



Original Article

Protective Effect of Neonatal Hepatitis B Vaccine Against HBV Breakthrough Infection in Children with Leukemia: A Real-world Study

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Abstract

Background and Aims: Hepatitis B vaccine is the most effective preventive measure against hepatitis B virus (HBV) infection. However, the risk of HBV breakthrough infection in fully immunized children (neonatal hepatitis B immunization) who receive immunosuppressive therapy and transfusion of blood components is not well characterized. In this real-world study, we aimed to investigate the immune protection conferred by neonatal hepatitis B vaccine in children with acute lymphoblastic leukemia (ALL) who were treated with immunosuppressive therapy and blood component transfusions. **Methods:** Children with ALL who had received all three doses of neonatal hepatitis B vaccine were included in this study. HBV seromarkers were detected before and after the initiation of immunosuppressive therapy. **Results:** A total of 1,011 children with ALL who were fully vaccinated against hepatitis B in infancy before the initiation of immunosuppressive therapy were eligible for inclusion. HBV infection was detected in four of 410 children (0.98%) with an HBsAg test after the initiation of immunosuppressive therapy. The median interval from treatment initiation was 19 months. **Conclusions:** Three doses of neonatal hepatitis B vaccine conferred adequate protection. In endemic regions, there is a low risk of HBV breakthrough infection in fully immunized children with immunosuppressive therapy.

Keywords: Hepatitis B vaccine; Immune protection; Children; Breakthrough infection.

Abbreviations: ALL, acute lymphoblastic leukemia; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CCLG, Children's Cancer and Leukemia Group in China; CQMU, Hospital of Chongqing Medical University; CMIA, chemiluminescence microparticle immunoassay; FFP, fresh frozen plasma; HBcAb, hepatitis B core antibody; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HepB, hepatitis B; IQR, interquartile range; LOQ, limit of quantitation; PC, platelet concentrate; RCC, red cell concentrate; TBIL, total bilirubin.

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Introduction

Hepatitis B (HepB) vaccine is safe and highly effective in protecting against hepatitis B virus (HBV) infection. According to estimates, 90% coverage with HepB vaccine is likely to prevent 84% of HBV-related deaths.¹ In a study conducted in China, completion of three dose regime of HepB vaccine in infancy was shown to elicit a protective antibody response defined as hepatitis B surface antibody (HBsAb) ≥ 10 mIU/mL in more than 95% of infants.² China has made concerted efforts to establish a universal immunization program for newborns with a 99% coverage rate,³ which has led to a remarkable reduction in the prevalence of HBV infection in children.

The positivity rate of hepatitis B core antibody (HBcAb) in the general population directly correlates with HBV prevalence. HBcAb positivity in intermediate or highly endemic areas (e.g., China) ranges from 13.5% to 60%.^{4–6} HBcAb positivity is indicative of previous HBV exposure,^{7–10} and HBcAb-positive patients undergoing immunosuppressive therapy (IT) may develop HBV breakthrough infections.^{11–14} However, blood donors in intermediate or highly endemic areas are usually not screened for HBcAb, as exclusion of these individuals from the blood donor pool would lead to a substantial shortage of blood and blood products. Transfusion of HBcAb-positive blood products is inevitable in areas with intermediate-high prevalence of HBV infection.

The routine treatment for acute lymphoblastic leukemia (ALL) includes IT (chemotherapy) and supportive care, including transfusion of various blood components. HBV infection is a global public health concern, with an estimated

case load of 2 billion people worldwide.¹⁵ Children receiving IT are immunocompromised and dependent on multiple blood product transfusions, which increase the risk of HBV infection. In HBcAb endemic areas, the risk of HBV breakthrough infection after IT and blood transfusion in children who had received HepB vaccine in infancy is not well characterized. HepB vaccine is recommended for all newborns to prevent HBV infection, and has been widely implemented. To the best of our knowledge, this is the first large real-world study to evaluate the immune protection conferred by neonatal HepB vaccine in children who received IT and blood transfusion in HBcAb epidemic areas.

