passive and active immunization of infants of HBsAg carrier mothers can almost completely prevent perinatal infection.

This thesis focuses on hepatitis B infection in pregnancy, passive-active immunization in infants of HBsAg-positive mothers and the program for the prevention of perinatal infection in the Netherlands and its initial results (**chapter 1**).

Hepatitis B in pregnancy

Hepatitis B infection, whether acute or chronic, generally does not affect the course of pregnancy or the disease nor the outcome. Pregnant women who are HBsAg-positive mostly transmit the hepatitis B virus to their offspring at the time of delivery. The virus can cross the placenta during labour by maternal-fetal transfusion. In addition, infants may swallow maternal blood, amniotic fluid, vaginal secretions and breast milk containing HBsAg. The risk of perinatal infection is related to the presence of hepatitis B e antigen (HBeAg) and HBV DNA, indicators of viral replication in the mother's serum. The rate of intrauterine infection is estimated from 1% to 10% and considered to occur mainly in the third trimester of pregnancy as a result of transplacental leakage (**chapter 2.1**).

In 1982 a multicenter study was started in the Netherlands to detect HBsAg-positive mothers and to immunize their newborns. The aim was to determine whether screening for HBsAg could be introduced successfully (i.e. high compliance) into prenatal care in and outside the hospital setting.

In the seven-year period 99,706 pregnant women were tested for HBsAg; the coverage of screening varied between 87% and 97%. The overall rate of HBsAg was 0.7% with a marked variation between urban centers (1.8%) and the rural area (0.3%). A considerable number of Dutch HBsAg carriers would have escaped detection if screening would have been limited to women considered at high risk for infection. If pregnant women are not screened before delivery, they should be screened at delivery since the prevalence of infection was more than twice as high in this group. It has been concluded that screening all pregnant women for HBsAg can be introduced effectively at reasonable cost in a country with a low prevalence for HBsAg and a high proportion of home deliveries (**chapter 2.2**).

Early prenatal diagnosis was performed for 18 pregnant HBsAg-positive women because of age above 36 years or because of previous fetal malformations; 17 women underwent amniocentesis and 1 chorionic villus sampling. Fifteen patients delivered a healthy infant without signs of intrauterine infection; pregnancy was terminated in three cases. Since numbers are small and all but two women were HBeAg-negative, it cannot be concluded that early invasive prenatal diagnosis in HBsAg-positive pregnant women is safe. Data from literature, however, suggest that HBV transmission in the first stages of pregnancy does not harm the fetal liver (**chapter 2.3**).

On the basis of limited experience there is no apparent risk of adverse effects on developing fetuses when hepatitis B vaccine is administered to pregnant women. HBV exposure in pregnancy may result in severe disease for the mother and chronic infection for the newborn. We studied the results of post-exposure prophylaxis with hepatitis B immune globulin (HBIg) and recombinant vaccine in 16 pregnant women after their inadvertent exposure to the HBV. No adverse consequences to the fetus were observed and all women developed protective levels of antibodies. The immune response to the vaccine was (s)lower in pregnant women than in nonpregnant controls of similar age (**chapter 2.4**).

Prevention of perinatal hepatitis B infection

HBV infections acquired soon after birth are most likely to become persistent. Passive immunization by the administration of hepatitis B immune globulin (HBIg) soon after birth has been shown to be partially (50%) effective in preventing hepatitis B infection. The efficacy is raised to 70% if several doses of HBIg are given. With the introduction of hepatitis B vaccine, which provides enhanced and longer lasting protection, a nearly complete prevention of HBV infection among infants became a reality with combined passive-active immunization. Passive-active immunization is highly effective (> 90%) in preventing perinatal HBV infection and up to 95% of infants respond to the vaccine (**chapter 3.1**).

A second aim of the Dutch multicenter study was to investigate whether active immunization given concomitantly with the diphtheria-tetanus-pertussis-poliomyelitis (DTP-polio) vaccination is superior to hepatitis B vaccination that is started immediately after birth as is advised by most researchers and producers of hepatitis B vaccines, as far as protective efficacy, immunogenicity and compliance are concerned.

