International Journal of Infectious Diseases 66 (2018) 153–156

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Letter to the Editor

Response to Letter to the Editor re 'Be cautious for exceptional results in evaluating the effect of adolescent booster of hepatitis B vaccine'

Brief introduction of hepatitis B vaccination program in China

In 1982, two plasma-derived hepatitis B vaccines, which were prepared from plasma of chronic HBsAg carriers from France and the United States, were licensed. From Jan 30 to Feb 4, 1983 a WHO Scientific Group meeting was convened on the discussion of HBV vaccination for prevention of primary liver cancer (Zuckerman et al., 1983). Millions of the first-generation plasma-derived vaccines have been administrated in neonates, infants, children and adults and the effectiveness and safety records are excellent. With the maturation of recombinant DNA technology, the plasma-derived hepatitis B vaccines were replaced with the HBsAg vaccines prepared from yeasts, or mammalian cells (Chen, 2009). In China, a recombinant vaccine was manufactured in early 1993 and in 1997 the plasma-derived vaccine was entirely replaced by the recombinant vaccine nationwide (Sun et al., 2002; Zhu et al., 2000; Liang et al., 2009, 2013). From 1st January 2002 the vaccination program was integrated into the national Expended Program of Immunization (EPI), with the vaccine being provided entirely by the government. With support from Global Alliance on Vaccine and Immunization (GAVI) the HBV vaccination program extended quickly to reach the resources-poor areas of China. Therefore, the nationwide HBV serosurvey conducted in 2006 showed that HBsAg seroprevalence was 0.96% in the population aged 1-4 years, 2.32% in those aged 5-14 years, 5.4% in persons aged 15-19 years, and more than 8.0% in individuals aged 20-59 years (Liang et al., 2009). In 2014, the HBsAg seroprevalence declined to 0.32% in the 1-4 age group, 0.94% in the 5-14 age group and 4.38% in those aged 15-29 years (Hepatology CSo, Diseases CSoI, 2015). Nationwide neonatal HBV vaccination dramatically decreased the HBV infection in children and young adults.

The participants and intervention of the Qidong Hepatitis B Intervention Study

The Qidong Hepatitis B Intervention Study (QHBIS) is a population-based, cluster randomized, controlled trial of HBV vaccination conducted in Qidong, China during 1983-1990 a window period when the vaccine was not available in any rural areas of China. At the time, Qidong had a population of 1·1 million and approximately 13,000 births each year. Approximately 80,000 newborns who were involved were randomly assigned into the vaccination or control groups (Sun et al., 1991, 2002; Qu et al., 2014). The study information has been described previously (Sun et al., 1991, 2002; Qu et al., 2014) and study protocol was posted in the Text S1 under the publication (Qu et al., 2014) (https://doi.org/10.1371/journal.pmed.1001774.s003). To reflect the real world with the hepatitis B vaccines, our analysis did not include the participants who were involved in the pilot study (Qu et al., 2014).

All neonates in the vaccination towns (N = 39,292) were vaccinated regardless of maternal HBsAg status. The first dose (5 μg) of HBV vaccine was administered within 24 hours after birth, followed by two doses (5 μg/dose) at one and six months of age, respectively. A total of 38,366 (97.64%) participants completed the three-dose, 5 μg-plasma-derived HBV vaccination series, which were manufactured and donated by the Merck Company through the WHO. Maternal HBsAg status of each vaccinee was determined by reverse passive hemagglutination assay. Neonates in the control towns (N = 34,441) received neither vaccine nor placebo.

This study could not be conducted currently, it was considered ethically justifiable during the time period of randomization given that the recombinant vaccine was not universally available in China. Therefore, in June 2000, the Qidong Center for Disease Control and Prevention (CDC) issued a Notification (File No. 2000-010) regarding HBV catch-up vaccination and booster. This information was posted in the Text S2 under the publication (Qu et al., 2014) (https://doi.org/10.1371/journal.pmed.1001774.s004).

Is a booster effective or not in the community population?