Methods

Study design and participants

We performed a hospital-based study to assess the HBV-specific immune protection conferred by neonatal HepB vaccine in children with ALL during IT. The diagnosis and treatment of ALL followed the guidelines of the Children's Cancer and Leukemia group in China (CCLG)-2008/CCCG-2015. Children with ALL who were between 1 and 14 years of age who tested negative for HBsAg and HbcAb before initiation of IT at the Children's Hospital of Chongqing Medical University (CQCMU) (the National Clinical Research Center for Child Health and Disorders) between September 2012 to August 2018 were eligible for inclusion. The exclusion criteria were: (1) not completing the three doses neonatal HepB vaccination schedule; (2) the presence of active chronic hepatitis or liver cirrhosis; carrying hepatitis C or human immunodeficiency virus; and (3) without HBV seromarker tests performed before and after the initiation of IT. A group of age- and/or sex-matched healthy children were recruited as controls at the physical examination center of CHCMU. The control group had no history of major illness, blood transfusion, or family history of HBV (determined by the attending physician at each visit). HBV seromarker results were collected from healthy adults from 18–35 years of age at the Second Affiliated Hospital of Chongqing Medical University; this age-group accounts for the vast majority of blood donors in China. The study was approved by the Ethics Review Committee of the Children's Hospital of Chongqing Medical University. Written informed consent was obtained from all subjects or their parent and/or legal guardian for subjects younger than 18 years of age).

HBV screening

HBV seromarkers were measured before- and after the initiation of IT using chemiluminescence microparticle immunoassay (CMIA) kits (Abbott GmbH & Co. KG, Wiesbaden, Germany) following the manufacturer's instructions. Subjects were considered HBsAg-positive at values ≥ 0.05 IU/mL, HBsAb-positive, or seroprotected at values ≥ 10 mIU/mL, HBeAg-positive at a sample rate/cut off rate ≥ 1 S/CO, HBeAb-positive at values ≤ 1 S/CO, and HBcAb-positive at values ≥ 1 S/CO. Occurrence of HBV infection was confirmed HBsAg seroconversion HBV DNA assay with a quantitative diagnostic kit for HBV DNA with a limit of quantitation (LOQ) of 500 IU/mL (Shengxiang, Hunan, China).

Statistical analysis

The statistical analysis was performed with SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Continuous variables

were reported as medians and interquartile range (IQR) and between-group differences were assessed using Mann-Whitney U tests. Categorical variables were reported as numbers and percentages (%) and between-group differences were assessed using chi-squared tests. All statistical tests were two-tailed and p -values < 0.05 were considered statistically significant.

Results

Characteristics of the study population

A total of 1,308 children with full neonatal HepB vaccination and diagnosed with ALL received IT at the CHCMU between September 2012 and August 2018 (Fig. 1). Transfusion of fresh frozen plasma (FFP), red cell concentrate (RCC), and platelet concentrate (PC) was administered as supportive care during IT, 229 children had not been tested for HBV seromarkers and 68 had tested HBsAg-positive or HBcAb-positive prior to the initiation of IT. The remaining 1,011 children were eligible before the initiation of IT. Of those, 703 had a record of HBV test at a median of 2 (IQR, 1.3–2.2) months after the initiation of IT, while entering the second-stage of chemotherapy. With the progress of IT, 297 children were tested for HBV seromarkers (HBsAb and HBcAb) at a median interval of 10 (IQR, 8–11) months after the initiation of IT, while entering the follow-up phase (maintenance treatment phase). To follow-up as many eligible children as possible, 410 were tested for HBsAg to assess HBV infection at a median interval of 19 (IQR, 14–26) months after the initiation of IT.

Occurrence of HBV breakthrough infection

Out of the 410 children with tests for HBV seromarkers at a median interval of 19 months after initiation of IT, four children (patients 1, 2, 3, and 4) were found to have developed HBV breakthrough infection with continuous HBsAg-positivity and detectable HBV DNA (Table 1, Fig. 2). Patient 1 died of leukemia recurrence. The father was confirmed to have HBV infection. The other three patients are alive and well (Supplementary Table 1). The changes in HBeAg, HBeAb, HBcAb, and liver biochemistry are shown in Supplementary Fig. 1. HBV status was not routinely monitored during IT, and the median time for confirmation of HBV infection in the four children was 14 (IQR, 13–15) months after the initiation of IT. The characteristics of children with or without HBV breakthrough infection are shown in Table 2. The HBsAb titers in the four children and others are shown in Supplementary Fig. 2. The HBsAb titers of patients 1, 2, and 4 were below the median level of the age- and sex-matched healthy children, but were within the level of the control children with ALL who did not have HBV infection.

