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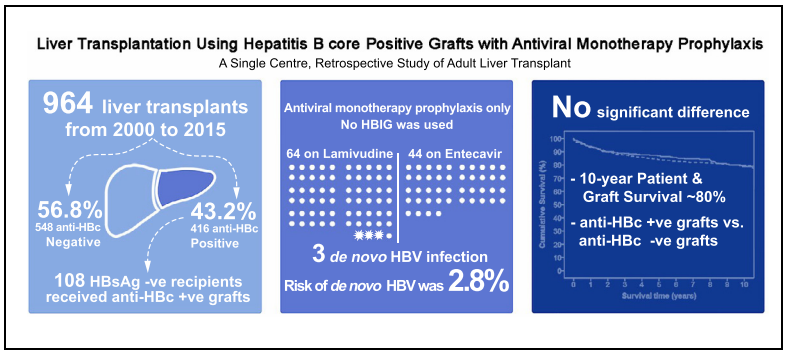
Liver transplantation using hepatitis B core positive grafts with antiviral monotherapy prophylaxis

Graphical abstract



Highlights

- Anti-HBc positive liver grafts did not lead to inferior survival after liver transplantation.
- Donor anti-HBc status did not impact on graft and patient survival, or HCC recurrence.
- De *novo* HBV infection was extremely rare with entecavir monoprophylaxis.

Authors

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Lay summary

The risk of *de novo* hepatitis B infection after liver transplantation was rare when using hepatitis B core positive liver grafts with entecavir monotherapy prophylaxis. Hepatitis B core antibody status did not impact on perioperative and long-term outcomes after liver transplantation. This provides support for the clinical use of hepatitis B core positive liver grafts when required.

http://dx.doi.org/10.1016/j.jhep.2019.03.003
 J. Hepatol. 2019, 70, 1114–1122

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Liver transplantation using hepatitis B core positive grafts with antiviral monotherapy prophylaxis

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Background & Aims: The impact of hepatitis B core antibody (anti-HBc) positive liver grafts on survival and the risk of *de novo* hepatitis B virus (HBV) infection after liver transplantation (LT) remain controversial. Therefore, we aimed to analyze this risk and the associated outcomes in a large cohort of patients.

Methods: This was a retrospective study that included all adults who underwent LT at Queen Mary Hospital, Hong Kong, between 2000 and 2015. Data were retrieved from a prospectively collected database. Antiviral monotherapy prophylaxis was given for patients receiving grafts from anti-HBc positive donors.

Results: A total of 964 LTs were performed during the study period, with 416 (43.2%) anti-HBc positive and 548 (56.8%) anti-HBc negative donors. The median follow-up time was 7.8 years. Perioperative outcomes (hospital mortality, complications, primary nonfunction and delayed graft function) were similar between the 2 groups. The 1-, 5- and 10-year graft survival rates were comparable in anti-HBc positive (93.3%, 85.3% and 76.8%) and anti-HBc negative groups (92.5%, 82.9% and 78.4%, $p = 0.944$). The 1-, 5- and 10-year patient survival rates in anti-HBc positive group were 94.2%, 87% and 79% and were similar to the anti-HBc negative group (93.5%, 84% and 79.7%, $p = 0.712$). One-hundred and eight HBsAg negative recipients received anti-HBc positive grafts, of whom 64 received lamivudine and 44 entecavir monotherapy prophylaxis. The risk of *de novo* HBV was 3/108 (2.8%) and all occurred in the lamivudine era. There were 659 HBsAg-positive patients and 308 (46.7%) received anti-HBc positive grafts. The risk of HBV recurrence was similar between the 2 groups. Donor anti-HBc status did not impact on long-term patient and graft survival, or the risk of hepatocellular carcinoma recurrence after LT.

Conclusions: *De novo* HBV was exceedingly rare especially with entecavir prophylaxis. Anti-HBc positive grafts did not impact on perioperative and long-term outcomes after transplant.

Lay summary: The risk of *de novo* hepatitis B infection after liver transplantation was rare when using hepatitis B core positive liver grafts with entecavir monotherapy prophylaxis. Hepatitis B core antibody status did not impact on perioperative and long-term outcomes after liver transplantation. This provides support for the clinical use of hepatitis B core positive liver grafts when required.

Introduction

Liver transplantation (LT) has become the standard of care for patients with end-stage liver disease and early non-resectable hepatocellular carcinoma (HCC). The growth in demand for LT has not been paralleled by a similar increase in organ supply.[1] Efforts have been made to promote organ donation, to develop surgical innovations such as living donor liver transplantation (LDLT), and to promote the use of extended criteria donor (ECD) organs.[1]

The use of hepatitis B surface antigen (HBsAg) negative donors with positive antibody against hepatitis B core antigen (anti-HBc) is an example of ECD graft.[2] There is potential for anti-HBc positive donors to harbor occult hepatitis B virus (HBV) infection, defined as the presence of liver and/or serum HBV DNA without serological evidence of chronic infection (HBsAg negative). Hence, there is a risk of transmitting HBV infection when these grafts are transplanted to HBsAg negative recipients (*de novo* HBV infection).[3,4] Nonetheless, anti-HBc positive donors represent an important source of organs in HBV endemic areas, including countries in the Asia-Pacific and Mediterranean region. The prevalence of anti-HBc positivity varies worldwide, and is related to the population prevalence of HBV infection.[5,6] In addition, HBV genotype strains vary in different geographic regions, with genotype B/C being the predominate strains in southeast Asia and China while genotype A/D is more commonly found in Europe.[7] As a result, studies have shown conflicting outcomes regarding the use of anti-HBc positive grafts, with inferior survival reported in some studies, and varying risk of *de novo* HBV infection ranging from <10% to 25%.[6,8–11] Prophylaxis against *de novo* HBV infection also varies among transplant centers worldwide, with different types of nucleos(t)ide analogue being used, with/without hepatitis B

Keywords: *De novo* HBV; *De novo* hepatocellular carcinoma; Long-term survival; Extended criteria organ; Entecavir.
Received 11 October 2018; received in revised form 20 February 2019; accepted 4 March 2019; available online 12 March 2019
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immunoglobulin (HBIG).[3,10–12] In this study, we analyzed the long-term outcomes of a large cohort of patients receiving anti-HBc positive grafts using nucleos(t)ide analogue monotherapy as prophylaxis, and evaluated the risk and outcomes of *de novo* HBV infection.

