MAJOR ARTICLE

Durability of Antibody Response Against the Hepatitis B Virus in Kidney Transplant Recipients: A Proposed Immunization Guideline From a 3-Year Follow-up Clinical Study

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Background. Despite the importance of hepatitis B virus (HBV) immunization in kidney transplantation (KT), data are lacking on fluctuations in hepatitis B surface antibody (anti-HBsAb) levels and optimal levels for KT recipients.

Methods. The study consisted of anti-HBsAb-positive recipients aged 18–70 years at the time of the KT. Recipients with anti-HBsAb <100 IU/L received a single booster HBV vaccination, and anti-HBsAb was measured at baseline and 3, 6, 12, 18, and 24 months post-KT. Anti-HBsAb, quantitative HBV deoxyribonucleic acid testing (12 and 24 months post-KT), and hepatitis B core-related antigen (24 months post-KT) were evaluated in recipients with anti-HBsAb >100 IU/L who received a hepatitis B surface antigen positive renal allograft.

Results. Seventy-six of 257 (29.6%) KT recipients with anti-HBsAb <100 IU/L at the time of enrollment received a single booster of HBV vaccination. Anti-HBsAb levels increased (≥100 IU/L) 1 and 3 months post-booster dose in 86% and 93% of cases, respectively. Anti-HBsAb levels were ≥100 IU/L in 95% of these recipients 6 months post-booster dose. Among 181 (70%) recipients with anti-HBsAb ≥100 IU/L without a booster dose, anti-HBsAb gradually decreased after the KT from 588 IU/L at baseline to 440 and 382 IU/L 3 and 6 months post-KT, respectively (*P* < .01).

Conclusions. To ensure optimal immunity against HBV, KT recipients should first be stratified according to their risk of HBV reactivation. Kidney transplantation recipients of renal allografts from HBV nonviremic or viremic donors should be reimmunized when their anti-HBsAb titers are <250 IU/L. A cutoff level of 100 IU/L is recommended in other cases.

Keywords. hepatitis B virus; kidney transplantation; vaccination.

The incidence of hepatitis B virus (HBV) infection remains high in solid organ transplantation [1], with highest mortality in kidney transplant (KT) HBV-positive recipients [2, 3]. Hepatitis B reactivation can be found in recipients with resolved HBV infection (hepatitis B surface antigen [HBsAg] negative and hepatitis B core antibody [anti-HBcAb] positive) [4, 5], especially in recipients with a low level of anti-hepatitis B surface antigen antibody (anti-HBsAb) [6, 7]. Large

observational studies in healthy volunteers and immunocompromised patients have pointed to the effectiveness of HBV immunization in terms of increasing the level of anti-HBsAb [8, 9]. Although a previous study reported that 3 intramuscular injections of recombinant hepatitis B vaccine had successful immunization in a healthy host [10], the Kidney Disease: Improving Global Outcomes (KDIGO) Chronic Kidney Disease Work Group recommends a double dose of HBV vaccination and more frequent injections in all patients with chronic kidney disease (CKD) [11]. Vaccination with anti-HBsAb (≥10 IU/L: the recommended level to provide protective immunity) is required in both healthy hosts and CKD patients after the recommended vaccination schedule as primary prevention [9, 12]. Anti-HBsAb levels should be evaluated every decade in the healthy population and annually in CKD patients due to the risk of anamnestic responses [8, 13]. In post-KT patients, anamnestic responses due to the use of immunosuppressive drugs present a greater risk to the patient than CKD [14]. Although there is limited evidence on HBV immunization for KT recipients, the KDIGO clinical practice guideline 2009

recommends reimmunization in post-KT patients with an anti-HBsAb titer <10 IU/L, in addition to annual anti-HBsAb assessments [15]. This guideline suggests an optimum anti-HBsAb titer ≥100 IU/L because HBV reactivation is more frequent in recipients with anti-HBsAb titer <100 IU/L before the transplant [6, 7].

In addition to HBV reactivation, KT recipients are at risk of de novo HBV infection transmitted by the renal allograft, blood transfusion, and hemodialysis. As such, most KT protocols recommend that renal allografts from HBV carriers should be avoided [16–21] due to the risk of HBV reactivation in transplant recipients in the long term [6, 7, 22]. There is a high rate of HBsAg-positive (HBsAg(+)) deceased donors (30%) in our institute. In a previous study, we report the success of KTs from HBsAg(+) donors to serology-matched recipients in whom the anti-HBsAb titer was ≥100 IU/L [23]. Therefore, based on KDIGO guidelines on KT, which suggest reactivation if anti-HBsAb titer falls <10 IU/L and suggest booster vaccination to raise a titer >100 IU/L in whose initial anti-HBsAb <100 IU/L [15], our institute uses a titer of anti-HBsAb <100 IU/L as a threshold for HBV revaccination in all KT recipients.

Due to the concern of HBV reactivation and fluctuations in anti-HBsAb levels after renal engraftment, several types of immune monitoring are performed, although all current tests have some limitations [24]. Because liver biopsies are invasive, among current tests, dynamic monitoring of covalently closed circular deoxyribonucleic acid (cccDNA), a 3.2-kilobase double-stranded episomal DNA (a template for all viral genomic transcription), is likely the best method to detect potential HBV proliferation [25] and the risk of HBV reactivation in KT recipients. The cccDNA pool is also correlated with serum HBsAg levels. Another highly sensitive test is the semiautomated immune complex transfer chemiluminescence enzyme immunoassay (ICT-CLEIA). This test can be used to detect hepatitis B core-related antigen (HBcrAg) in serum [26–29]. Thus, the HBcrAg level measured in all of the KT recipients who received renal allograft from HBV nonviremic or viremic donors in the present study.

Knowledge of the durability of the anti-HBsAb response and its long-term protective effects would be useful to prevent HBV reactivation after KT. In addition, a proper HBV immunization for KT recipients is important, not only for HBV recipients but also for the recipient with renal allograft from a HBV-positive donor.

The aim of the present study was to investigate fluctuations in anti-HBsAb titers in KT recipient's posttransplantation and amnestic responses after HBV immunization. An additional aim was to determine the potential use of the ICT-CLEIA for HBcrAg in detecting HBV reactivation in KT recipients.

METHODS

### Recipient Selection and Study Design

This retrospective, longitudinal study was conducted during January 2012 through December 2017 at King Chulalongkorn Memorial Hospital, Bangkok, Thailand, and the study was approved by Chulalongkorn University Institutional Review Board. All of the participants provided written informed consent.