The long-term protective efficacy of passive-active immunization in infants born to HBsAg- and HBeAg-positive mothers was evaluated. Infants received HBIg within two hours after birth and four doses of hepatitis B plasma vaccine. In group A (n=38) active immunization began at birth (0, 1, 2, 11 months). In group B (n=42) vaccination was started at the age of 3 months in combination with a second dose of HBIg (3, 4, 5, 11 months). During the study period of five years, 3 infants in each group became HBsAg carriers corresponding to an incidence of infection of 9% and 8%, respectively. Subclinical infections were diagnosed in 4 infants in each group. Delayed active immunization starting at 3 months of age may be an effective and an attractive alternative to early active immunization because of compliance and low cost; it can be incorporated into the existing DTP-polio vaccination program in the Netherlands (**chapter 3.2**).

In addition, a study was performed to investigate the need for the second dose of HBIg if active immunization with recombinant hepatitis B vaccine is initiated at the age of 3 months. 112 infants received HBIg at birth and four doses of vaccine at 3, 4, 5, and 11 months (group E). Group F (n=98) received the same schedule with an additional dose of HBIg at 3 months of age. In both groups over 90% of infants had protective levels of antiHBs from

month 3 onwards although antiHBs titers during the first six months were significantly higher in infants receiving additional HBIg. The geometric mean titers in infants receiving recombinant vaccine were significantly lower than those achieved with the plasma vaccine in historic controls (group B). For programs with delayed active hepatitis B immunization, additional HBIg injections in infants receiving a high dose of HBIg (1 ml) at birth are not advised (**chapter 3.3**).

The protective efficacy and long-term immunogenicity of six passive-active immunization schedules (groups A-F) administered to 705 neonates of HBsAg-positive mothers were evaluated over a ten-year period. 118 (17%) mothers were also HBeAg-positive. The immunization schedules varied in time of beginning of vaccination, type and doses of HBIg and/or vaccine.

During follow-up 9 infants became HBsAg carriers: 8 infants, born to HBeAg-positive mothers, within the first year of life and one infant, born to an HBeAg-negative mother, at the age of 5 years. The protective efficacy rate (PER) of passive-active immunization at 12 months of age for the entire group was 92%. There were no significant differences between groups that began immunization at birth or at 3 months, between groups receiving one or two doses of HBIg and between groups receiving plasma or recombinant vaccine. However, the PER at month 12 in the group with maternal HBV DNA levels below 150 pg/ml was 100% and significantly higher than the PER (68%) for the group with maternal HBV DNA levels above 150 pg/ml. After a follow-up of 5 years, the group that began immunization at 3 months had significantly less (2%) unprotected infants with antiHBs levels < 10 IU/l than the corresponding group that started at birth (15%). The geometric mean titer was significantly higher in the group starting immunization at 3 months of age with plasma vaccine than in the corresponding group starting at birth and also higher than in the corresponding group using recombinant vaccine. The program showed that hepatitis B vaccine is highly effective and immunogenic. Evaluation of vaccination programs according to maternal HBV DNA levels is needed and important to further improve results of intervention for those infants at highest risk of infection (**chapter 3.4**).

National prevention program

Based on the results of the multicenter study a national program for the prevention of perinatal hepatitis B infection was initiated in October 1989 (**chapter 4.1**).