Patients and methods

This was a retrospective analysis of a prospective collected database, from Queen Mary Hospital, the University of Hong Kong, Hong Kong. The study protocol was approved by Institutional Review Board (Ref: UW18-358) and was conducted according to the Declaration of Helsinki. All adult patients who underwent LT from January 2000 to December 2015 were included. None of the donors were positive for HBsAg or antibody to hepatitis C virus. All deceased donors were brain death donors. No donor organs were obtained from executed prisoners or other institutionalized persons. All living donations were voluntary and all donors underwent evaluation by transplant surgeons, hepatologists, clinical psychologists, and nurse specialists. HBV serology including HBsAg, anti-HBc and hepatitis B surface antibody (anti-HBs) were checked for all donors. Pre-transplant blood samples from all anti-HBc positive donors were retrieved from tissue bank to test for circulating HBV DNA. Prioritization of deceased donor liver transplantation (DDLT) and graft allocation were based on the model for end-stage liver disease (MELD) score, irrespective of the anti-HBc status of donors and recipients.

Surgery and graft biopsy

LT was performed under standard techniques as described previously.[13] Post perfusion graft biopsy was performed routinely in all patients. A core of liver tissue was taken using 14-gauge trucut biopsy needle and was fixed in 10% formalin and processed with hematoxylin and eosin stained sections. All liver biopsies were read by specialist pathologists and the degree of macrovesicular steatosis and fibrosis was reported.[14]

Immunosuppression protocol

The immunosuppression regimen was standardized. All recipients received induction therapy with basiliximab. The maintenance regimen consisted of calcineurin inhibitors (CNIs) and mycophenolate mofetil. The latter would be discontinued 3 months after LT. Mammalian target of rapamycin (mTOR) inhibitors were available from 2006 as CNI sparing agents. The majority of patients were maintained on tacrolimus monotherapy. Maintenance steroid was only prescribed to patients who had autoimmune liver disease or a history of rejection or as CNI sparing agents.

Follow-up protocol

All patients were followed up regularly in clinic. Standard liver and renal biochemistry, blood count, clotting profile and immunosuppressant level were monitored at every visit. Hepatitis B serology including HBsAg, hepatitis B e-antigen (HBeAg), anti-HBs and HBV DNA were monitored at 3 monthly intervals.

HBV prophylaxis

Nucleos(t)ide analogue monotherapy was used as HBV prophylaxis after LT for HBV recipients. Before 2007, lamivudine was used for all patients, with subsequent switch to entecavir thereafter. For patients with evidence of lamivudine resistance, a combination of nucleoside and nucleotide analogue was used. No HBIG was used before, during or after LT. In non-HBV-related recipients who had anti-HBc positive grafts, nucleoside analogue monotherapy was given, *i.e.* lamivudine prior to 2011, and entecavir thereafter. *De novo* HBV infection was defined as HBsAg seropositivity and/or detectable HBV DNA in a non-HBV recipient. HBV DNA was measured using COBAS Taqman assay (Roche Molecular Systems, Branchburg, NJ) with a lower limit of quantitation of 10 IU/ml.

Statistical analysis

Continuous parameters were presented as median with range. Comparison between groups was performed using chi-squared test for categorical variables, or Mann-Whitney *U* test for continuous variables. Patient survival was measured from the time of transplantation to death from any cause. Graft loss was defined as recipient death, listing for re-transplantation or re-transplantation. Survival was analyzed using the Kaplan-Meier method and compared using log-rank test. Cox regression analysis was used to defined variables that predicted patient and graft survival. Univariate analysis was performed using demographics data, graft size, operative details and postoperative events; significant factors from univariate analysis (p <0.1) were entered for multivariate analysis. Statistical significance was defined as p <0.05 and all tests were performed 2-tailed. All calculations were done using SPSS version 22.0.

Results

A total of 964 adult LTs were performed during the study period, which included 416 (43.2%) anti-HBc positive and 548 (56.8%) anti-HBc negative donors. The median follow-up time for the cohort was 7.8 (0–18) years; it was 8.1 (0–18) years for the anti-HBc positive group and 7.3 (0–17.9) years for the anti-HBc negative group.

Among the non-HBV recipients, 125/305 (41%) patients had detectable anti-HBs before transplant and 82 (26.9%) remained anti-HBs positive. In HBV recipients, a minority of them (95/659 [14.4%]) developed anti-HBs transiently after transplant and only 35 patients (5.3%) remained anti-HBs positive at the last follow-up. The allocation of anti-HBc positive grafts in our cohort are illustrated (Fig. 1); 108(26%) anti-HBc positive grafts were allocated to non-HBV recipients. Of these, 62 were transplanted to anti-HBs positive recipients and 46 were transplanted to anti-HBs negative recipients. Thirty-eight patients had detectable anti-HBs and anti-HBc before transplant, while 24 patients were anti-HBs positive but anti-HBc negative. Out of these 24 patients, only 5 (20.8%) received HBV vaccination. In the remaining anti-HBs negative recipients, 22 were positive for anti-HBc while 24 were negative for both.

The clinical details of all patients grouped by donor anti-HBc status were summarized (Table 1). Anti-HBc positive donors were less likely to be live donors (49 *vs.* 60.2%, p = 0.001) and were older on average (47.5 *vs.* 35 years, p <0.001) but donor body mass index (BMI) and donor gender were similar.