Passive transfer of HBV antibody

During IT, the positivity rate for HBcAb increased to 37.7% (265/703) after supportive blood transfusion (2 months after initiation of IT); 62.3% (438/703) of patients remained HBcAb-negative (group I, HBcAb-/-), and 37.7% had detectable HBcAb after transfusion (Group II, HBcAb-/+). There was a significant difference between the pretreatment HBsAb and the HBsAb 2 months after initiation of IT in Group I and Group II. There was also a significant difference between Group I and Group II in post-transfusion HBsAb ($p < 0.05$) (Table 3). HBsAb positivity increased

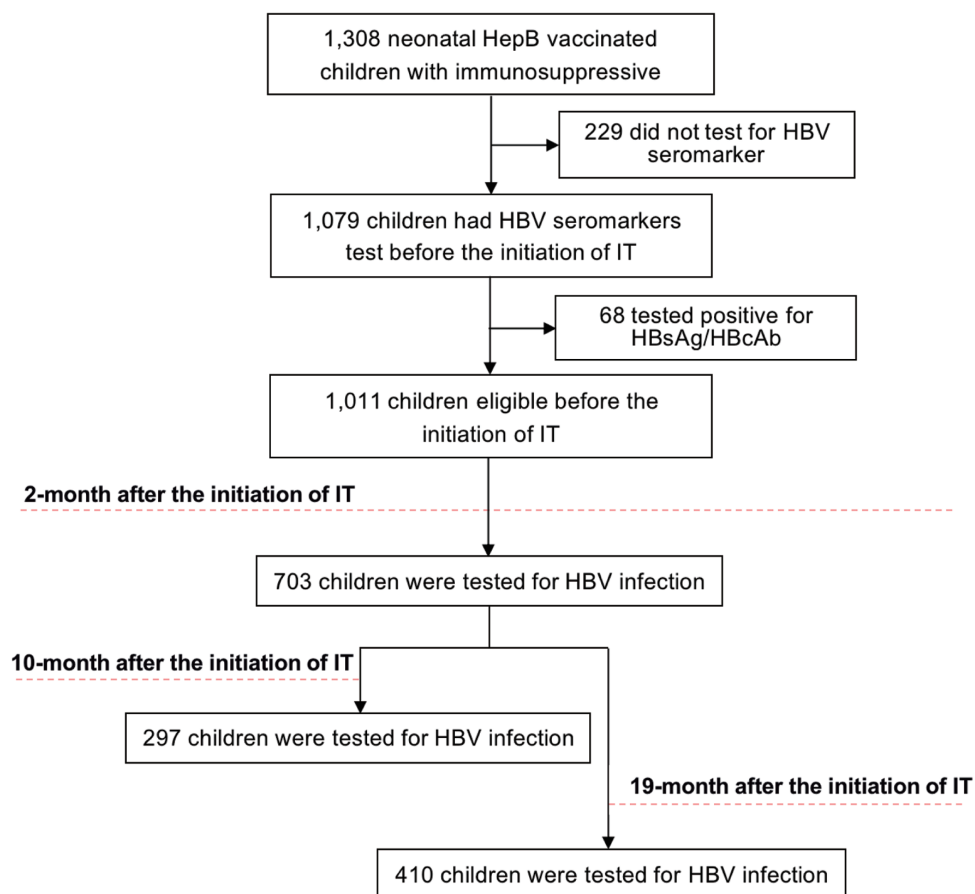


Fig. 1. Study sample selection. HepB, hepatitis B; HBV, hepatitis B virus; IT, immunosuppressive therapy; HBsAg, hepatitis B surface antigen; HBcAb, hepatitis B core antibody.

after transfusion from 63.4% to 79.5%, with HBsAb titers of 20 mIU/mL vs. 39 mIU/mL, and was higher in Group II (64.9% vs. 94.0%, $p=0.000$) and 22 mIU/mL vs. 73 mIU/mL, $p=0.000$) than in group I (62.6% vs. 70.8%, $p=0.01$) and 20 mIU/mL vs. 23 mIU/mL, $p=0.119$). With the progress of IT, passively acquired HBcAb and HBsAb gradually disappeared.