The inclusion criteria were all KT recipients who were anti-HBsAb(+) before transplantation. All recipients without documented HBV vaccination were immunized by a HBV vaccine after a serological assessment of their HBV status (Figure 1). In recipients with an anti-HBsAb titer <100 IU/L and a documented complete HBV vaccination history (ie, 4 shots of a double dose of recombinant HBV vaccine at 0, 1, 2, and 6 months), a single booster shot of a double dose of recombinant HBV vaccine was administered. Recombinant hepatitis B vaccine (Engerix-B; GlaxoSmithKline Biologicals, Rixensart, Belgium) was administered via an intramuscular injection on both sides of the deltoid muscle. Another full course of HBV vaccination was performed in patients with anti-HBsAb titers <100 U/L after a booster dose. Thereafter, patients with anti-HBsAb titers ≥100 IU/L and <100 U/L were considered responders and nonresponders, respectively. Only recipients with anti-HBsAb levels >100 IU/L were accepted as KT recipients of organs from HBsAg(+) donors to ensure that the anti-HBsAb level was as high as possible before the transplantation [23].

The exclusion criteria included KT recipients who were HBsAg(+) (chronic hepatitis B infection), in addition to recipients with chronic hepatitis C infection and nonresponders to HBV vaccination. Before organ donation, the risk of HBV transmission was evaluated by mandatory HBV viremia detection in cases of HBsAg(+) donors. As screening tests, both the KT recipients and donors underwent HBV serological testing, including HBsAg, anti-HBsAb, anti-HBcAb, and hepatitis B extracellular antigen (HBeAg), before transplantation.

In accordance with our institute's policy, recipients with anti-HBsAb >100 IU/L could receive an allograft from donors who were anti-HBcAb(+)/HBsAg(−) or HBsAg(+). Therefore, the recipients were classified into 4 groups according to the HBV status of the donors (Figures 1 and 2). Group 1 comprised non-HBV donors: HBsAg(−)/anti-HBcAb(−). Group 2 comprised negative HBV serology donors: anti-HBcAb(+)/anti-HBsAb(+). Group 3 comprised HBV nonviremic donors: anti-HBcAb(+)/anti-HBsAb(−)/HBV DNA quantitative(−). Finally, group 4 comprised HBV viremic donors: HBV DNA quantitative(+) or HBsAg(+) with HBeAg(−). More importantly, only allografts from deceased donors were included in group 4.

Posttransplantation, anti-HBsAb levels were monitored regularly. In brief, the anti-HBsAb levels of KT recipients who had excellent immunity to HBV (anti-HBsAb >1000 IU/L) were measured 12 months post-KT. If the anti-HBsAb titer
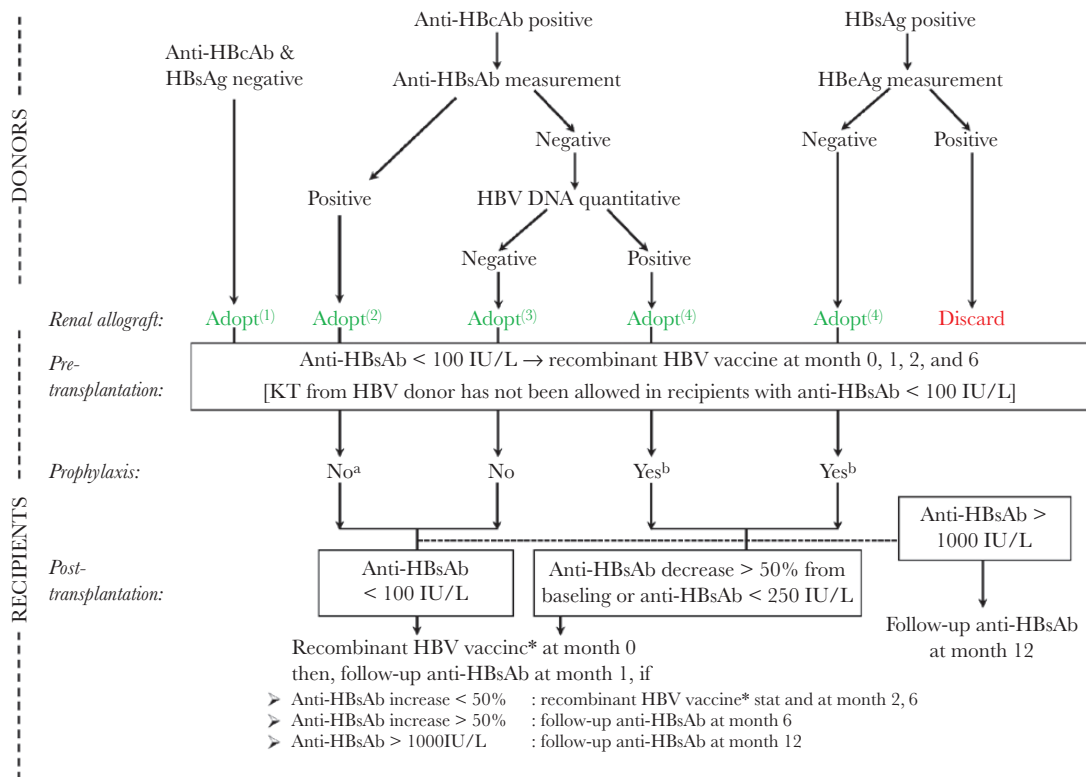
Figure 1. The management protocol for hepatitis B virus (HBV) immunization in kidney transplant (KT) recipients in terms of the donor's HBV status. [1]Group 1: non-HBV donors (anti-HBcAb[−]/HBsAg[−]); [2]group 2: past-resolved HBV donor (anti-HBcAb[+]/anti-HBsAb[+] donor); [3]group 3: isolated anti-HBcAb or HBV nonviremic donor (anti-HBcAb[+]/anti-HBsAb[−]/HBV DNA[−] donor); and [4]group 4: HBV viremic donor (HBsAg[+] and/or HBV DNA[+]/HBeAg[−] donor). [a]No need for antiviral prophylaxis unless hepatitis B surface antigen (HBsAg) and quantifiable HBV DNA from the donor are positive. [b]Consider prophylaxis with lamivudine (150 mg/day) only or in conjunction with high-dose hepatitis B immunoglobulin if the recipient received a T-cell and/or B-cell depleting agent(s) for desensitization. Posttransplant: Check anti-HBsAb status 3, 6, 12, 18, and 24 posttransplantation. *, A double intramuscular (both sides of deltoid muscle) injection of 20 grams of recombinant HBV vaccine. Abbreviations: DNA, deoxyribonucleic acid; HBcAb, hepatitis B core antibody; KT, kidney transplant.