There was no significant difference in the cause of death for deceased donors. There was no difference in recipient age, gender, BMI, disease etiology, transplant indication and MELD between the 2 groups. The majority of recipients had HBV-related liver disease, but the proportion was higher in the anti-HBc positive group. There were 534 (55.4%) LDLTs and 430 (44.6%) DDLTs in the cohort. There was no difference in

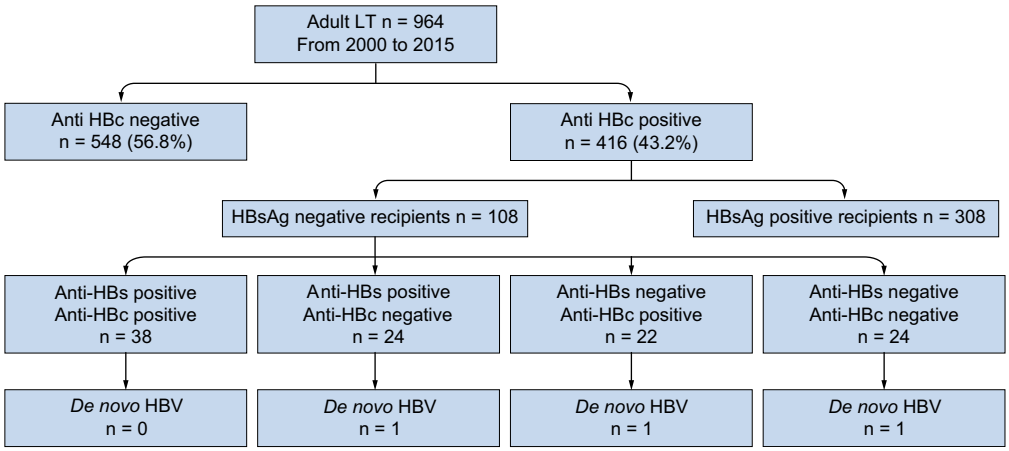


Fig. 1. Flow chart of allocation of anti-HBc positive grafts. Anti-HBc, hepatitis B core antibody.

Table 1. Clinical details of donors and recipients.

	Anti-HBc positive group (n = 416)	Anti-HBc negative group (n = 548)	*p* value
Donor age (years)	47.5 (14–77)	35 (2–84)	<0.001
Donor gender (n, % male)	192 (46.2)	262 (47.8)	0.610
Donor BMI (kg/m^2)	22.6 (15.6–35.2)	22.2 (12.8–35.6)	0.052
Live donors (n, %)	204 (49)	330 (60.2)	0.001
Cause of death (n, %) (if deceased donor)			0.100
Trauma	39 (9.4)	30 (5.5)	
Cerebrovascular accident	160 (38.5)	162 (29.6)	
Hypoxia	13 (3.1)	25 (4.6)	
Others	0 (0)	1 (0.2)	
Recipient age (years)	51 (17–69)	52 (16–73)	0.425
Recipient gender (n, % male)	307 (73.8)	393 (71.7)	0.473
Recipient BM I (kg/m^2)	23.9 (15.1–47.7)	24.3 (15.4–42.9)	0.946
MELD	21.5 (6–56)	21.3 (6–53)	0.876
HBV:HCV (n, %)	306 (73.6):24 (5.8)	343 (62.6):50 (9.1)	0.016
Transplant indication (n, %)			0.987
Acute	68 (16.3)	90 (16.4)	
Acute on chronic	75 (18)	103 (18.8)	
Decompensated cirrhosis	246 (59.1)	319 (58.2)	
Retransplantation	17 (4.1)	21 (3.8)	
Others	10 (2.4)	15 (2.7)	
Warm ischemic time (mins)	51 (25–146)	50 (24–151)	0.143
Cold ischemic time (mins)	256 (60–924)	134 (53–742)	<0.001
Day 0 graft biopsy (n, %)			
Steatosis > 10%	45 (10.8)	70 (12.7)	0.630
Fibrosis	24 (5.8)	49 (8.9)	0.163
Primary nonfunction (n, %)	0 (0)	0 (0)	–
Delayed graft function (n, %)	1 (0.2)	1 (0.2)	1.000
Recipient complication rate (n, %)	244 (58.7)	355 (64.8)	0.052
Recipient severe postoperative complications[†] (n, %)	100 (24)	175 (31.9)	0.073
Recipient hospital mortality (n, %)	9 (2.2)	18 (3.3)	0.296

Group comparisons were performed using chi-squared/Mann-Whitney *U* test, as appropriate. Anti-HBc, hepatitis B core antibody; BMI, body mass index; MELD, model of end-stage liver disease; HBV, hepatitis B virus; HCV, hepatitis C virus. [†]Clavien-Dindo Classification ≥Grade IIIa.

time on waitlist for LDLT (13 [0-1,701] days for anti-HBc positive *vs.* 11 [1-2,129] days for anti-HBc negative grafts, $p = 0.602$) and DDLT (117 [0-3,849] days for anti-HBc positive *vs.* 77.5 [0-3,668] days for anti-HBc negative grafts, $p = 0.621$). Pre-transplant plasma samples of anti-HBc donors were retrieved to test for circulating HBV DNA and 158/416 (38%) donors had samples available. Six (3.8%) donors (4 live donors and 2 deceased donors) had detectable HBV DNA but the level was <10 IU/ml. Graft biopsies from these donors did not show any abnormal histological finding and immunohistochemistry stains for HBsAg and HBcAg were negative.

The operative outcomes, hospital mortality, overall complication, and severe complication (defined as Clavien-Dindo grade ≥3a) were similar between the 2 groups.[15] Cold ischemic time was significantly longer in the anti-HBc positive groups, likely related to a higher percentage of DDLT in the group. On liver graft biopsy, graft steatosis >10% was comparable (10.8 *vs.* 12.7%, $p = 0.630$) and the presence of fibrosis was very low

(5.8 *vs*. 8.9%, $p = 0.163$). No patient in either group had significant fibrosis (F2 or greater).