Changes in HBsAb level during IT

HBsAb positivity rates significantly decreased in children during IT from 63.0% to 37.4%. The HBsAb titers in group

I and Group II patients before and after the initiation of IT are shown in Fig. 3A–C. Compared with the age-matched healthy controls, the levels of protective HBsAb were always lower in children undergoing IT (Fig. 3B–D). With the progress of IT, HBsAb titers decreased significantly in both group I and Group II. At a median interval of 10 months after the initiation of IT, the HBsAb titers were 61.8% vs. 39.4% and 21.7 mIU/mL vs. 13.5 mIU/mL in group I and 63.6% vs. 34.9% and 18.3 mIU/mL vs. 12.6 mIU/mL in Group II, significantly lower than those in age-matched healthy children (70.4% and 43.2 mIU/mL, Supplementary Table 1); similar changes were observed in children in different age groups (Supplementary Fig. 3).

Table 1. Characteristics of children who developed HBV infection during IT

Patient	Sex	Age (years)	Baseline					HBV breakthrough infection				Outcome
			HepB vaccine	Diagnosis	HB-sAg	HBsAb (mIU/mL)	HB-cAb	HBV DNA (IU/mL)	HB-sAg	HBsAb (mIU/mL)	HB-cAb ^a	
1	F	2	Yes	ALL	Neg	27.34	Neg	3.3×10^6	Pos	1.32	Neg	Died-leukemia
2	F	3	Yes	ALL	Neg	0.06	Neg	3.0×10^6	Pos	0	Neg	Alive and well
3	F	3	Yes	ALL	Neg	Pos	Neg	5.6×10^6	Pos	0.55	Neg	Alive and well
4	M	9	Yes	ALL	Neg	1.77	Neg	2.9×10^8	Pos	0	Neg	Alive and well

Normal values: HBsAg (<0.05); HBsAb (≥ 10); HBeAg (<1); HBeAb (>1); HBcAb (<1). ^aStatus of HBcAb at HBV breakthrough infection. Pos, positive; Neg, negative; NA, not available; IT, immunosuppressive therapy; HBsAg, hepatitis B surface antigen; HBsAb, hepatitis B surface antibody; HBeAb, hepatitis B e antibody; HBeAg, hepatitis B e antigen; HBcAb, hepatitis B core antibody.