HBV vaccination has been recommended universally for prevention of HBV infection and liver diseases and is very efficacious in decreasing the HBsAg seroprevalence in children and in preventing the devastating complications of HBV infection in young adults (Qu et al., 2014; Romano et al., 2011; Chiang et al., 2013; Trepo et al., 2014). Since after 10-15 years of the vaccination the HBV vaccine-induced neutralizing antibodies to HBsAg (anti-HBs) have been found to disappear in many individuals, the long-lasting immunity to prevent the HBV infection was determined by booster test of anamnestic response (Zanetti et al., 2005). The conclusion was controversial because of the enrolled population who were born to mothers with unknown or different HBsAg status (Zanetti et al., 2005; McMahon et al., 2005; But et al., 2008; Lu et al., 2004; Lu et al., 2008; Jan et al., 2010; Chinchai et al., 2009; Zhu et al., 2011). The benefit of adolescent booster was unknown.

Built upon the QHBIS, we previously notified that the vaccinated infants had an approximately 16-fold increased risk of being chronic HBsAg carriers in adulthood if they were born to HBsAg-positive mothers as compared with those born to HBsAg-negative mothers (OR = 15.94, 95% CI 12.63–20.12). However, if participants were born to HBsAg-positive mothers, receiving one-dose adolescence booster decreased HBsAg seroprevalence (HBsAg

http://dx.doi.org/10.1016/j.ijid.2017.11.002

positive rate 10.72% versus 14.78%, respectively, OR = 0.66, 95% CI 0.46–0.95) (Qu et al., 2014). Meanwhile, several studies reported that infection with mutated HBV in the HBV-S genes, where the vaccination conferred neutralizing antibodies target, had increased in the general population (Bian et al., 2013; Lai et al., 2012). Due to various reasons, the HBIG was not administrated to the high-risk infants who were born to HBsAg-positive mother in many low- and middle-income countries and areas, and it was only administrated to some of the neonates after year of 2002 in China (Shao et al., 2011). An estimated 1,000,000 infants were born to HBsAg(+) mothers in 2015 of China (Zhang et al., 2010). We then continued to recall more individuals who originally participated in the QHBIS to provide some evidence related to adolescent booster with HBV vaccine (Wang et al., 2017). Only the individuals who were HBsAg-negative at childhood and donated blood both in childhood and in adulthood from the vaccination group in the cohort of QHBIS were further analyzed (Figure 1 in Ref Wang et al. (2017)). Indeed, the adolescent booster in the study was not randomized because some participants or the participants' guardians were unwilling to receive the booster. To control the bias, we further analyzed the distributions of gender, family income, and maternal HBsAg status. The participants who did not receive a booster had similar distribution to the participants who received a booster. Our results suggest that the adolescent booster might be necessary for the high-risk individuals who were born to HBsAg-positive mothers when they have no detectable serum anti-HBs (Wang et al., 2017). We also sequenced the PreS-S genes in each of the chronic HBV-infected adults. No increase of HBV mutants in the S gene and the 'a' epitope was found. Currently, all the sequences were filed in GenBank (Carmody, 2017).

Responses to some concerns raised by Dr. Zhou regarding the work (Wang et al., 2017)

1. Did the two surveys (2010–2014 and 1996–2000 respectively) in Wang's study (Wang et al., 2017) include the same participants? Our answer is YES! (Figure in Ref Wang et al. (2017)).

Based on our previous observation (Qu et al., 2014; Zhu et al., 2011; Xu et al., 2010), we tried to provide some evidence in Wang's study that whether the vaccination protected children/adolescents who were born to HBsAg-positive mothers are still resistant against HBV infection in their adulthood (Wang et al., 2017). The follow-up on HBsAg-negative children in the vaccination group was extended to December 31, 2014 and their HBV markers were updated by their age 23-28 years.

In two surveys, serum levels of alanine aminotransferase and serum HBsAg were tested on the same day as the blood was drawn as described in the methods section. All the results were recorded in our database system. One copy of the printed forms was given to the participant's guardian (10–11 year time-point) or the participant himself or herself (23–28 year time-point), one copy was filed. In order to protect the participants and have the participants get benefit from our study, only the remaining blood samples after the clinical laboratory tests were frozen were used for future analysis (Methods section in Ref Wang et al. (2017)). Therefore, among the 9793 participants only "a total of 6132 and 6067 individuals had stored serum samples for 10–11 year time-point and 23–28 year time-point respectively" for the analysis of the status of anti-HBc, anti-HBs and serum HBV-DNA.