Patient and graft survival in relation to anti-HBc status

There was no difference in patient survival between the 2 groups; the 1-, 5- and 10-year patient survival rates in the anti-HBc positive group were 94.2%, 87% and 79% and they were 93.5%, 84% and 79.7% for the anti-HBc negative group ($p = 0.712$) (Fig. 2A). The 1-, 5- and 10-year graft survival rates were also comparable in the anti-HBc positive (93.3%, 85.3% and 76.8%) and the anti-HBc negative groups (92.5%, 82.9% and 78.4%, $p = 0.944$) (Fig. 2B).

There were 212 graft losses at the time of analysis; a total of 93/416 (22.4%) graft losses in the anti-HBc positive group and 119/548 (21.7%) graft losses in the anti-HBc negative group. The majority of graft losses were due to HCC recurrence or co-morbidities (such as infection or cardiovascular events). There was no graft loss due to *de novo* HBV infection (Table 2).

Risk and outcomes of *de novo* hepatitis B infection and *de novo* hepatocellular carcinoma

The overall incidence of *de novo* HBV infection was 3/108 (2.8%), with all instances occurring in the lamivudine era. Sixty-four patients were on lamivudine prophylaxis and 3/64(4.7%) developed *de novo* HBV infection. Forty-four patients were given entecavir as prophylaxis and none developed *de novo* HBV, but it was not statistically significant ($p = 0.269$). The risk of *de novo* HBV was similar irrespective of recipient anti-HBs status. There was 1 *de novo* HBV infection out of 62 (1.6%) anti-HBs positive recipients (with pre-transplant anti-HBs at 187 IU/ml), while the risk of *de novo* HBV infection was 2/46 (4.3%) in anti-HBs negative recipients ($p = 0.792$) (Fig. 1).

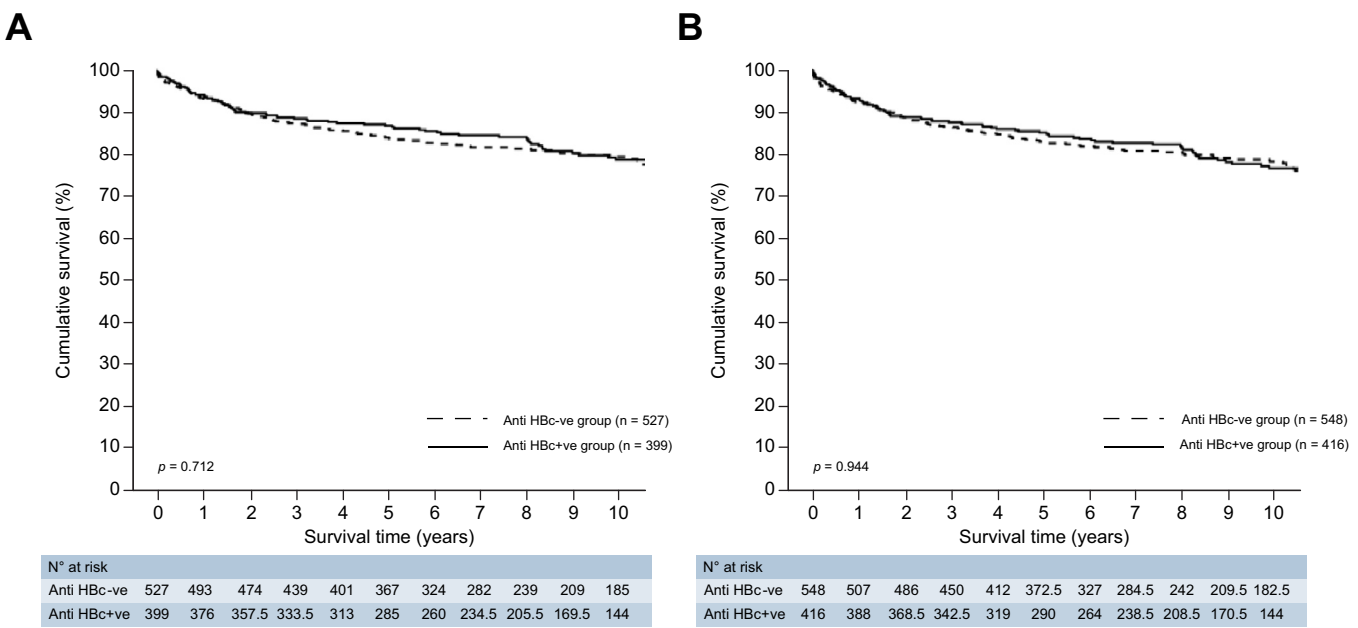
The characteristics of patients who developed *de novo* HBV infection are illustrated (Table 3). All 3 patients were found to be HBsAg-positive on routine monitoring without evidence of graft dysfunction at the time. Two patients had positive HBsAg transiently and subsequent HBsAg seroclearance again with the development of anti-HBs which was maintained until the time of last follow-up. The third patient was found to be HBsAg seropositive 1 month after transplant and serial HBV DNA every 3 months remained negative. She developed graft dysfunction 2 years after transplant and 3 liver graft biopsies that showed evidence of non-specific hepatitis; immunohistochemical stains were negative for HBsAg and hepatitis B core antigen (HBcAg). Her HBV DNA, hepatitis C virus (HCV)/hepatitis E virus (HEV) RNA and autoimmune markers were also negative. She subsequently developed graft cirrhosis and *de novo* HCC 9 years afterwards and underwent re-transplantation. Among the 3 patients with *de novo* HBV, 2 were anti-HBs negative before transplant and 1 was anti-HBs positive with a titer of 187 IU/ml. No patient developed *de novo* HBV infection from 2011 onwards with the use of entecavir as prophylaxis.

Among the 416 patients who were transplanted with anti-HBc positive grafts, there was 1 *de novo* HCC. The clinical details of the patient were described previously. There was neither virological nor histological evidence that *de novo* HBV infection was the cause of the graft cirrhosis and HCC, and the latter was most likely due to cirrhosis.