had decreased (>50%) from baseline or an absolute value (ie, <250 IU/L), the recipients were offered a booster dose of a recombinant HBV vaccine (month 0), and their anti-HBsAb levels were then measured again 1 month later. The remaining 3 booster doses (from a total of 4 doses) were omitted unless the anti-HBsAb titer was <50% that of the baseline level or the anti-HBsAb level was approximately 100–250 IU/L. Due to the high risk of HBV reactivation in patients treated with rituximab [30], a full course of recombinant HBV vaccination before drug administration was administered, regardless of anti-HBsAb levels. In groups 3 and 4, after informing each recipient of the potential risks and benefits of KTs from the donors, lamivudine (150 mg/day) was prescribed for a 12-month period according to HBV risk stratification, as shown in Figure 1.
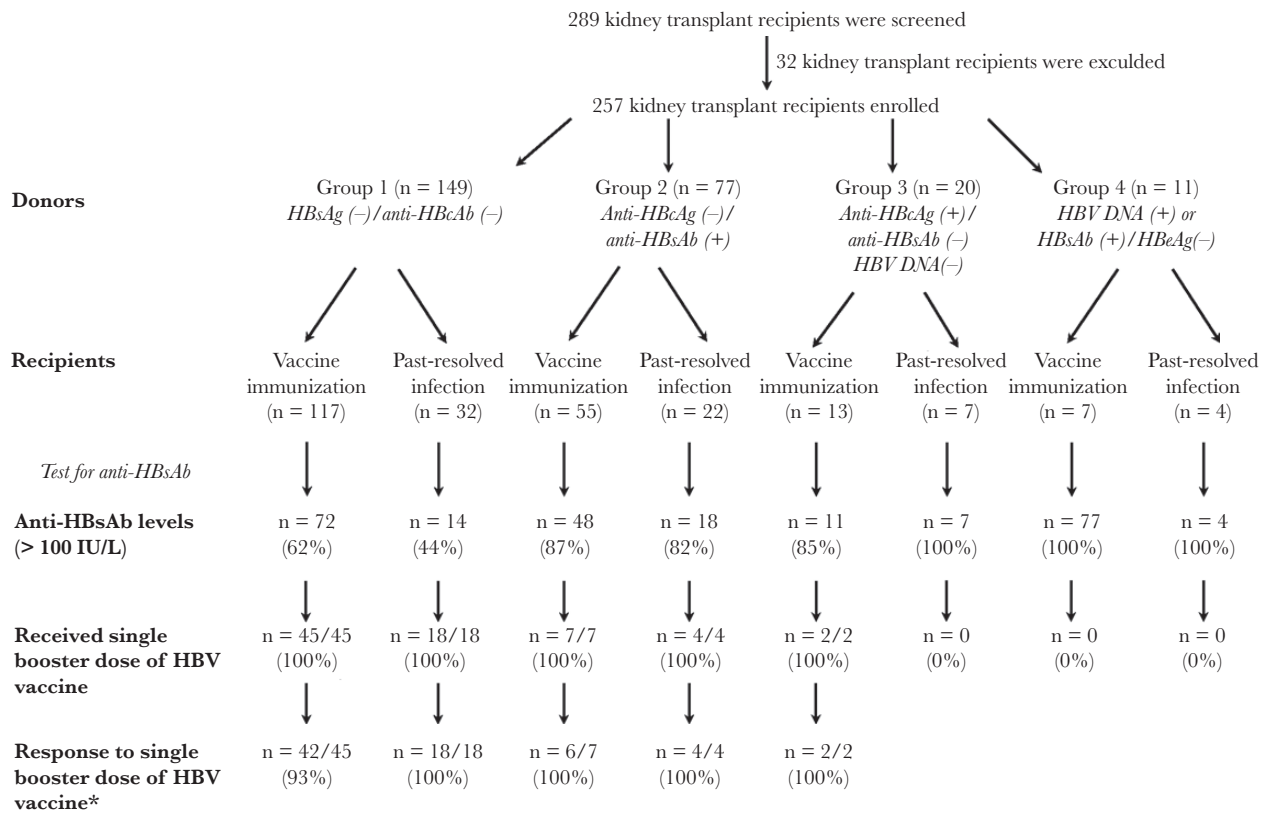
The primary endpoint was the loss of protective immunity, defined as an anti-HBsAb level <100 IU/L. All KT recipients were followed up until loss of protective immunity against HBV or censoring. Each recipient was censored according to death or nonavailability of HBV serology data 24 months after the KT (lost to follow-up or moved to other centers). The secondary endpoints included the following: (1) anti-HBsAb

seroconversion (undetectable anti-HBsAb); (2) HBV reactivation as determined by HBV DNA >20 IU/mL and HBsAg or HBcrAg; (3) acute hepatitis or liver failure, as determined by an increase in alanine aminotransferase more than twice the upper limit of normal; and (4) survival of the allograft and recipient.

Regarding the immunosuppressive regimen, immunosuppression was induced with basiliximab or antithymocyte globulin and maintained with calcineurin inhibitors, mycophenolate mofetil (1.5–2 g/day), and corticosteroids. Calcineurin inhibitors were administered at the time of the surgery. The target tacrolimus trough level was 8–10 ng/mL for the first 6 months and reduced to 5–8 ng/mL thereafter. The target C_2 cyclosporine levels were 1000–1200 and 800–1000 mg/dL for the first 0–2 and 3–6 months, respectively, and the target levels were then gradually decreased based on renal allograft function.