The design and the organization of the prevention program are described in greater detail. During their first antenatal visit a test for HBsAg is added to the routine prenatal blood testing of pregnant women in 16 Regional Public Health Laboratories. HBsAg-positive test results are forwarded to the person assisting in the delivery of the pregnant woman and to the Provincial Immunization Administration maintaing a database of immunization records for each infant in the region. HBIg is administered to all infants of HBsAg-positive mothers soon after birth. When at the Child Health Clinic, infants receive

DTP-polio immunization hepatitis B vaccine is also administered during the same visit to infants of HBsAg-positive mothers. Registration of vaccination coverage and reminders, if necessary, are taken care of by the Provincial Immunization Administration. Screening for HBsAg takes place at national cost but parents should obtain the HBIg and the hepatitis B vaccine. Between 1989 and 1992, the screening coverage increased from 46% to 84%. The overall prevalence for HBsAg was 0.44%. 1,645 infants were born to HBsAg-positive mothers of whom 85% received HBIg after birth. In 1991, respectively 96%, 95%, 94% and 87% of infants received the first, second, third and fourth dose of hepatitis B vaccine. There was considerable delay in vaccine administration: in 17% of infants the first dose was given more than two weeks later than scheduled. It is our conclusion that the hepatitis B prevention program was successfully incorporated into the existing health care systems for antenatal management of pregnant women and good baby care. More attention, however, should be focused on adherence to the vaccination schedule (**chapter 4.2**).

Factors associated with incomplete screening coverage for HBsAg and deficient immunization of infants of HBsAg-positive mothers that were achieved in 1990, were further analyzed. Interviews were carried out among the participating health care professionals to evaluate their experience and future actions. It appeared that for logistical and financial reasons 10% of screening for HBsAg took place in other than the appointed laboratories and the HBIg injection to infants of HBsAg-positive mothers identified elsewhere, was not guaranteed. Shortcomings of the immunization program were poor functioning of the registration system and lack of motivation on the part of both parents and professionals. Missed opportunities to immunize included absence of vaccine and frequent departure of infants of ethnic origin to their native countries. It is recommended that more extensive educational programs be organized. Restriction of screening to a limited number of laboratories should be enhanced to be able to monitor the coverage and quality. The appointment of coordinators in every region should increase the program coverage as would the storage of hepatitis B vaccine at the Child Health Clinics. The effect of the prevention program will have to be dertermined by means of continuous epidemiological study of the incidence of hepatitis B infection among pregnant women and their offspring (**chapter 4.3**).

In chapter 5 a general discussion on the prevention of perinatal hepatitis B virus infection is given, including propositions for further research. It is concluded that in developed countries all pregnant women should be routinely screened for HBsAg during an early prenatal visit in each pregnancy, preferably at the same time other routine prenatal laboratory testing is done. Prenatal screening should be performed in a limited number of laboratories and tests for additional HBV markers (HBeAg and HBV DNA) are necessary to identify those women who are highly infectious (HBV DNA > 150 pg/ml). For women admitted for delivery who have not had prenatal testing an HBsAg test "on the spot" should become available.

Infants born to HBsAg-positive mothers should receive the appropriate doses of HBIg

(1 ml) at birth and hepatitis B recombinant vaccine (adult dose) concurrently with DTP-polio vaccine at 3, 4, 5, and 11 months of age.

Special efforts are necessary to ensure that infants will receive all their vaccinations and the efficacy of the present program should be assessed continuously. Future directions for hepatitis B vaccine research include: the development of combination vaccines with DTP-polio and *Haemophilus influenzae* type b conjugate and the need for booster doses at a later age. Further research is needed for the optimal protection against hepatitis B infection in infants born to HBsAg-positive mothers with high levels of HBV DNA.

SAMENVATTING

Perinatale besmetting van pasgeborenen met het hepatitis B virus (HBV) afkomstig van moeders die draagster zijn van het hepatitis B surface antigeen (HBsAg) is een belangrijke oorzaak van het ontstaan van hepatitis B infecties en van het in stand houden van het hepatitis B virusreservoir. Immunisatie van pasgeborenen van HBsAg draagsters kan de gevolgen van de perinatale besmetting vrijwel volledig voorkomen.

De in dit proefschrift beschreven studies richten zich op een drietal onderwerpen: hepatitis B infectie tijdens de zwangerschap, passieve en actieve immunisatie van de pasgeborenen van HBsAg-positieve moeders en het programma voor de preventie van perinatale hepatitis B in Nederland (**hoofdstuk 1**).