Risk of hepatitis B recurrence in HBsAg-positive recipients

In our cohort, there were 659 patients with HBV infection, of whom 308 (46.7%) received anti-HBc positive grafts and 351 (53.3%) anti-HBc negative grafts. In the former group, pre-transplant HBV DNA was positive in 173/308 (56.2%) recipients with a median level of 3×10^5 (211.7–5.5×10^9) IU/ml. All recipients received antiviral treatment before transplant.

HBsAg positivity after LT can occur as a reappearance of HBsAg after initial seroclearance or persistent HBsAg positivity without HBsAg seroclearance. There were 73 patients with



N° at risk											
Anti HBc-ve	527	493	474	439	401	367	324	282	239	209	185
Anti HBc+ve	399	376	357.5	333.5	313	285	260	234.5	205.5	169.5	144

N° at risk											
Anti HBc-ve	548	507	486	450	412	372.5	327	284.5	242	209.5	182.5
Anti HBc+ve	416	388	368.5	342.5	319	290	264	238.5	208.5	170.5	144

Fig. 2. Patient and graft survival stratified by donor anti-HBc status. (A) Patient survival stratified by donor anti-HBc status. Group comparisons were performed using Kaplan-Meier and log-rank test. (B) Graft survival stratified by donor anti-HBc status. Group comparisons were performed using Kaplan-Meier and log-rank test. Anti-HBc, hepatitis B core antibody.

Table 2. Cause of graft losses in the whole cohort.

	Anti-HBc positive group (n = 416)	Anti-HBc negative group (n = 548)	Total no.
Total number of graft losses	93 (22.4%)	119 (21.7%)	212
Liver complications			
Hepatic artery thrombosis	3	3	6
Portal vein thrombosis	3	1	4
Hepatic vein thrombosis	0	2	2
Rejection	4	3	7
Biliary complications	7	4	11
De novo HBV infection	0	0	0
Alcoholic cirrhosis	1	0	1
Others	1	0	1
Recurrence of original disease			
HBV infection	2	1	3
HCV infection	1	5	6
Wilson's disease	0	1	1
Others	0	1	1
Malignancy			
Recurrence of original tumor	19	27	46
De novo solid tumor	8	4	12
Lymphoproliferative disease	3	4	7
Patient's death from other causes	41	63	104

No analysis was made for the causes of graft loss. HBV, hepatitis B virus; HCV, hepatitis C virus.

Table 3. Characteristics and outcomes of patients who developed *de novo* hepatitis B infection.

Patient/sex	Age at LT/year	Indication for LT	Time to *de novo* HBV/months	Treatment before *de novo* HBV	HBV DNA	Mutant	Recipient anti-HBs	Recipient anti-HBc	Current status
1/F	28	Drug induced	0.2	Lamivudine	–	–	–	–	Normal graft function
2/F	54	PBC	6	Lamivudine	–	–	+ 187 IU/ml	–	Normal graft function
3/F	45	HCV	1	Lamivudine	–	–	–	+	Retransplanted due to graft cirrhosis and HCC

LT, liver transplantation; anti-HBs, hepatitis B surface antibody; anti-HBc, hepatitis B core antibody; PBC, primary biliary cirrhosis; HBV, hepatitis B virus; HCV, hepatitis C virus; HCC, hepatocellular carcinoma.

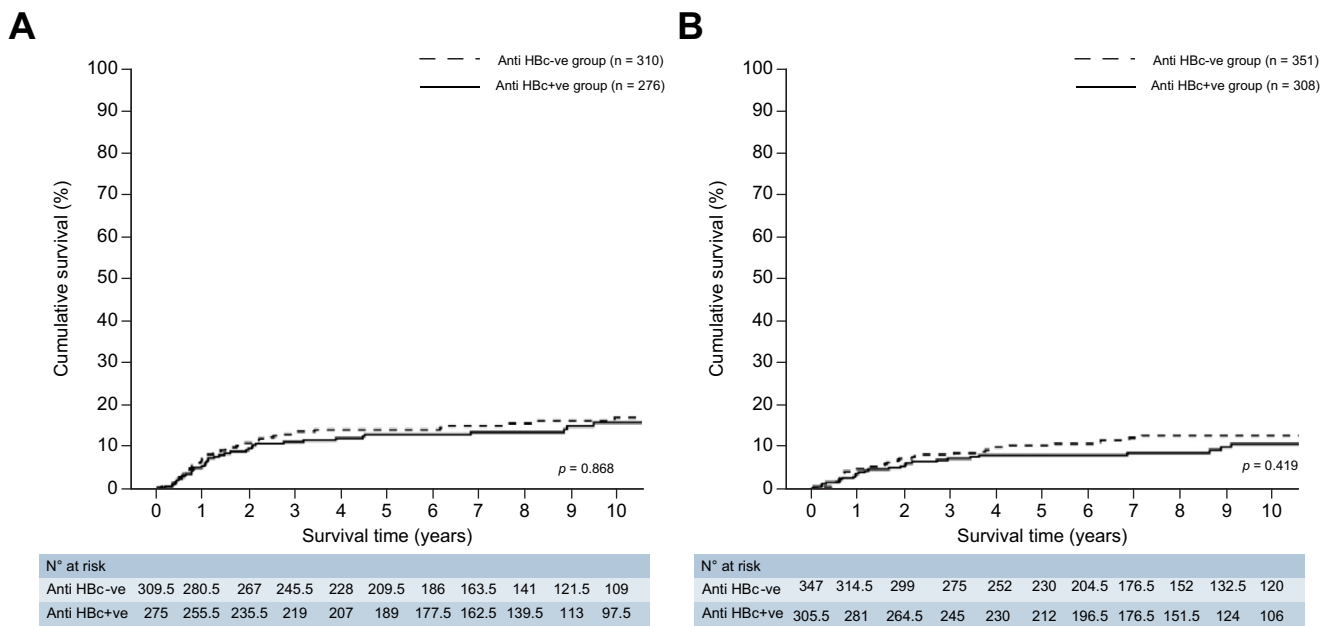


Fig. 3. Cumulative rate of HBsAg positivity after seroclearance and virological rebound after liver transplantation, stratified by anti-HBc status. (A) Cumulative rate of HBsAg positivity after initial seroclearance. Group comparisons were performed using Kaplan-Meier and log-rank test. (B) Cumulative rate of virological rebound after liver transplantation in HBV recipients. Group comparisons were performed using Kaplan-Meier and log-rank test. Anti-HBc, hepatitis B core antibody; HBV, hepatitis B virus; HBsAg, HBV surface antigen.