Definitions

The presence of anti-HBsAb in the absence of anti-HBcAb and HBsAg indicated vaccine immunity. Positive anti-HBsAb and anti-HBcAb without HBsAg indicated past-resolved HBV infection (natural immunity). Loss of protective immunity

289 kidney transplant recipients were screened

32 kidney transplant recipients were excluded

257 kidney transplant recipients enrolled

Donors

Group 1 (n = 149)
HBsAg (–)/anti-HBcAb (–)

Group 2 (n = 77)
*Anti-HBcAg (–)/
anti-HBsAb (+)*

Group 3 (n = 20)
*Anti-HBcAg (+)/
anti-HBsAb (–)
HBV DNA(–)*

Group 4 (n = 11)
*HBV DNA (+) or
HBsAb (+)/HBeAg(–)*

Recipients

	Vaccine immunization (n = 117)	Past-resolved infection (n = 32)	Vaccine immunization (n = 55)	Past-resolved infection (n = 22)	Vaccine immunization (n = 13)	Past-resolved infection (n = 7)	Vaccine immunization (n = 7)	Past-resolved infection (n = 4)
Test for anti-HBsAb								
Anti-HBsAb levels (> 100 IU/L)	n = 72 (62%)	n = 14 (44%)	n = 48 (87%)	n = 18 (82%)	n = 11 (85%)	n = 7 (100%)	n = 77 (100%)	n = 4 (100%)
Received single booster dose of HBV vaccine	n = 45/45 (100%)	n = 18/18 (100%)	n = 7/7 (100%)	n = 4/4 (100%)	n = 2/2 (100%)	n = 0 (0%)	n = 0 (0%)	n = 0 (0%)
Response to single booster dose of HBV vaccine*	n = 42/45 (93%)	n = 18/18 (100%)	n = 6/7 (100%)	n = 4/4 (100%)	n = 2/2 (100%)			

Figure 2. Flow chart showing the number of kidney transplant (KT) recipients screened and included in the study (at the time of enrollment). *, A response to a single booster dose of hepatitis B virus (HBV) vaccine means that the anti-hepatitis B surface antibody (HBsAb) levels of the recipients were >50% of baseline levels before immunization at 6 months posttransplant. Abbreviations: DNA, deoxyribonucleic acid; HBcAb, hepatitis B core antibody; HBeAg, hepatitis B extracellular antigen; HBsAg, hepatitis B surface antigen.

against HBV was defined by a decrease in anti-HBsAb <10 IU/L. Protective immunity against HBV was considered anti-HBsAb >100 IU/L. Hence, the duration of protective immunity was defined by the persistence of anti-HBsAb >100 IU/L. Booster (or revaccination) refers to an additional dose of recombinant hepatitis B vaccine as postprimary vaccination to induce immune memory and improve protection against HBV infection. Hepatitis B virus reactivation was defined as HBsAg reversion with serum HBV DNA >2000 IU/L [31].

Laboratory Measurements

The HBsAg, anti-HBsAb, anti-HBcAb immunoglobulin (Ig) G and IgM, and HBeAg were measured using a chemiluminescence microparticle immunoassay (Architect Qualitative System; Abbott, Delkenheim, Germany). The detection threshold of anti-HBsAb assay was 10 IU/L, with a quantification range of 10–1000 IU/L. The HBcrAg quantification was evaluated using an automated Lumipulse CLEIA analyzer (Fujirebio Inc., Tokyo, Japan) with an analytic measurement range from 1000 to 10 000 000 IU/mL (3–7 log IU/mL). Serial dilutions of serum samples were performed when the serum qHBcrAg level exceeded the detection limit of the assay. The HBV DNA was quantified using real-time polymerase chain reactions (PCRs) (COBAS AmpliPrep/COBAS TaqMan; Roche Diagnostics, Mannheim, Germany). The HBV DNA test has a dynamic range from 20 to 170 000 000 IU/mL (1.30–8.23 log IU/mL) (sensitivity of 99.8% and specificity of 100%). Both HBcrAg and HBV DNA were tested only in the recipients in groups 3 and 4.

Data Analyses

The baseline characteristics of the recipients are presented as mean ± standard deviation. Proportions were compared using the Yates χ^2 corrected test or Fisher's exact test. Continuous variables were compared either by an analysis of variance test (data with a normal distribution) or by Friedman, Mann-Whitney, and Wilcoxon tests (data with a nonnormal distribution). To assess the endpoints after complete immunization, the off-immunization follow-up time was calculated from the last HBV vaccine until an endpoint or the last follow-up visit. For the loss of protective anti-HBsAb (anti-HBsAb level <100 IU/L), the last dose of prescribed vaccine was considered as time = 0. The decay rate of anti-HBsAb were estimated by using a longitudinal mixed-effects model as described elsewhere [32]. In brief, the KT recipients had to have at least 3 data points for a period of 2 years or more to be included in the final analysis. The outlier data or random components were assumed to be

uncorrelated and excluded. All data were logarithmically transformed as a natural log scale, resulting in a 1-phase decay model of the untransformed data. Half-life of anti-HBsAb titers were obtained by transforming the decay rate and the boundaries of 95% confidence interval obtained from the fixed-effects slope component of the mixed-effects model. Analyses were performed using SPSS, version 21.0 (IBM Corp., Armonk, NY). A Kaplan-Meier analysis and log-rank test were used to analyze the time-to-event endpoint. $P < .05$ was considered statistically significant.

RESULTS

Baseline Characteristics of the Study Group

As shown in Figure 2, in total, 289 KT recipients were screened for enrollment. Thirty patients were excluded due to the absence of a vaccination record (or lost to follow-up), and 2 patients were excluded because they were nonresponders after complete HBV vaccination, respectively. The determination of HBV vaccination status in the remaining 257 recruited recipients was based on (1) complete documentation (n = 213, 83%), (2) a signed written affidavit indicating the dates of the vaccination (n = 26, 10%), and (3) confirmation by primary physicians

(n = 18, 7%). All of the enrolled KT recipients tested negative for anti-HCV and antihuman immunodeficiency virus. Of the enrolled recipients, there were 149 (58%) recipients in group 1 (non-HBV donors), 77 recipients (30%) in group 2 (negative HBV serology donors), 20 (8%) recipients in group 3 (HBV nonviremic donors), and 11 (4%) recipients in group 4 (HBV viremic donors) There were differences in donor ages, types of donors, dialysis vintages, and cold ischemic times between the groups (Table 1).