2. Nature HBsAg clearance and breakthrough HBV infection

Nature HBsAg clearance was frequently reported in the population from different areas worldwide. A total of 1,271 Alaska Native Americans with chronic HBV infection, whose median age at initial HBsAg-positive result was 20.9 years, were followed for an average of 19.6 years. It was found that the HBsAg loss occurred in 158 persons for a rate of HBsAg clearance of 0.7%/year (Simonetti et al., 2010). Another study included 315 patients with a median age of 35 years were followed for a mean period of 5.7 ± 3.9 years. The patients were different ethnic origins but most of them were Caucasian. It was found that inactive HBsAg carriers showed an annual HBsAg clearance incidence rate of 23.4 cases per 1000 persons-years, which was higher than that of patients with chronic hepatitis B (Habersetzer et al., 2015). Overall, it was previously reported that the annual incidence of HBsAg seroprevalence varied from 0.12% to 2.38% in cohorts from Asian countries and from 0.54% to 1.98% in cohorts from Western countries.

Natural HBsAg clearance was also observed in children. A total of 405 chronic carriers (95% genotype E), recruited at a median age of 10.8 years in two Gambian villages, were followed for a median length of 28.4 years. It was found that 7.4% (95% CI 6.3% to 8.8%) cleared HBeAg and 1.0% (0.8% to 1.2%) cleared HBsAg annually (Shimakawa et al., 2016). In other reports, a total of 349 children (205 male) from Taiwan were followed for 20.6 ± 4.4 years with initial ages of 8.4 ± 3.9 years; 42 (12.0%) cleared HBsAg spontaneously. The spontaneous HBsAg clearance was found more likely to occur in a 'patient' born to a non-HBsAg carrier mother (Chiu et al., 2014). Based on data obtained from three national serosurveys of hepatitis B on the local residents living in 160 disease surveillance points in 31 provinces of China, the recent analysis found that the estimated annual rates of HBsAg seroclearance in chronic HBV infections of Chinese population varied in different age groups. From age 10 to age 24, the annual rate was 0.56% (age 10-14), 1.44% (age 15-19), and 4.37% (age 20-24) (Zu et al., 2017). We previously found that the children had increased annual rates of HBsAg seroclearance who received catch-up vaccination (1.46%) than those who did not receive catch-up vaccination at age 10-14 years. Nerveless, the annual rate of HBsAg seroclearance was much less (0.36%) among the vaccinated children (Qu et al., 2014).

3. Did HBV breakthrough infection happen among vaccinated individuals?

A case of a 50-year-old homosexual Caucasian man who received the plasma-derived HBV vaccination at age 28 years was reported to have suffered from acute hepatitis B in 2009 (Boot et al., 2009). It was also reported that a child from Taiwan was documented to have received 4 doses of plasma-derived HB vaccines and acquired an anti-HBs titer of 21 mIU/mL at the age of 18 months. He remained HBsAg-negative at 7 years of age but HBsAg-positivity was detected at age 15 years and the infected HBV had no G145 mutation (Lu et al., 2004). We previously followed a total of 806 vaccinated individuals at their ages 5, 10, 20 and 24 years old (Figure 2 of Ref Zhu et al. (2011)). Their HBV markers were determined at each time point. We noticed natural HBV infection did happen, 2 of them became positive for HBsAg during the period between age 5 and age 10, 1 happened between age 10 and age 20, and 1 happened between 20 and age 24 years old (Zhu et al., 2011). We assumed the lesser vaccination protection might be due to limited herd immunity barrier because the vaccination coverage was very low in the 1980s (Sun et al., 2002; Liang et al., 2013).