persistent HBsAg positivity after LT without HBsAg seroclearance and 86 patients with reappearance of HBsAg after initial seroclearance. Of these, 9/159 (5.7%) were HBeAg positive. Although the proportion of HCC in the HBsAg-positive group was similar to that in the HBsAg-negative group (54/159 [34%] *vs.* 182/500 [36.4%], $p = 0.577$), HBsAg-positive patients experienced significantly more HCC recurrence (25/54 [46.3%] *vs.* 15/182 [8.2%], $p < 0.001$] and the risk of death from HCC was also significantly higher (30/54 [55.6%] *vs.* 24/182 [13.2%], $p < 0.001$).

The 1-, 5- and 10-year cumulative HBsAg positivity rates, after initial seroclearance, for patients who had anti-HBc positive donors were 5.1%, 12.6% and 15.4%. Among patients who had anti-HBc negative donors, the 1-, 5- and 10-year cumulative HBsAg positivity rate was 6.9%, 13.7% and 16.5% ($p = 0.868$). (Fig. 3A) The 1-, 5- and 10-year cumulative rate of virological rebound (defined as detectable HBV DNA) was 3.7% *vs.* 4.5%, 8.0% *vs.* 10.3% and 10.7% *vs.* 12.6% in anti-HBc positive group and anti-HBc negative group, respectively ($p = 0.419$) (Fig. 3B).

Outcomes between donor anti-HBc and hepatocellular carcinoma

Out of 659 HBsAg-positive patients, 290 (44%) had concomitant HCC. Approximately half received anti-HBc positive grafts (130/290 [44.8%]) and 160/290 (55.2%) received anti-HBc negative grafts, respectively ($p = 0.660$). The 1-, 5- and 10-year patient survival rates in HCC recipients who were transplanted with anti-HBc positive grafts were 96.2%, 83.9% and 73.5%, compared with 95.6%, 75.8% and 70.4% for those with anti-HBc negative grafts ($p = 0.271$) (Fig. S1A). The risk of HCC recurrence was also the same irrespective of donor anti-HBc status. The 1-, 5- and 10-year disease-free survival rates of patients with HCC were 90.8%, 80.8% and 71.3% in the anti-HBc positive group and 88.1%, 74.7% and 69.7% in the anti-HBc negative group ($p = 0.395$) (Fig. S1B).

Outcomes of anti-HBc positive grafts in living donor *vs.* deceased donor liver transplantation

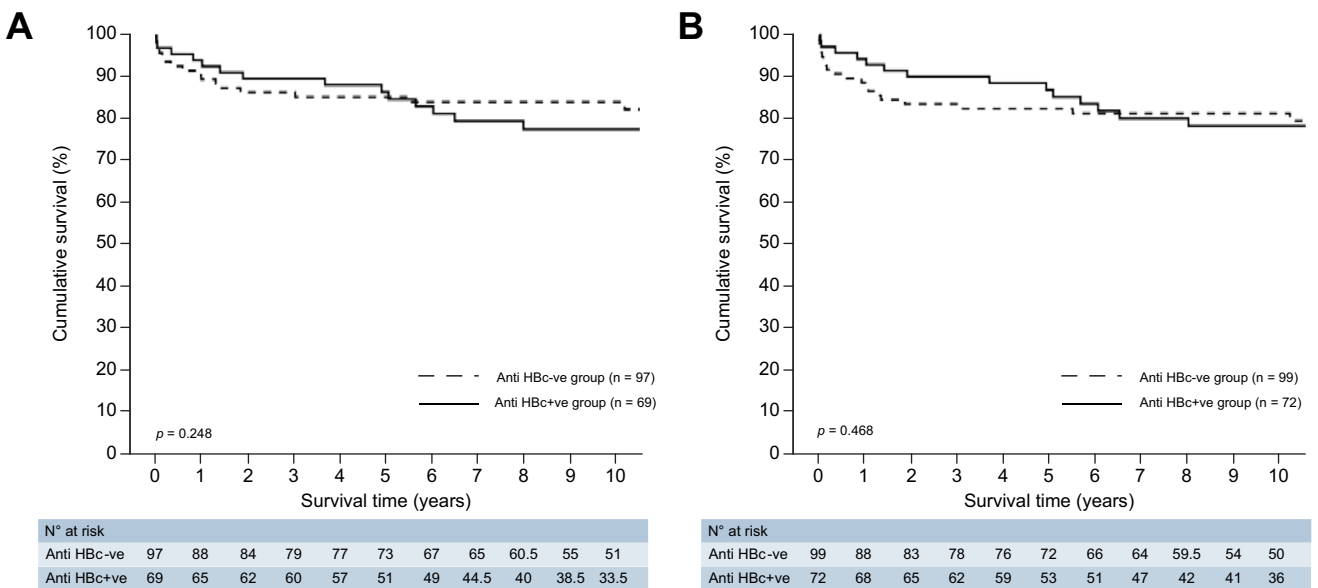
Use of anti-HBc positive grafts showed comparable outcomes in LDLT *vs.* DDLT. Patient and graft survival up to 10 years were similar between anti-HBc positive and anti-HBc negative groups, for both LDLT and DDLT (Fig. S2A,B).