Analyses of the Initial Response and Rate of Antihepatitis B Surface Antibody Decline

In 86 of 149 (58%) recipients in group 1, 66 of 77 (86%) recipients in group 2, 18 of 20 (90%) recipients in group 3, and all of the recipients in group 4, the baseline anti-HBsAb level was >100 IU/L (Figure 2 and Table 2). All of the recipients had anti-HBsAb levels >10 IU/L before engraftment. Seventy-six (30%) recipients had an inadequate anti-HBsAb (<100 IU/L) level at the time of enrollment and required a new course of HBV vaccination (double-dose series of a recombinant HBV vaccine at 0, 1, 2, and 6 months), and anti-HBsAb levels were ≥100 IU/L (average 338; range 55–536) 1 month after the booster dose in 65 (86%) recipient. Seven of 11 KT recipients who did not

Table 1.   Demographic, Clinical, and Immunological Baseline Data of the Study Population

Parameters	Group 1 (n = 149)	Group 2 (n = 77)	Group 3 (n = 20)	Group 4 (n = 11)	P Value
Donor age (years)	37.6 ± 10.3	39.8 ± 12.0	42.1 ± 11.1	49.5 ± 8.1	**.002**
Donor sex (F/M)	74/75	31/46	4/16	0/11	**.001**
Recipient age (years)	44.7 ± 11.9	44.0 ± 12.1	47.9 ± 12.7	43.7 ± 9.3	.61
Recipient sex (F/M)	76/73	39/38	7/13	5/6	.59
Dialysis vintage (months)	42.4 ± 44.6	53.9 ± 46.3	72.2 ± 39.9	74.3 ± 25.7	**.006**
Type of donor (living/deceased)	90/59	42/35	3/17	0/11	**<.0001**
Total ischemic time (min)	471.8 ± 540.6	639.9 ± 553.9	1117.2 ± 447.1	1116.4 ± 246.7	**<.0001**
HLA mismatch	2.7 ± 1.6	2.8 ± 1.4	2.6 ± 1.9	3.0 ± 1.9	.80
Highly sensitized PRA (PRA > 30), n (%)	24 (16.1)	18 (23.4)	4 (20.0)	3 (27.3)	.52
Natural immunity against HBV, n (%)	32 (21.5)	22 (28.6)	7 (35.0)	4 (36.4)	.35
Vaccinated immunity against HBV, n (%)	117 (78.5)	55 (71.4)	13 (65.0)	7 (63.6)	.35
Native kidney disease, n (%)					
ADPKD	3 (2.0)	3 (3.9)	0 (0)	1 (9.1)	.40
Chronic glomerulonephritis	50 (33.5)	35 (45.4)	6 (30.0)	3 (27.3)	.26
Diabetic nephropathy	22 (14.8)	9 (11.7)	1 (5.0)	0 (0)	.34
Obstructive nephropathy	3 (2.0)	2 (2.6)	0 (0)	0 (0)	.85
Unknown	71 (47.7)	28 (36.4)	13 (65.0)	7 (63.6)	.06
Immunosuppression, n (%)					
Basiliximab	132 (88.6)	55 (71.4)	15 (75.0)	9 (81.8)	**.01**
Antithymocyte globulin	6 (4.0)	14 (18.2)	2 (10.0)	2 (18.2)	**.004**
Cyclosporine A-based	6 (4.0)	3 (3.9)	1 (5.0)	1 (9.1)	.87
Tacrolimus-based	143 (96.0)	74 (96.1)	19 (95.0)	10 (90.9)	.87
Rapamycin-based	0 (0)	0 (0)	0 (0)	0 (0)	-
Everolimus-based	0 (0)	0 (0)	0 (0)	0 (0)	-
Azathioprine	0 (0)	0 (0)	0 (0)	0 (0)	-
Mycophenolate mofetil	149 (100.0)	77 (100.0)	20 (100.0)	11 (100.0)	-
Corticosteroids	142 (95.3)	75 (97.4)	19 (95.0)	11 (100.0)	.77

Bold values indicate statistically significant.

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; F, female; HBV, hepatitis B virus; HLA, human leukocyte antigen; M, male; PRA, panel reactive antibody.

Table 2. Hepatitis B Viral Status Outcomes

Parameters, N (%)	Group 1 (n = 149)	Group 2 (n = 77)	Group 3 (n = 20)	Group 4 (n = 11)
The Recipients' Serological Status at the Time of Enrollment				
Time from HBV vaccine series completion to transplantation (median, IQR) (months)	7.5 (1.8–13.5)	5.6 (2.2–9.5)	3.6 (1.0–5.5)	2.5 (0.4–3.8)
Anti-HBsAb titer (range, IU/L)	34 to >1000	94 to >1000	574 to >1000	437 to >1000
Posttransplant Recipients' Clinical and Serological Status				
Duration of follow up (median, IQR) (months)	39.2 (11–38.4)	44.4 (14.1–54)	40.6 (13.3–45)	38.2 (15.2–40)
Number of anti-HBsAb performed per recipient (mean ± SD)	3.5 ± 2.2	3.4 ± 1.3	4.8 ± 0.3	4.2 ± 0.7
Anti-HBsAb(+)	149 (100)	77 (100)	20 (100)	11 (100)
Anti-HBsAb titer (range, IU/L)	64 to >1000	119 to >1000	388 to >1000	507 to >1000
Anti-HBcAb (de novo)	0 (0)	0 (0)	0 (0)	0 (0)
HBsAg(+) (de novo)	0 (0)	0 (0)	0 (0)	0 (0)
HBcrAg(+)[a]	Not tested	Not tested	0 (0)	0 (0)
Detectable HBV DNA viral load[b]	Not tested	Not tested	0 (0)	0 (0)
Acute hepatitis	0 (0)	0 (0)	0 (0)	0 (0)
Acute liver failure	0 (0)	0 (0)	0 (0)	0 (0)

Abbreviations: anti-HBcAb, hepatitis B core antibody; anti-HBsAb, hepatitis B surface antibody; DNA, deoxyribonucleic acid; HBcrAg, hepatitis B core-related antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IQR, interquartile range; SD, standard deviation.

NOTE: All parameters were assessed 3, 6, 12, 18, and 24 months after kidney transplantation unless otherwise specified.

[a]Data 24 months posttransplantation.

[b]Data 12 and 24 months posttransplantation.

achieve anti-HBsAb levels ≥100 IU/L 1 month after the booster dose showed vaccine immunity (anti-HBcAb(−)). However, 3 months postvaccination (3 HBV vaccine shots), 71 of 76 (93%) recipients had anti-HBsAb ≥100 IU/L (average, 492; range, 138–774). Six months postvaccination, 72 of 76 (95%) recipients had anti-HBsAb levels ≥100 IU/L (average, 584; range, 186–1000). Thus, delayed anti-HBsAb production occurred in 1 recipient after 3 shots of HBV vaccination. After completion of our protocol (ie, 4 shots of HBV vaccine), only 4 (5%) recipients had inadequate anti-HBsAb levels (<100 IU/L) after a full course of HBV vaccination 1 month after the last injection (Figure 2). It

is interesting to note that the success rate of the anti-HBsAb booster (ie, in increasing the anti-HBsAb titer to ≥100 IU/L) 12 months after 1 course of HBV vaccination was 2-fold higher in anti-HBcAb(+) recipients (n = 36) than in anti-HBcAb(−) recipients (n = 16), indicating an amnestic immune response of past-resolved HBV infection in these KT recipients.