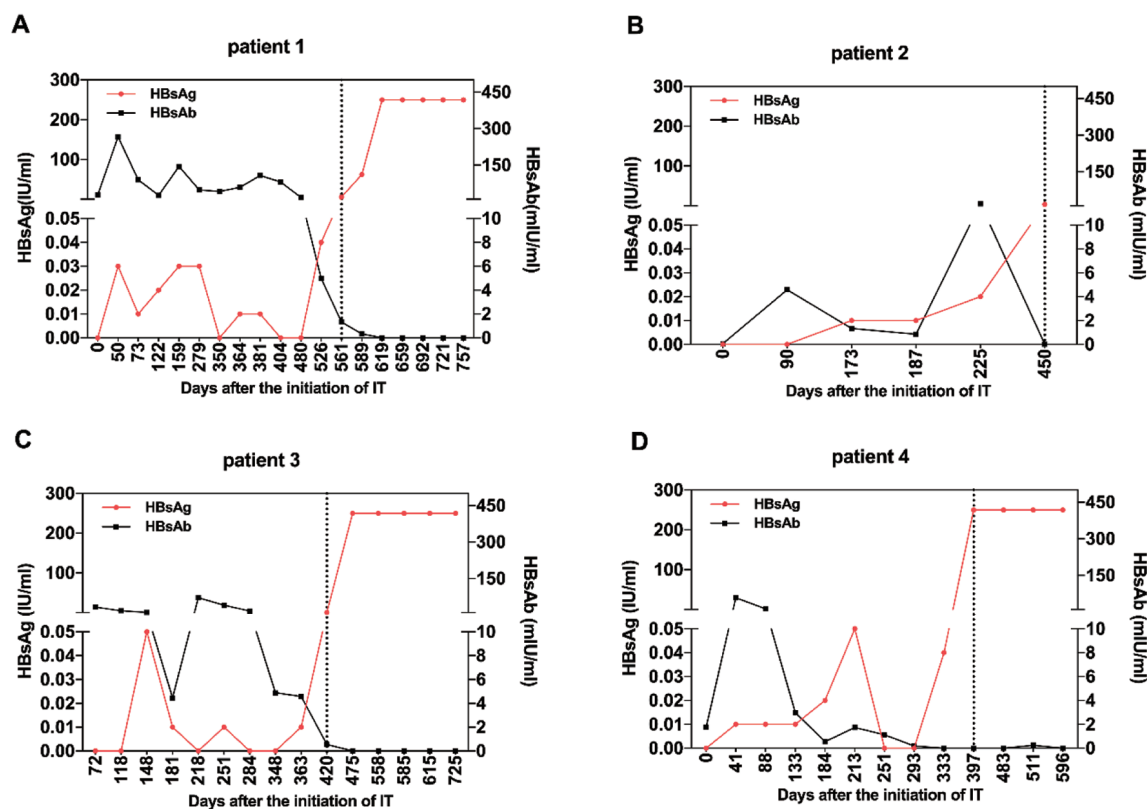


Fig. 2. Changes in HBsAg and HBsAb in children who developed HBV breakthrough infection. Profile of HBsAg and HBsAb in the four children who developed HBV breakthrough infection during IT (A) Patient 1; (B) Patient 2; (C) Patient 3; (D) Patient 4)]. Vertical dashed lines indicate the time of detection of HBV infection.

Discussion

The immune protection conferred by neonatal HepB vaccine against HBV in children receiving IT is not clear. In this study, 0.98% (4/410) of neonatal HepB vaccinated children developed HBV breakthrough infection during IT, and a

Table 2. Characteristics of children with and without HBV breakthrough infection during IT

Characteristic	HBV infection	Without HBV infection
Number	4	406
Age ^a (years)	3 (3, 5)	4 (3, 8)
Sex (Male: Female)	1:3	250:156
Diagnosis	ALL	ALL
HBsAg test		
--	0	406
-/+	4	0
HBCAb test		
--	1	234
-/+	3	172
HBsAb titer ^a (mIU/mL)	2 (1, 15)	20 (4, 86)

^aData are medians (interquartile range). IT, immunosuppressive therapy; HBsAg, hepatitis B surface antigen; HBsAb, hepatitis B surface antibody; HBCAb, hepatitis B core antibody.

large proportion of children lost protective HBsAb titers with progression of IT. In areas of high and intermediate HBV endemicity, blood transfusion is likely to result in passive transfer of both HBCAb and HBsAb.

ALL is the most common pediatric malignancy, accounting for approximately 25% of cancer diagnoses in children <15 years of age.^{16,17} Advances in IT and improved supportive care including transfusion of blood components have helped improve the survival of ALL patients with ALL.¹⁸ The immunity against HBV infection is likely to be compromised in patients undergoing IT. In previous studies, the reported rate of HBV breakthrough infection rate in HBsAg-negative adult patients undergoing IT ranged from 8.3% to 41.5%.¹¹⁻¹⁴ In this study, only four of 410 children (0.98%) developed HBV breakthrough infections during IT. After comprehensive consideration of other research evidence, breakthrough infections are primarily attributable to occult HBV. Occult HBV infection (OBI) is regarded as the fifth phase of the natural history of chronic HBV infection, characterized by the presence of HBV DNA in HBsAg-negative individuals.¹⁹ Although there are sensitive screening assays to screen for HBV infection, OBI is notoriously difficult to detect because of the low-viral-load. The occurrence of occult HBV breakthrough infection can be attributed to one of the following two reasons. First, the children may have been infected with HBV previously, albeit only occult infections with a very-low-level of viral replication and HBsAg expression. Therefore, no serological markers were detected until the occurrence of HBV breakthrough infection. Despite universal infant HepB immunization in China since 1992, vaccinated children with OBI are not uncommon.^{20,21} The second reason could be transmission of HBV during transfusion from OBI donors.