Outcomes of anti-HBc grafts by MELD score

Seventy-two high MELD patients (defined as MELD ≥35) were allocated with anti-HBc positive grafts and 99 high MELD patients had anti-HBc negative grafts. The 1-, 5- and 10-year graft survival rates were 94.4 *vs.* 88.9%, 87.2 *vs.* 82.8% and 78.7 *vs.* 81.6% in the anti-HBc positive and anti-HBc negative groups, respectively ($p = 0.468$). The 1-, 5- and 10-year patient survival rates were 94.2%, 86.7% and 77.8% in anti-HBc positive donors and 90.7%, 85.5% and 84.3% in anti-HBc negative donors, respectively ($p = 0.248$) (Fig. 4). Comparable outcomes were also seen among patients with MELD <35 (Fig. S3).

Predictors of patient and graft survival at multivariate analysis

Anti-HBc positive graft was not a significant factor associated with survival. In multivariate analysis, the following variables were associated with poorer patient survival: male recipient (hazard ratio [HR]; 95% CI 1.04–2.11; $p = 0.03$), HCC (HR = 1.64; 95% CI 1.22–2.21; $p = 0.001$), prolonged cold ischemic time (HR = 1.002; 95% CI 1.001–1.003; $p < 0.001$) and grade ≥3a complications (HR = 1.90; 95% CI 1.42–2.54; $p < 0.001$) were associated with poorer patient survival (Table 4). Similar factors were found in multivariate analysis for graft survival; HCC (HR = 1.55; 95% CI 1.16–2.07; $p = 0.003$), prolonged cold ischemic time (HR = 1.002; 95% CI 1.001–1.002; $p < 0.001$) and grade ≥3a complications (HR = 2.08; 95% CI 1.57–2.75; $p < 0.001$) were associated with poorer graft survival (Table 5).



N° at risk											
Anti HBc-ve	97	88	84	79	77	73	67	65	60.5	55	51
Anti HBc+ve	69	65	62	60	57	51	49	44.5	40	38.5	33.5

N° at risk											
Anti HBc-ve	99	88	83	78	76	72	66	64	59.5	54	50
Anti HBc+ve	72	68	65	62	59	53	51	47	42	41	36

Fig. 4. Patient and graft survival between anti-HBc positive *vs.* anti-HBc negative donors in MELD ≥35 patients. Group comparisons were performed using Kaplan-Meier and log-rank test. Anti-HBc, hepatitis B core antibody; MELD, model for end-stage liver disease.

Table 4. Univariate and multivariate analyses of prognostic factors for patient survival.

	Univariate		Multivariate	
Variables	**HR (95% CI)**	***p* value**	**HR (95% CI)**	***p* value**
Recipient age	1.03 (1.01–1.04)	0.001		
Male recipient	1.50 (1.05–2.14)	0.026	1.48 (1.04–2.11)	0.030
Recipient BMI	0.97 (0.93–1.001)	0.055		
Donor age	1.02 (1.004–1.03)	0.006		
Male donor	0.81 (0.60–1.08)	0.150		
Donor BMI	1.00 (0.95–1.05)	0.965		
MELD	1.00 (0.98–1.004)	0.150		
Hepatocellular carcinoma	1.57 (1.17–2.12)	0.003	1.64 (1.22–2.21)	0.001
LDLT	0.73 (0.55–0.98)	0.035		
Anti-HBc positive graft	0.95 (0.71–1.27)	0.712		
Recipient HBsAg positive	0.88 (0.64–1.21)	0.424		
Recipient HCV positive	1.39 (0.86–2.23)	0.177		
Graft–to–recipient weight ratio <0.8%	0.81 (0.56–1.18)	0.281		
Cold ischemic time	1.001 (1.001–1.002)	0.001	1.002 (1.001–1.003)	<0.001
Warm ischemic time	1.00 (0.99–1.005)	0.338		
Grade ≥3a postoperative complication	1.80 (1.33–2.42)	<0.001	1.90 (1.42–2.54)	<0.001

Analysis using Cox regression model. HR, hazard ratio; BMI, body mass index; MELD, model of end-stage liver disease; LDLT, living donor liver transplantation; anti-HBc, hepatitis B core antibody; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus infection.

Table 5. Univariate and multivariate analyses of prognostic factors for graft survival.

	Univariate		Multivariate	
Variables	**HR (95% CI)**	***p* value**	**HR (95% CI)**	***p* value**
Recipient age	1.01 (1.00–1.27)	0.078		
Male recipient	1.42 (1.02–1.97)	0.039		
Recipient BMI	0.97 (0.94–1.002)	0.063		
Donor age	1.01 (1.004–1.02)	0.006		
Male donor	0.82 (0.62–1.08)	0.152		
Donor BMI	1.006 (0.96–1.05)	0.781		
MELD	0.99 (0.98–1.002)	0.097		
Hepatocellular carcinoma	1.37 (1.03–1.82)	0.030	1.55 (1.16–2.07)	0.003
LDLT	0.72 (0.55–0.94)	0.017		
Anti-HBc positive graft	0.99 (0.76–1.30)	0.944		
Recipient HBsAg positive	1.30 (0.97–1.72)	0.078		
Recipient HCV positive	1.37 (0.86–2.17)	0.185		
Graft-to-recipient weight ratio <0.8%	1.20 (0.84–1.72)	0.318		
Cold ischemic time	1.001 (1.001–1.002)	<0.001	1.002 (1.001–1.002)	<0.001
Warm ischemic time	1.00 (0.99–1.005)	0.398		
Grade ≥3a postoperative complication	2.02 (1.53–2.66)	<0.001	2.08 (1.57–2.75)	<0.001

Analysis using Cox regression model. HR, hazard ratio; BMI, body mass index; MELD, model for end-stage liver disease; LDLT, living donor liver transplantation; anti-HBc, hepatitis B core antibody; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus infection.