On the other hand, the means of anti-HBsAb levels among the 4 groups of KT recipients were inverse correlation with the likelihood of waning responses at the posttransplant time points, at least in the first 6 months after the KTs (Figure 3). The anti-HBsAb levels of KT recipients with baseline titers ≥100
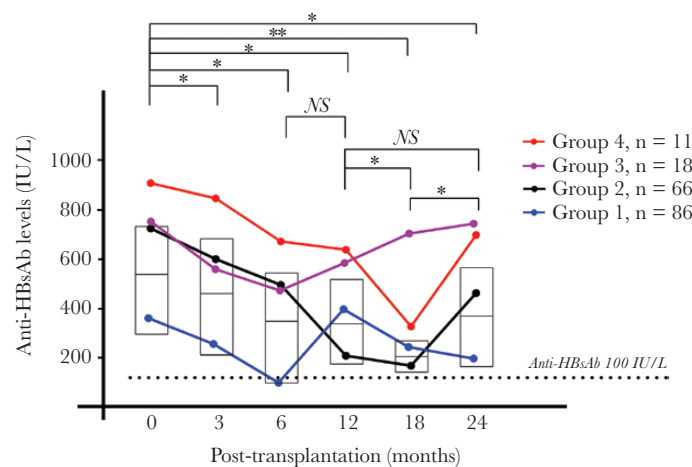

Figure 3. The evolution of hepatitis B surface antibody (anti-HBsAb) levels among the 4 groups of kidney transplant recipients based on quantifiable anti-HBsAb titers (between 10 and 1000 IU/L). The solid blue, black, purple, and red circles represent means of anti-HBsAb levels in groups 1, 2, 3, and 4, respectively. There was a correlation between the evolution of anti-HBsAb levels and the timing of posttransplantation (r^2 = 0.998, 0.929, 0.987, and 0.954 in group 1, 2, 3 and 4, respectively; $P < .001$ all). Each box represents all kidney transplant recipients in the study period; the 25th (bottom border), 50th (middle border), and 75th (top border) percentiles. *, $P < .05$; **, $P < .01$. Abbreviations: *NS*, nonsignificant.

IU/L (n = 181, 70%) decreased significantly during the follow-up, falling from 588 IU/L at baseline (month 0) to 440 and 382 IU/L 3 and 6 months post-KT, respectively (*P* < .01) (Figure 3). Among these 181 recipients (high baseline anti-HBsAb), anti-HBsAb fell to a nonprotective level (ie, <100 IU/L in 15% (n = 27) and 22% (n = 40) of recipients 3 and 6 months post-KT, respectively. The anti-HBsAb titer of all the anti-HBcAb(−) recipients with an anti-HBsAb titer of 250–500 IU/L at baseline fell to <100 IU/L 6 months post-KT, but all of them developed anti-HBsAb >100 IU/L after receiving a booster dose. The approximate rate of anti-HBsAb decline was 85 IU/L per month (data not shown). Moreover, in the recipients with anti-HBsAb levels >1000 IU/L at baseline, the anti-HBsAb levels decreased significantly 12 and 18 months post-KT (*P* < .001 and *P* < .0001, respectively) (Figure 3). However, none of these recipients had anti-HBsAb titers <100 IU/L after a median duration of 446 days (range, 378–507 days).

In the recipients with a high risk of HBV transmission from donors (groups 3 and 4), reimmunization was performed if the anti-HBsAb levels fell >50% from the baseline or they were <250 IU/L. The median anti-HBsAb level in these groups was 769.5 IU/L (range, 210 to >1000) at baseline. Seventeen of 31 (55%) recipients (groups 3 and 4) required reimmunization 6 months post-KT. However, 24 months post-KT, none of these recipients had lost protective immunity against HBV, and they tested negative for HBcrAg and HBV DNA (Table 2). In addition, all of the recipients in groups 3 and 4 had anti-HBsAb levels >100 IU/L throughout the study period (Figure 3).

To determine the half-life of anti-HBsAb as a function of time after the last received vaccination, the duration of protective immunity (anti-HBsAb >100 IU/L) was assessed as a function of the magnitude of the anti-HBsAb response and the time taken for antibody titers to decline to under protective levels (anti-HBsAb <100 IU/L). We found that 68 from 72 (95%) of the KT recipients who received full course of vaccination (4 shots of a double dose of recombinant HBV vaccine at 0, 1, 2, and 6 months) would be >100 IU/L for up to 43 months, without the need for further booster vaccinations. Meanwhile, the half-life of anti-HBsAb, which calculated from 225 recipients, was a nonlinear regression pattern with 8.9 months half-life decay rate (Figure 4).

The duration of protective immunity (anti-HBsAb >100 IU/L) among KT recipients with anti-HBsAb levels >1000 IU/L at baseline (month 0) was significantly longer than among those with anti-HBsAb levels of 500–1000 and 250–500 IU/L, respectively (log-rank test, *P* < .0001) (Figure 5A). Moreover, in all the recipients with anti-HBsAb levels >1000 IU/L, the anti-HBsAb levels were >250 IU/L at the next 12-month assessment. Furthermore, 96% of recipients with anti-HBsAb increases >50% maintained anti-HBsAb levels >100 IU/L, and only 37% of recipients with anti-HBsAb increases <50% had anti-HBsAb levels >100 IU/L 6 months after the booster HBV vaccination.
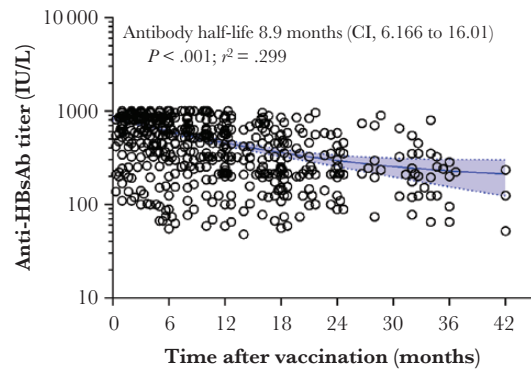
Figure 4. Humoral immunity to hepatitis B virus with time after vaccination. The solid blue line is the fitted nonlinear regression representing the antibody half-life decay rate, and the dashed blue line represents the upper and lower bound of 95% confidence interval (CI) for the antibody half-life estimation. The antibody half-life decay rate was 8.9 months (95% CI, 6.166–16.01) (n = 951 data from 225 recipients).