Table 3. Characteristics of group I (HBcAb–/HBcAb–) and group II (HBcAb–/HBcAb+) children before and after the initiation of IT

Characteristic	Total	Group I (HB-cAb–/HBcAb–)	Group II (HB-cAb–/HBcAb+)	p-value ^b
Number	703	438	265	–
Age ^a (years)	4 (3, 8)	5 (3, 8)	4 (2, 7)	0.004
Sex (Male: Female)	410:293	260:178	150:115	0.472
HBsAb positivity rate (%)				
Before initiation of IT	63.4	62.6	64.9	0.531
2 months after initiation of IT	79.5	70.8	94.0	0.000
p-value ^c	–	0.01	0.000	–
HBsAb ^a (mIU/mL)				
Before IT	20 (4, 94)	20 (4, 96)	22 (4, 91)	0.561
2 months after initiation of IT	39 (13, 116)	23 (8, 78)	73 (33, 160)	0.000
p-value ^c	–	0.119	0.000	–

^aData are medians (interquartile range); ^bBetween-group difference; ^cWithin group difference. IT, immunosuppressive therapy.

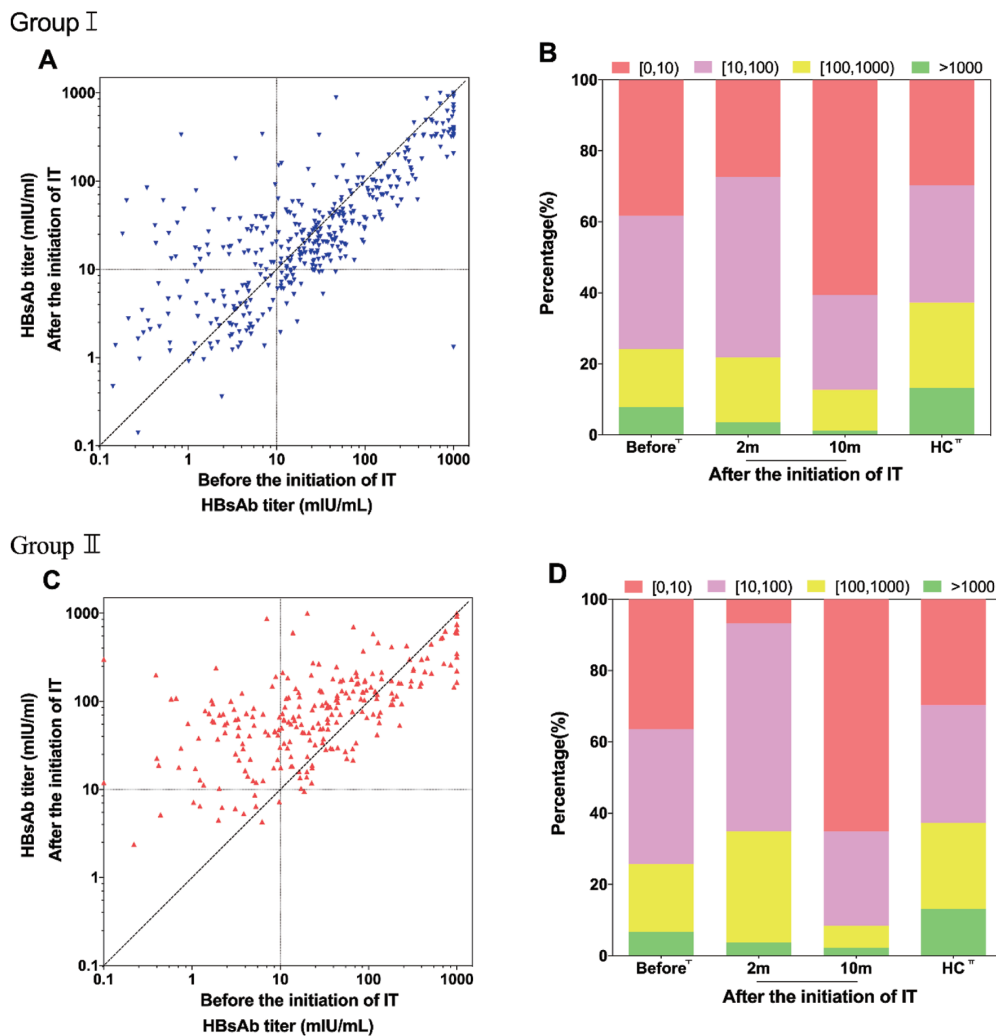


Fig. 3. Distribution of HBsAb level with the progress of IT. Children were divided into two groups by their HBcAb status 2 months after the initiation of immunosuppressive therapy (IT). (A, B) Group I, HBcAb–/–; (C, D) Group II, HBcAb–/+. 3A–3C show the HBsAb titer before IT and 2 months after the initiation of IT. (3B–D) Antibody concentration percentage during immunosuppressive therapy compared with age-matched healthy children (HC).