Hepatitis B infectie tijdens de zwangerschap

Niet alleen moeders die HBsAg draagster zijn maar ook moeders die een acute hepatitis B infectie tijdens de zwangerschap doormaken, kunnen het HBV overbrengen. Algemeen wordt aangenomen dat de overdracht voornamelijk perinataal, durante partu plaatsvindt door materno-foetale transfusie. HBsAg-positieve zwangeren die tevens hepatitis B e antigeen (HBeAg)-positief zijn, worden als veel besmettelijker beschouwd dan zwangeren bij wie HBeAg niet aanwezig is. Intrauteriene overdracht van HBV vindt bij 1-10% van de pasgeborenen plaats, voornamelijk in het derde trimester van de zwangerschap (**hoofdstuk 2.1**).

In 1982 werd in drie centra in Nederland een studie opgezet om vast te stellen of zwangeren door middel van systematisch onderzoek als HBsAg draagsters konden worden geïdentificeerd. Tot 1 oktober 1989 werden 99.706 zwangeren op de aanwezigheid van HBsAg onderzocht. Dit is 87-97% van alle zwangeren in deze centra. Van de zwangeren was 0,7% HBsAg-positief. Bij het ontbreken van een HBsAg uitslag ten tijde van de partus werd alsnog HBsAg bepaald. In deze tijdens de partus onderzochte groep was de prevalentie van HBsAg meer dan twee maal zo hoog. Slechts een beperkt deel van de uit Nederland afkomstige HBsAg-positieve vrouwen behoorde tot de bekende risicogroepen met verhoogde kans op HBV infectie. Derhalve is screening van alle zwangeren aangewezen; de HBsAg bepaling kan efficiënt worden verricht in hetzelfde bloedmonster dat wordt afgenomen ter bepaling van bloedgroep, Rhesusfactor en luesserologie (**hoofdstuk 2.2**).

Bij 18 HBsAg-positieve zwangeren werd invasieve prenatale diagnostiek verricht wegens gevorderde maternale leeftijd of genetische indicatie. Slechts twee vrouwen waren HBsAg- en HBeAg-positief. Vijftien vrouwen kregen een gezonde baby zonder tekenen van een intrauterien verworven HBV infectie; bij 3 vrouwen werd de zwangerschap afgebroken. Ook blijkens de literatuur is de kans op intrauteriene overdracht van HBV tijdens het eerste en tweede trimester van de zwangerschap gering maar de veiligheid van deze ingrepen bij HBsAg- en HBeAg-positieve zwangeren moet worden bevestigd (**hoofdstuk 2.3**).

Over het resultaat en de risico's van hepatitis B vaccinatie tijdens de zwangerschap is weinig bekend. Zestien zwangeren kregen hepatitis B immunoglobuline (HBIg) en recombinant hepatitis B vaccin toegediend na mogelijke besmetting met HBV.

Er werden geen nadelige gevolgen van deze postexpositie profylaxe geconstateerd bij moeder en kind. Alle zwangeren ontwikkelden beschermende antistof titers (antiHBs) maar de immuunreactie was trager en lager dan de immuunreactie in een controle groep van gevaccineerde niet zwangere vrouwen (**hoofdstuk 2.4**).

Passieve en actieve immunisatie van pasgeborenen

Na een perinatale besmetting met HBV worden de meeste pasgeborenen hepatitis B drager. Gecontroleerde studies hebben aangetoond dat eenmalige toediening van HBIg direct na de geboorte HBV infectie bij 50% van de zuigelingen kan voorkomen. Herhaald toedienen van HBIg leidde tot een verdere reductie van het aantal gevallen van perinataal besmette HBV dragers van 70%. Met het beschikbaar komen van een hepatitis B vaccin deed zich de mogelijkheid voor gedurende langere tijd beschermende antiHBs titers te verwezenlijken. Hepatitis B vaccin bij kinderen bleek in hoge mate immunogeen en gecombineerde passieve-actieve immunisatie kon in meer dan 90% van de gevallen perinatale infectie voorkomen (**hoofdstuk 3.1**).