The cutoff anti-HBsAb level of 168 IU/L was effective in determining anti-HBsAb(+) status at the 6-month assessment after the booster vaccination.

Hepatitis B Surface Antigen Detection, Transplantation Outcomes, and Predictors of Hepatitis B Virus Immunity

At a median follow-up of 43 months (interquartile range, 11–54 months) after the KTs, none of the participants had developed de novo or positive HBsAg, HBcrAg, anti-HBcAb, and quantifiable HBV DNA was not detected (Table 2). None of the recipients with renal allografts from HBsAg(+) donors had elevated alanine transaminase levels (classified as an increase more than twice that of the upper limit) throughout the study period. As shown by the log-rank analysis, there were no significant differences in graft (Figure 5B) and recipient survival among the 4 groups (Figure 5C) and no incidences of HBV-associated glomerulonephritis.

To identify factors influencing HBV immunity, data from all 257 KT recipients were evaluated. The sustainable HBV immunity (anti-HBsAb >100 IU/L) in KT recipients was associated with a shorter dialysis vintage and positive anti-HBcAb (*P* < .0001) (Table 3). There were no significant differences in the age, sex, body mass index, administration of T cell-depleting agents or salvage therapy for allograft rejection, and concurrent chronic medical conditions (diabetes mellitus and hypertension) of these KT recipients.

DISCUSSION

We report the results of a 3-year longitudinal analysis of the HBV status of KT recipients and a HBV immunization program. Seroconversion of anti-HBsAb from <100 IU/L to ≥100 IU/L was observed in 95% of the KT recipients after an additional course of HBV vaccination (ie, 4 shots of a double dose of recombinant HBV vaccine at 0, 1, 2, and 6 months). The estimated anti-HBsAb half-life was 8.9 months. In the follow-up,
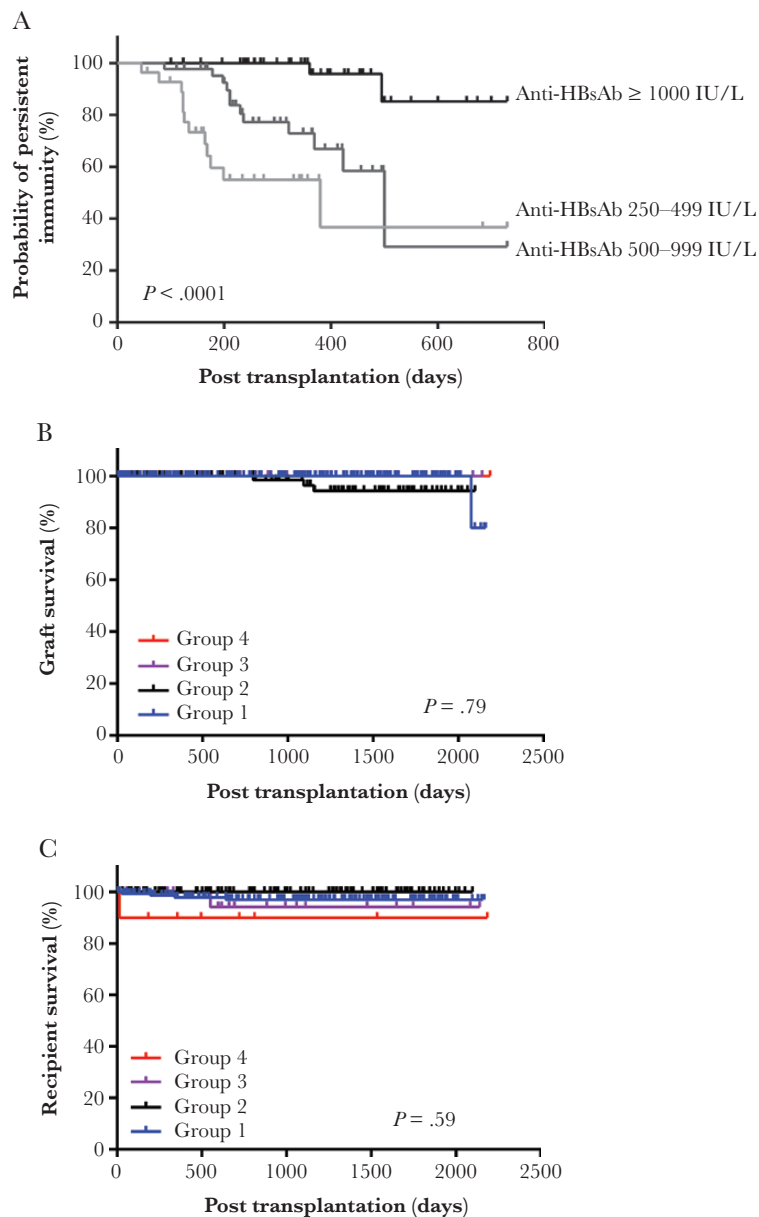
Figure 5. Kaplan-Meier and log-rank test. (A) Duration of protective hepatitis B surface antibody (anti-HBsAb) >100 IU/L using the Kaplan-Meier and log-rank test based on baseline anti-HBsAb levels (anti-HBsAb 250–499 IU/L, 500–999 IU/L, and 1000 IU/L). Kidney transplant recipients were followed-up until they lost protective immunity (defined as anti-HBsAb levels <100 IU/L) or they were censored. (B) Renal allograft survival and (C) recipient survival at the end of the study in the 4 groups.

none of the KT recipients with anti-HBsAb titers >100 IU/L who received HBV-positive renal allografts tested positive for hepatitis or HBV.