4. Did occult HBV infection (OBI) in the vaccinated children or adults happen?

In Wang's study (Wang et al., 2017) there was the possibility that the observed infections in adulthood were already acquired at childhood (Carmody, 2017). Mu et al. (2009) had reported among the vaccinated children in Taiwan the OBI in all the anti-HBc positive participants was as high as 10.9% and all the HBV isolates had no G145R mutation. OBI is characterized by "the persistence of HBV-DNA in the liver tissue (and in some cases also in the serum) in absence of HBsAg". OBI may be anti-HBc(+) alone or together with anti-HBs(+). In addition, an individual infected at birth might not necessarily be positive for HBV-DNA in serum

when he or she was positive for anti-HBc (Raimondo et al., 2010). Indeed some of the individuals with anti-HBc(+) status at childhood converted to HBsAg-positive status in 13-17 years. The detailed evolution of the serologic status of children with isolated anti-HBc(+) is provided in the supplementary file (http://www.sciencedirect.com/science/article/pii/S1201971217301315). Stratified by the maternal HBsAg status, the individuals who were born to healthy mothers tend to be able to become negative for anti-HBc in 13-17 years. Recent literature has shown that the immune responses to microbial production stimulation in HBV prenatal exposed neonates were very different from the healthy ones (Hong et al., 2015).

Currently, we are still unclear why the immune responses to the HBV vaccine in infants/children are different. Although the murine immune system is different from that of humans, we could understand the potential implication from the murine immune responses to model antigen. The experiments using the I-Ek-restricted helper T cell response of B10.BR mice to pigeon cytochrome c, the tractable protein vaccination model for studying different TCR affinities, have demonstrated that significantly more T cells with high affinity TCR developed into "resident" CD4+ T (Tfh) cells *in vivo* than the T cells with low affinity TCR, and the low affinity clonotypes of T cells failed to form memory (McHeyzer-Williams and Davis, 1995; McHeyzer-Williams et al., 1999; Fazilleau et al., 2009). Immunology advances have revealed that follicular helper CD4+ T cells (Tfh) are the specialized providers of B cell helper to regulate B cell differentiation (Crotty, 2011). HBsAg presence was documented in amniotic fluid, cord blood, breast milk, vaginal fluids, and infant gastric content (Lee et al., 1978; Lu et al., 2014). Therefore, T cells that recognize the epitopes of HBsAg with high-affinity T cell antigen receptors (TCR) might be deleted during immune system development (Starr et al., 2003). We need more experimental evidence to elaborate the mechanisms in infants with HBsAg pre-exposure before or just after the birth.

Limitations and implications

Certain limitations should be considered when interpreting the study results. As was stated in the Wang study (Wang et al., 2017), there was a limited sample size of participants who were born to HBsAg-positive mothers and had anti-HBs(−) status at baseline. The difference of adolescent booster in decreasing HBsAg was statistically borderline significant (P = 0.074). In addition, the booster was not randomized and anti-HBc status at baseline was not determined in all the participants. Our study findings should be replicated in other populations.

Plasma-derived HBV vaccine has been totally replaced nowadays by recombinant HBV vaccines. There was no clear documentation of whether the vaccination-protected children/adolescents born to HBsAg-positive mothers are still resistant against HBV infection in their adulthood after being vaccinated two decades earlier. More evidence based on cohort studies is needed. Our study provided a piece of data for HBV vaccination program in controlling its chronic infection.

References

Bian T, Yan H, Shen L, Wang F, Zhang S, Cao Y, et al. Change in hepatitis B virus large surface antigen variant prevalence 13 years after implementation of a universal vaccination program in China. J Virol 2013;87:12196–206.

Boot HJ, van der Waaij LA, Schirm J, Kallenberg CG, van Steenbergen J, Wolters B. Acute hepatitis B in a healthcare worker: a case report of genuine vaccination failure. J Hepatol 2009;50:426–31.

But DY, Lai CL, Lim WL, Fung J, Wong DK, Yuen MF. Twenty-two years follow-up of a prospective randomized trial of hepatitis B vaccines without booster dose in children: final report. Vaccine 2008;26:6587–91.

Carmody E. Time to re-evaluate the effect of the adolescent booster of hepatitis B vaccine. Int J Infect Dis 2017;60:88–90.

Chen DS. Hepatitis B vaccination: the key towards elimination and eradication of hepatitis B. J Hepatol 2009;50:805–16.

Chiang CJ, Yang YW, You SL, Lai MS, Chen CJ. Thirty-year outcomes of the national hepatitis B immunization program in Taiwan. JAMA 2013;310:974–6.