De vraagstelling van dit deel van de studie was: welk schema van gecombineerde passieve-actieve immunisatie is praktisch toepasbaar, voorkomt hepatitis B infectie en leidt tot het actief ontstaan van voldoende antiHBs in meer dan 90% van de gevallen?

Aanvankelijk werden twee immunisatieschema's toegepast bij kinderen van HBsAg- en HBeAg-positieve moeders: in schema A (n=38) werd direct na de geboorte HBIg toegediend, waarna op de leeftijd van 0, 1, 2 en 11 maanden plasma vaccin werd gegeven. In schema B (n=42) kregen de kinderen HBIg bij de geboorte waarna plasma vaccin werd toegediend op de leeftijd van 3, 4, 5 en 11 maanden. De kinderen in schema B kregen op de leeftijd van 3 maanden nogmaals HBIg toegediend.

Tijdens de follow-up van vijf jaar werden 6 kinderen HBsAg drager, respectievelijk 9% en 8% van de kinderen in groep A en B. Vier kinderen in elke groep ontwikkelden een subklinische HBV infectie. Beide schema's waren even effectief in het voorkomen van HBV infectie; de kinderen in groep B ontwikkelden gemiddeld hogere antiHBs titers. Schema B is ook beter uitvoerbaar in de praktijk aangezien de vaccintoedieningen tegelijk met de DKTP-vaccinaties kunnen worden gegeven (**hoofdstuk 3.2**)

Aansluitend werd een studie verricht bij kinderen van HBsAg-positieve moeders om te bepalen of een tweede dosis HBIg wel noodzakelijk is wanneer een hoge dosis HBIg bij de geboorte wordt gegeven. Kinderen in groep E (n=112) kregen HBIg bij de geboorte en hepatitis B recombinant vaccin op de leeftijd van 3, 4, 5, en 11 maanden. Kinderen in groep F (n=98) kregen hetzelfde vaccinatieschema aangevuld met een tweede dosis HBIg op de leeftijd van 3 maanden.

Het percentage kinderen met beschermende antistof titers (antiHBs $\geq$ 10 IE/L) was in beide groepen gelijk evenals het aantal kinderen dat HBsAg drager werd, een in elke groep. De antiHBs titers na de toediening van recombinant vaccin waren significant lager dan de antiHBs titers na de toediening van plasma vaccin aan kinderen in groep B. De conclusie is

dat een tweede dosis HBIg niet noodzakelijk is wanneer kinderen van HBsAg-positieve moeders een hoge dosis HBIg bij de geboorte krijgen gevolgd door de eerste vaccintoediening tegelijk met de DKTP-vaccinatie op de leeftijd van 3 maanden (**hoofdstuk 3.3**).

Van 1982 tot 1992 werden de effectiviteit en de duur van de beschermende antiHBs titers na toediening van zes verschillende passieve-actieve immunisatieschema's (groep A-F) aan totaal 705 kinderen van HBsAg-positieve moeders geëvalueerd. 118 van deze moeders waren tevens HBeAg-positief. De schema's varieerden in tijdstip en dosering van de vaccintoedieningen, type vaccin en het aantal doses HBIg. Tijdens de follow-up werden 9 kinderen HBsAg drager. De effectiviteit van passieve-actieve immunisatie op de leeftijd van 12 maanden was 92%; er werd geen verschil in de effectiviteit gevonden tussen de diverse vaccinatieschema's. De effectiviteit bleek echter wel afhankelijk van de hoeveelheid maternaal HBV DNA in serum: bij kinderen van moeders met lage HBV DNA titers (< 150 pg/ml) was de effectiviteit van vaccinatie 100% ongeacht het toegepaste schema. Bij kinderen van moeders met hoge HBV DNA titers (≥150 pg/ml) was de effectiviteit 68%. De gemiddelde antiHBs titers waren significant hoger voor de groepen waarbij vaccintoediening op de leeftijd van 3 maanden begon in plaats van direct na de geboorte.