Renal allografts from donors with extended criteria are used because of an organ shortage and the benefits of kidney transplantation over dialysis. Accordingly, the transplantation of HBV-positive allografts into HBV-immunized recipients is common, and comparable outcomes, including graft and recipient survival, have been reported [16–21]. However, data on HBV vaccination in KT recipients and the impact of HBV infection on these recipients are limited [15]. Recent retrospective cohort studies demonstrated that the transplantation of

HBV-positive renal allografts was safe in recipients with adequate protective anti-HBsAb levels (10–100 IU/L) [20, 21, 23]. Nevertheless, several relevant issues remain unclear in terms of KTs. These include the optimal anti-HBsAb protective level, optimal immunization program, maintenance of antibody levels, and nucleoside analog (NA) prophylaxis. In the present study, we found that anti-HBsAb titers significantly decreased 3 months after KTs in most of the recipients and anti-HBsAb levels <100 IU/L in 10% of recipients at the engraftment KT stage, despite anti-HBsAb levels >100 IU/L pre-KT. The level of anti-HBsAb at baseline was associated with the time to a drop in the titer, with higher levels at baseline associated with

Table 3. Comparisons of the Recipients With Adequate Maintenance of HBV⁺ Immunity (a Sustainable Hepatitis Anti-HBsAb >100 IU/L) Versus Those With Inadequate Maintenance of HBV Immunity[a]

Parameters	Anti-HBsAb >100 IU/L (n = 146)	Anti-HBsAb <100 IU/L (n = 111)	P Value
Age, years, mean ± SD	42.5 ± 10.9	41.2 ± 11.3	.35
Sex, female	70 (47.9)	57 (51.4)	.62
BMI, kg/m², mean ± SD	22.1 ± 3.4	21.9 ± 2.9	.62
Dialysis vintage, months, mean ± SD	2.8 ± 1.2	3.8 ± 2.3	**<.0001**
Diabetes mellitus	14 (9.6)	18 (16.2)	.13
Hypertension	43 (29.5)	31 (27.9)	.89
Anti-HBcAb(+)	60 (41.1)	5 (4.5)	**<.0001**
T-cell depleting agents (induction)	14 (9.6)	10 (9)	.87
Salvage treatment for rejection	1 (0.7)	0	.38

Bold values indicate statistically significant.

Abbreviations: anti-HBcAb, hepatitis B core antibody; anti-HBsAb, hepatitis B surface antibody; HBV, hepatitis B virus; BMI, body mass index; SD, standard deviation.

[a]Data are presented as numbers (%) unless otherwise specified.

longer durations to a decline in anti-HBsAb titers below 100 IU/L. Therefore, the anti-HBsAb decay rate in recipients with anti-HBsAb titers 250–500 IU/L was 85 IU/L per month, with possible loss of protective immunity (<100 IU/L) 6 months post-KT. In recipients with baseline anti-HBsAb levels >1000 IU/L, the protective immunity should persist 12 months posttransplantation. Reimmunization should be performed within 6 months once anti-HBsAb levels fall to ≤250 IU/L. The lowest level of anti-HBsAb that can maintain sustainable immunity (all-time anti-HBsAb >100 IU/L) is 168 IU/L. More importantly, although there is less information of anti-HBsAb affected by the potent immunosuppressive therapy from the present study, more frequent anti-HBsAb monitoring is recommended particularly in recipients with potent immunosuppressive regimens (for rejection treatment or relapsed glomerular diseases) [30].

The HBV immunization protocol in this study (Figure 1) is a modified version of the immunization of CKD. We demonstrated that only 5% of KT recipients were unable to achieve anti-HBsAb protective levels (anti-HBsAb >100 IU/L) after a full course of this vaccination protocol. In addition, the median of anti-HBsAb half-life decay rate was 8.9 months (Figure 4), and 60% of the KT recipients with baseline anti-HBsAb 250–499 IU/L showed significantly decreased anti-HBsAb at 6 months (Figure 5A). These findings were particularly clear among recipients with a short dialysis vintage, anti-HBcAb(+), and high baseline anti-HBsAb levels. Accordingly, we suggest that anti-HBsAb need to be monitored at 6 months post-KT and the threshold of anti-HBsAb at <250 IU/L should be an action level for reimmunization. In addition, in our protocol, a lower threshold of reimmunization (anti-HBsAb at <250 IU/L), together with intensive monitoring, is used in recipients of HBV-positive allografts (HBsAg positive, with or without positive HBV DNA). Prophylactic treatment with NAs in recipients of HBV-positive allografts and in recipients with anti-HBsAb levels >100 IU/L is controversial [23]. In the present study, after treatment with 150 mg/day of the NA lamivudine for at least 12 months post-KT, none of the KT recipients showed HBV infection or reactivation. There are only a few case reports worldwide of HBV infection after KT, despite prophylaxis [6, 7, 22]. However, potential resistance of viral strains to lamivudine is a concern, although there is no risk of NA resistance in a 12-month prophylactic regimen [33].

This 3-year follow-up study demonstrates that kidney allografts from donors with all the spectrum of HBV transmission risk can be safely used. Organs from HBV viremic donors (the highest HBV transmission risk group) and HBV nonviremic donors (the standard HBV transmission risk group) were only used in recipients with anti-HBsAb levels >100 IU/L at the time of the KT. Of interest, these recipients were negative for HBV antigenemia by 3 molecular methods: a chemiluminescence microparticle immunoassay for detecting HBsAg, an automated CLEIA for detecting HBcrAg, and a real-time PCR for detecting HBV DNA.

Guidelines on the management of HBV in KTs are diverse [22, 34]. In the present study, we demonstrated that transplant recipients with anti-HBsAb levels >100 IU/L with HBV-DNA monitoring should be recommended as an excellent result. Although HBcrAg might be the most sensitive test for HBV reactivation, HBcrAg detection is not suitable for high-throughput screening in routine practice. Serum HBV-DNA remains a crucial test in clinical practice because it can detect positive HBV-DNA in serum with negative or low titer serum HBsAg [24, 35]. Due to the gradual decrease in the anti-HBsAb post-KT and the tendency toward improved survival of renal allografts, HBV reactivation might occur in long-term post-KT. Therefore, we underline the importance of sequential monitoring of liver transaminase, HBsAg, anti-HBsAb, and HBV-DNA levels in recipients throughout their lifetimes. Nevertheless, the cost-effectiveness following the guideline need to be further addressed. The use of 2 doses of HEPALISAV-B, a new HBV vaccine with a synthetic oligonucleotide immunostimulatory adjuvant, may be compatible and merge well with the guideline because of the cost-effectiveness and the advantages of the higher and earlier seroprotection rate than 3 doses of Engerix-B [36–38]. However, the immunogenicity and long-term safety of HEPALISAV-B in KT population remains to be established.

CONCLUSIONS

In conclusion, the HBV immunization protocol described herein provided excellent protective immunity in terms of anti-HBsAb levels and enabled renal allografts from HBV-positive donors to be safely used in HBV-positive or HBV-negative KT recipients. Our data can enhance scientific confidence in how

to maintain the optimal protective immunity against HBV in KTs, and the protocol described herein might be transferable to standard care in the near future.

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