Chinchai T, Chirathaworn C, Praianantathavorn K, Theamboonlers A, Hutagalung Y, Bock PH, et al. Long-term humoral and cellular immune response to hepatitis B vaccine in high-risk children 18-20 years after neonatal immunization. Viral Immunol 2009;22:125–30.

Chiu YC, Liao SF, Wu JF, Lin CY, Lee WC, Chen HL, et al. Factors affecting the natural decay of hepatitis B surface antigen in children with chronic hepatitis B virus infection during long-term follow-up. J Pediatr 2014;165:767–72 e1.

Crotty S. Follicular Helper CD4 T Cells (TFH). Annu Rev Immunol 2011;29:621–63.

Fazilleau N, McHeyzer-Williams LJ, Rosen H, McHeyzer-Williams MG. The function of follicular helper T cells is regulated by the strength of T cell antigen receptor binding. Nat Immunol 2009;10:375–84.

Habersetzer F, Moenne-Loccoz R, Meyer N, Schvoerer E, Simo-Noumbissie P, Dritsas S, et al. Loss of hepatitis B surface antigen in a real-life clinical cohort of patients with chronic hepatitis B virus infection. Liver Int 2015;35:130–9.

Hepatology CSo, Diseases CSoI. Guidelines for prevention of chronic hepatitis-Chronic Hepatitis B. 2015.

Hong M, Sandalova E, Low D, Gehring AJ, Fieni S, Amadei B, et al. Trained immunity in newborn infants of HBV-infected mothers. Nat Commun 2015;6:6588.

Jan CF, Huang KC, Chien YC, Greydanus DE, Davies HD, Chiu TY, et al. Determination of immune memory to hepatitis B vaccination through early booster response in college students. Hepatology 2010;51:1547–54.

Lai MW, Lin TY, Tsao KC, Huang CG, Hsiao MJ, Liang KH, et al. Increased seroprevalence of HBV DNA with mutations in the s gene among individuals greater than 18 years old after complete vaccination. Gastroenterology 2012;143:400–7.

Lee AK, Ip HM, Wong VC. Mechanisms of maternal-fetal transmission of hepatitis B virus. J Infect Dis 1978;138:668–71.

Liang X, Bi S, Yang W, Wang L, Cui G, Cui F, et al. Epidemiological serosurvey of hepatitis B in China–declining HBV prevalence due to hepatitis B vaccination. Vaccine 2009;27:6550–7.

Liang X, Cui F, Hadler S, Wang X, Luo H, Chen Y, et al. Origins, design and implementation of the China GAVI project. Vaccine 2013;31(Suppl 9):J8–14.

Lu CY, Chiang BL, Chi WK, Chang MH, Ni YH, Hsu HM, et al. Waning immunity to plasma-derived hepatitis B vaccine and the need for boosters 15 years after neonatal vaccination. Hepatology 2004;40:1415–20.

Lu CY, Ni YH, Chiang BL, Chen PJ, Chang MH, Chang LY, et al. Humoral and cellular immune responses to a hepatitis B vaccine booster 15-18 years after neonatal immunization. J Infect Dis 2008;197:1419–26.

Lu LL, Chen BX, Wang J, Wang D, Ji Y, Yi HG, et al. Maternal transmission risk and antibody levels against hepatitis B virus e antigen in pregnant women. Int J Infect Dis 2014;28:41–4.

McHeyzer-Williams M, Davis M. Antigen-specific development of primary and memory T cells in vivo. Science 1995;268:106–11.

McHeyzer-Williams LJ, Panus JF, Mikszta JA, McHeyzer-Williams MG. Evolution of antigen-specific T cell receptors In vivo: preimmune and antigen-driven selection of preferred complementarity-determining region 3 (CDR3) motifs. J Exp Med 1999;189:1823–38.

McMahon BJ, Bruden DL, Petersen KM, Bulkow LR, Parkinson AJ, Nainan O, et al. Antibody levels and protection after hepatitis B vaccination: results of a 15-year follow-up. Ann Intern Med 2005;142:333–41.

Mu SC, Lin YM, Jow GM, Chen BF. Occult hepatitis B virus infection in hepatitis B vaccinated children in Taiwan. J Hepatol 2009;50:264–72.