Hepatitis B vaccin blijkt zeer effectief in het voorkomen van perinatale hepatitis B infecties en leidt tot het ontstaan van langdurige bescherming. Voor kinderen met zeer besmettelijke moeders (HBV DNA ≥ 150 pg/ml) is onderzoek naar aanvullende preventieve maatregelen noodzakelijk (**hoofdstuk 3.4**).

Landelijk programma ter voorkoming van perinatale hepatitis B

Op grond van de bovengenoemde resultaten is per 1 oktober 1989 een landelijk programma voor de preventie van perinatale hepatitis B ingevoerd (**hoofdstuk 4.1**).

Bij een van de eerste zwangerschapscontroles wordt tegelijk met bloedgroep, Rhesusfactor en luesserologie een HBsAg bepaling aangevraagd. De Streeklaboratoria verrichten de bepaling en melden een HBsAg-positieve uitslag aan de inzender van het bloedmonster en aan de Provinciale Entadministratie. Direct na de geboorte wordt HBIg aan de pasgeborene gegeven door degene die de zwangere begeleidt. De actieve immunisaties worden verricht door degene die ook de DKTP-vaccinaties uitvoert. Alle vaccinaties worden geregistreerd door de Provinciale Entadministratie die rappelleren indien ze geen vaccinatiegegevens ontvangen. In de periode van 1 oktober 1989 tot 31 december 1992 steeg het jaarlijks bereik van de screening van 46% naar 84%. Van de gescreende zwangeren was 0,44% HBsAg-positief.

In dezelfde periode werden 1645 kinderen van HBsAg-positieve moeders geboren, hiervan kreeg 85% HBIg bij de geboorte. In 1991 kreeg respectievelijk 96%, 95%, 94% en 87% van de kinderen de 1e, 2e, 3e en 4e dosis hepatitis B vaccin toegediend.

Bij 17% van de kinderen vond de eerste vaccintoediening echter wel meer dan twee weken

te laat plaats.
Uit de resultaten blijkt dat het programma goed uitvoerbaar is alhoewel meer aandacht voor de tijdige toediening van het hepatitis B vaccin noodzakelijk is (**hoofdstuk 4.2**).

Op grond van het matige bereik (< 75% in 1990) van de landelijke screening van zwangeren in de Streeklaboratoria, werd een procesevaluatie uitgevoerd om bestaande knelpunten van het programma te inventariseren. Diverse betrokkenen werd gevraagd naar hun ervaringen en suggesties voor verbetering van het programma. Het bleek dat in 1990 nog eens 10% van de HBsAg bepalingen om logistieke en financiële redenen niet in de Streeklaboratoria werd uitgevoerd. De HBIg toediening aan de pasgeborenen van de in andere laboratoria opgespoorde HBsAg-positieve moeders was in die gevallen niet gegarandeerd. Het bereik van de vaccinaties was onder andere incompleet door onbekendheid met het programma, ontbreken van het hepatitis B vaccin op de consultatiebureau's, migratie van de veelal buitenlandse kinderen en gebrekkige registratie van de vaccinaties. Op grond van de bevindingen kan geconcludeerd worden dat screening buiten de Streeklaboratoria niet wenselijk is. Aanbevolen wordt om het hepatitis B vaccin onder beheer van de Provinciale Entadministraties te brengen en coördinatoren aan te stellen die het bereik van de screening en de vaccinaties op regionaal niveau bewaken.
Serologisch onderzoek van de kinderen moet de effectiviteit van het preventieprogramma bevestigen (**hoofdstuk 4.3**).

In hoofdstuk 5 wordt een algemene beschouwing gegeven over de preventie van perinatale hepatitis B en worden voorstellen voor toekomstig onderzoek geformuleerd.