The implementation of viral nucleic acid testing (NAT) has greatly reduced the risk of transfusion-transmitted HBV infection. However, residual risk of transfusion-transmitted OBI exists and anecdotal cases are occasionally reported.^{9,22–24} Candotti *et al.* found that the minimal infectious dose transmitted by blood transfusion was 100 to 16 copies of HBV DNA.⁹ The minimal HBV DNA infectious dose is below the limit of detection of the available nucleic acid test assays. OBI has been involved in different clinical contexts.²⁵ The clinical implications of OBI need to be more extensively investigated, especially in the context of immunosuppressed children receiving blood transfusion in HBV highly endemic areas.

Multiple blood component transfusions are given as routine supportive care in children with ALL who receive IT, which in China may be associated with the risk of transfusion of HBcAb-positive blood products. The children with ALL who acquired HBcAb had passive HBV-specific humoral immunity to transmission of blood products instead of true infection, and that gradually disappeared with the progression of IT. Previous studies indicated that frequent HBsAb limited transfusion risk.⁶ Increasing HBsAb titers accompanied with detectable HBcAb after transfusion was unexpectedly common in this study, but was also consistent with our previous HBV serological survey in healthy adults between 18 and 35 years of age (Supplementary Table 2). On the other hand, Yuen *et al.* reported occult HBV donors who were HBcAb-positive.²⁶ Transfusion of HBcAb-positive blood products is common in highly endemic regions, and HBcAb can be transferred from blood donors to receptors by transfusion. Therefore, the probability of HBV transmission by transfusion of HBcAb-positive blood cannot be ignored.

With the loss of protective HBsAb against HepB-preventable diseases, children undergoing IT have impaired humoral immune responses. In this study, the level of protective HBsAb in immunosuppressed children was always lower than that in their age-matched healthy controls. HBsAb target the HBV surface antigen, develop after vaccination and are maintained by the production of functionally efficient multispecific antiviral immune cells. Vaccine-specific antibody levels are maintained by terminally differentiated, nonproliferating, long-lived plasma cells.²⁷ The expression of these antibodies in the bloodstream is also associated with a T cell response, particularly CD4+ cells, which facilitates the induction and maintenance of a virus-specific B cell response and the production of anti-HBV neutralizing antibodies.²⁸ However, IT induces persistent depletion of lymphocyte subsets,²⁹ and the serological memory in HepB vaccinated children is often impaired. A previous study has shown that the HBsAb level has an important role in preventing HBV infection in patients undergoing IT or organ transplantation.³⁰ Soluble HBsAb in serum is the most reliable clinical marker of adequate immunity. Despite universal HBV vaccination after birth, children receiving IT have far lower levels of HBsAb than healthy children, which should be taken seriously. Whether compromised immunity accompanied with insufficient HBsAb would increase the risk of HBV breakthrough infection in children undergoing IT is worthy of further investigation.

Three doses of neonatal HepB vaccine may be adequate to protect children against HBV breakthrough infection because of the low risk even with IT in HBcAb epidemic areas. Clinically, there is no need to worry about the sudden appearance of HBcAb-positivity, which would likely reflect the passive transfer of antibodies by transfusion. A high proportion of children would lose the HBV-specific humoral immunity after initiation of IT; the role of HepB vaccine booster dose may be considered in this setting. Further studies are warranted to follow-up neonatal HepB vaccinated children over a longer time.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Study conception and design (YZ, AH), sample and data collection and performance of experiments and statistical analysis (YY), interpretation of results (YY, JX, YZ), and drafting of the manuscript (YY, YZ). All authors reviewed and approved the final version of the manuscript.

Data sharing statement

The datasets in this study are available from the corresponding author upon reasonable request.

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