Qu C, Chen T, Fan C, Zhan Q, Wang Y, Lu J, et al. Efficacy of neonatal HBV vaccination on liver cancer and other liver diseases over 30-year follow-up of the Qidong hepatitis B intervention study: a cluster randomized controlled trial. PLoS Med 2014;11:e1001774.

Raimondo G, Pollicino T, Romano L, Zanetti AR. A 2010 update on occult hepatitis B infection. Pathol Biol (Paris) 2010;.

Romano L, Paladini S, Van Damme P, Zanetti AR. The worldwide impact of vaccination on the control and protection of viral hepatitis B. Dig Liver Dis 2011;43(Suppl 1):S2–7.

Shao ZJ, Zhang L, Xu JQ, Xu DZ, Men K, Zhang JX, et al. Mother-to-infant transmission of hepatitis B virus: a Chinese experience. J Med Virol 2011;83:791–5.

Shimakawa Y, Lemoine M, Njai HF, Bottomley C, Ndow G, Goldin RD, et al. Natural history of chronic HBV infection in West Africa: a longitudinal population-based study from The Gambia. Gut 2016;65:2007–16.

Simonetti J, Bulkow L, McMahon BJ, Homan C, Snowball M, Negus S, et al. Clearance of hepatitis B surface antigen and risk of hepatocellular carcinoma in a cohort chronically infected with hepatitis B virus. Hepatology 2010;51:1531–7.

Starr TK, Jameson SC, Hogquist KA. Positive and negative selection of T cells. Annu Rev Immunol 2003;21:139–76.

Sun Z, Zhu Y, Stjernsward J, Hilleman M, Collins R, Zhen Y, et al. Design and compliance of HBV vaccination trial on newborns to prevent hepatocellular carcinoma and 5-year results of its pilot study. Cancer Detect Prev 1991;15:313–8.

Sun Z, Ming L, Zhu X, Lu J. Prevention and control of hepatitis B in China. J Med Virol 2002;67:447–50.

Trepo C, Chan HL, Lok A. Hepatitis B virus infection. Lancet 2014;384:2053–63.

Wang Y, Chen T, Lu LL, Wang M, Wang D, Yao H, et al. Adolescent booster with hepatitis B virus vaccines decreases HBV infection in high-risk adults. Vaccine 2017;35:1064–70.
Xu L, Wei Y, Chen T, Lu J, Zhu CL, Ni Z, et al. Occult HBV infection in anti-HBs-positive young adults after neonatal HB vaccination. Vaccine 2010;28:5986–92.
Zanetti AR, Mariano A, Romano L, D'Amelio R, Chironna M, Coppola RC, et al. Long-term immunogenicity of hepatitis B vaccination and policy for booster: an Italian multicentre study. Lancet 2005;366:1379–84.
Zhang H, Cui FQ, Gong XH. Staus of the hepatitis B virus surface antigen and e antigen prevalence among reproductive women in China. Chin J Vac Immun 2010;12:496–9.
Zhu X, Zhang XL, WL X. National EPI vaccination and hepatitis B vaccine coverage rate and the related factors: results from the 1999 nationwide survey. Chin J Vac Immun 2000;6:193–7.
Zhu CL, Liu P, Chen T, Ni Z, Lu LL, Huang F, et al. Presence of immune memory and immunity to hepatitis B virus in adults after neonatal hepatitis B vaccination. Vaccine 2011;29:7835–41.
Zu J, Zhuang G, Liang P, Cui F, Wang F, Zheng H, et al. Estimating age-related incidence of HBsAg seroclearance in chronic hepatitis B virus infections of China by using a dynamic compartmental model. Sci Rep 2017;7:2912.
Zuckerman AJ, Sun TT, Linsell A, Stjernsward J. Prevention of Primary Liver Cancer-Report on a Meeting of a W.H.O. Scientific Group. Lancet 1983;1:463–5.

Chunfeng Qu*
Yuting Wang
Department of Immunology, National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, China

Chunsun Fan
Department of Etiology, Qidong People's Hospital/Qidong Liver Cancer Institute, Jiangsu 226200, China

* Corresponding author.
E-mail address: quchf@cicams.ac.cn (C. Qu).

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Received 6 November 2017