Systematisch onderzoek van alle zwangeren naar de aanwezigheid van HBsAg, gelijktijdig met de bepaling van bloedgroep, Rhesusfactor en luesserologie in het eerste trimester van de zwangerschap, is geïndiceerd. Geadviseerd wordt de screening van zwangeren in een beperkt aantal (Streek)laboratoria te centraliseren; bij de HBsAg-positieve zwangeren moet nader onderzoek naar andere HBV merkstoffen (HBeAg en HBV DNA) plaatsvinden. Als een vrouw tijdens de zwangerschap niet is gescreend, moet HBsAg ten tijde van de bevalling alsnog met spoed worden bepaald; hiervoor moet een eenvoudige en snelle test beschikbaar komen.Voor de passieve-actieve immunisatie van kinderen van HBsAg-positieve moeders wordt het gebruik van 1 ml HBIg na de geboorte en het gebruik van de volwassen dosis (1 ml) recombinant vaccin, op de leeftijd van 3, 4, 5 en 11 maanden, aanbevolen. Meer aandacht is nodig voor de tijdige toediening van hepatitis B vaccin. Voorts moet de effectiviteit van het programma door serologisch onderzoek worden vastgesteld. In de nabije toekomst is onderzoek naar combinatievaccins met DKTP, *Haemophilus influenzae* type b en hepatitis B gewenst om het aantal injecties te kunnen beperken. Nader onderzoek moet uitsluitsel geven over de duur van de bescherming tegen HBV infectie na vaccinatie en de noodzaak tot revaccinatie op latere leeftijd. Voor de pasgeborenen van HBsAg-positieve moeders met hoge HBV DNA titers moet naar aanvullende preventieve maatregelen worden gezocht.

DANKWOORD

Bij het samenstellen van het dankwoord ben ik me meer dan ooit bewust dat dit proefschrift het resultaat is van een groot team. Het onderzoek van bijna 100.000 zwangere vrouwen en een aantal van hun kinderen in Rotterdam, Utrecht en de regio Twente-Gelderse Achterhoek was afhankelijk van de inzet van zeer velen. Een protocol ontwikkelen voor een landelijk preventieprogramma en de evaluatie daarvan, vereist een multidisciplinaire aanpak. Een aantal personen zijn al in dit boekje vermeld. Ik realiseer me dat zij mij de eer lieten een en ander op te schrijven.

Namen noemen is niet altijd nodig. Mijn dank gaat uit naar al diegenen die mij op de weg naar het "hora est" begeleidden met werklust en wijsheid, met wetenschappelijke of meer klantvriendelijke suggesties bij het samenstellen van voorlichtingsmateriaal.
Mijn dank gaat ook naar hen die vol vertrouwen in het onderzoek participeerden, de kleine bloedige bijdragen leverden en naar de helpende hand bij het afnemen daarvan tijdens de vaak hectische spreekuren. De vele rumoerige uren gezamenlijk doorgebracht tussen huilende baby's en rinkelende telefoons zijn onvergetelijk.
De snelheid waarmee het gewenste onderzoek werd uitgevoerd deed vermoeden dat het altijd om "rapid" screening ging en de valide interpretatie van de uitslagen alsook de immer opgeruimde zoekacties naar serummonsters bij temperaturen ver onder nul, heb ik zeer gewaardeerd.

Veler bereidheid belangeloos gegevens te verzamelen en af te staan mag niet onvermeld blijven. De verdraagzaamheid bij de steeds terugkerende verzoeken om aanvullende informatie en de vlotte beoordeling van talrijke concepten waren bijzonder.
Goede herinneringen heb ik aan de tomeloze ijver waarmee de verkregen gegevens vervolgens in de altijd onvermurwbare computer werden ingevoerd, kritisch gecontroleerd en nauwgezet gecorrigeerd. Onder muzikale begeleiding bood men hulp in nood.

Regelmatig werd ik bijgevoed: de nodige aanvullingen op de te magere lunch en het appeltje voor de dorst hebben mij zeer goed gedaan. Voor de niet aflatende persoonlijke inzet bij het beheren van mijn telefoon en overige kantoorartikelen ben ik zeer erkentelijk.

De getoonde normen en waarden bij de statistische bewerking van zowel 'ruwe' als 'cleane' data en het artistieke oog voor de grafische weergave van de resultaten hebben tot fraaie pres(en)taties geleid.
Het samenwerken met de vele betrokken instanties en het omgaan met even zoveel gevoeligheden is een aparte kunst; dank voor het geduld. De hulp bij het leren conformeren aan de ambtelijke regels van het spel, het begrip van alle misdeelde vrienden; ze waren allemaal onontbeerlijk. Correct words go a long way, thank you very much.

Tot slot kreeg ik bij een spaarzame dronk op de gezondheid van de lever veel morele steun van alle collegae/promovendi die wisten hoe de lang de weg zou zijn.

Toch een persoonlijk woord voor een enkeling. Mijn promotor, professor Solko Schalm wil ik allereerst bedanken voor de gelegenheid mij geboden om op de afdeling

Interne Geneeskunde II te kunnen werken. Zijn kritische kanttekeningen bij het onderzoek en voorstellen tot wijziging van de manuscripten hebben niet zelden tot de nodige frustratie aanleiding gegeven, maar zonder twijfel tot een beter resultaat geleid. De manier waarop promotor, professor Joop Huisman, mij voor de infectieuze ziekten heeft weten te interesseren, is besmettelijk gebleken; dank voor alle supervisie in de loop der jaren.

Zonder Riwka del Canho was het nooit tot een dubbelpromotie gekomen; fantastisch dat het zo heeft mogen zijn. Het verheugt mij dat Marja Bakker-Bendik en Adriaan Mazel, beiden pioniers op het terrein van het hier beschreven onderzoek, zich bereid hebben verklaard mij tijdens de verdediging van het proefschrift terzijde te staan. Zij lieten niet na mij tijdens dieptepunten te motiveren om toch vooral door te gaan.

Mijn ouders, wil ik op deze plaats graag bedanken voor de uitzonderlijke steun en belangstelling; weldadige vitaminen waardoor ik mijn bovenkamer goed heb kunnen gebruiken. Een geruststelling wellicht: ik ben voorlopig uitgestudeerd.

Lieve Arnold, dit boekje is voor jou, omdat je mij niet hinderde met aansporingen.
Je hebt wel de voorwaarden voor me geschapen die nodig waren og i de konmende år vil jeg gerne gøre gengæld og skabe de samme vilkår for dig...

ABOUT THE AUTHOR

The author of this thesis was born on August 18, 1956, in the Hague, the Netherlands. In 1974 she graduated from secondary school at the Christelijke Scholengemeenschap 'de Populier'. She started Medical School at the University of Leyden in 1976 and qualified in January 1984. From 1984 to 1986 she worked as a resident in the department of Surgery at the 'Rooms Katholieke Ziekenverpleging' in Hilversum. Until 1988 she was employed as a resident in the department of Obstetrics and Gynaecology at the 'Bronovo hospital' in the Hague. In 1988, she became a research assistant at the department of Internal Medicine II of the University Hospital Dijkzigt in Rotterdam; she participated in the hepatitis B vaccination trials described in this thesis under the supervision of professor dr. S.W. Schalm.

From 1990 up to 1994 she worked at the National Institute of Public Health and Environmental Protection (RIVM), Center for Infectious Disease Epidemiology (head: dr. M.J.W. Sprenger), in Bilthoven. Here, she was responsible for the evaluation of the national program on the prevention of perinatal hepatitis B infection, the results of which are also described in this thesis. During this period she started her training as a public health officer from the TNO Institute of Preventive Health Care (NIPG TNO) in Leyden and registered in 1992. In addition, she registered as an epidemiologist in 1993.