Discussion

Data in literature are conflicting regarding the risk of *de novo* HBV infection with the use of anti-HBc positive grafts and the possible inferior survival outcomes in long-term. This explains the wide variation in clinical practice. Almost half of the surveyed transplant physicians reported that they would not use anti-HBc positive grafts in HBV naïve recipients, and there were also different practices regarding HBV prophylaxis.[3,10,16] The present study demonstrated excellent long-term survival with the use of anti-HBc positive grafts. Graft survival at 10 years was 76.8% for the anti-HBc positive and 78.4% for the anti-HBc negative group, whereas patient survival at 10 years was almost 80% for both groups. In our region, the population prevalence of anti-HBc positivity is up to 50% and discarding anti-HBc positive organs would not be practical.[5] In the present study, we have demonstrated that the risk of primary nonfunction, delayed graft function, overall complication and hospital mortality were comparable between anti-HBc positive and negative grafts. There was no difference in long-term survival outcomes between anti-HBc positive and negative grafts in patients who received different graft types, had high MELD scores, or had HCC.

The overall incidence of *de novo* HBV infection was 2.8%. The existence of intrahepatic covalently closed circular DNA (cccDNA) in anti-HBc positive donors is likely the main reason for *de novo* HBV infection. Without proper prophylaxis, the risk of *de novo* HBV after LT could be up to 80%.[2,3] We have demonstrated the risk of *de novo* HBV remained very low with the use of antiviral prophylaxis. The 3 *de novo* HBV infections occurred in patients who received lamivudine monotherapy. More importantly, no *de novo* HBV infection occurred with the use of entecavir as antiviral prophylaxis, highlighting the importance of using an antiviral agent with a high barrier to resistance. HBV virological analyses were routinely monitored for all patients, therefore the exact incidence of recurrent HBV and *de novo* HBV infection can be identified in the absence of graft dysfunction. In the patient who developed cirrhosis, there was no evidence of HBV/HCV/HEV infection in the graft. The exact cause of graft failure was unclear, although it was postulated that *de novo* autoimmune hepatitis or chronic rejection may have contributed.

Our data provides strong evidence that the risk of *de novo* HBV infection is exceedingly rare using entecavir alone without

HBIG in HBV naïve recipients who received anti-HBc positive organs. Current international guideline still recommends lamivudine as first line therapy,[17] however, the current study showed that all *de novo* HBV infection occurred in those receiving lamivudine, whereas this was not observed in those receiving entecavir. Therefore, we recommend that agents with better resistance profiles should be adopted irrespective of recipient anti-HBs status for transplant recipients under the effect of immunosuppression. With the availability of generic entecavir, the cost issue will be less of a concern. The use of HBIG in the setting of occult HBV-infected graft would seem superfluous as it is primarily used for preventing graft infection.

Out of the 3 *de novo* HBV infection in our study, 1 patient had a pre-transplant anti-HBs titer at 187 IU/ml. In non-HBV patients, even in those who had acquired prior HBV immunity (*i.e.* detectable anti-HBs before LT), the presence of anti-HBs would not confer absolute immunity to *de novo* HBV infection, and the titer of anti-HBs tends to fall after LT.[18,19] In addition, whether pre-/post-transplant vaccination would be effective remains unclear.[20–22] In our center, there is no routine vaccination strategy. Response to HBV vaccination was low in immunosuppressed patients, but there was evidence to show that response rate would improve when multiple prolonged courses of vaccination were administered.[22,23] We have previously demonstrated that recipients who responded to HBV vaccination, and those who developed spontaneous anti-HBs after LT, would respond to further vaccination.[24] At the moment, lifelong indefinite antiviral prophylaxis is still considered necessary. Active immunization might serve as a potential therapeutic strategy that might enable cessation of antiviral therapy. The overall *de novo* HBV infection rate was extremely low, suggesting that antiviral prophylaxis may possibly be stopped in selected recipients such as those who are anti-HBs positive. The risk of reactivation arises from the presence of intrahepatic cccDNA. However, a liver biopsy is necessary to detect intrahepatic cccDNA, which limits its applicability for regular monitoring, coupled with the lack of standardization for measuring cccDNA. Recently, a highly sensitive method for quantitation of intrahepatic cccDNA by droplet digital PCR has been developed. Other markers, including serum anti-HBc IgG level, were shown to correlate with intrahepatic cccDNA in patients with occult HBV infection.[25] Therefore the quantitation of anti-HBc IgG, together with other novel markers such as hepatitis B core-related antigen (HBcAg) or HBV RNA, might serve as a surrogate for intrahepatic cccDNA.[26,27]

Meanwhile, the use of anti-HBc positive grafts in HBsAg-positive recipients is less of a concern because such recipients will require lifelong HBV prophylaxis, and there is no risk of transmitting a "new" infection. In our study, the risk of HBsAg positivity and virological rebound was similar irrespective of anti-HBc status.

There did not seem to be an increase risk of HCC recurrence and risk of *de novo* HCC in patients who were transplanted with anti-HBc positive grafts. Overall survival and risk of HCC recurrence were similar between the 2 groups. More importantly, we have demonstrated the risk of *de novo* HCC was minimal. The cause for the *de novo* HCC was likely due to graft cirrhosis. The concern that anti-HBc positive grafts might harbor occult HBV infection with increased risk of *de novo* HCC was not evident. However, for those who remain HBsAg positive, there was a higher rate of HCC recurrence, leading to a poorer outcome.

There is always concern regarding the use of ECD grafts in high MELD recipients. The use of anti-HBc positive organs in MELD ≥35 patients did not indicate a significant difference in long-term survival, with excellent 10-year survival reported when using anti-HBc positive grafts. The use of anti-HBc positive grafts also showed comparable results after LDLT and DDLT with 10-year survival around 80%.

At our center, anti-HBc status was not used for donor-recipient matching.[28] The need for donor-recipient matching was based on balancing benefit and risk of the individual recipient; in the setting of donor anti-HBc status, there does not seem to be a need for such matching as the outcomes of these grafts were equivalent to anti-HBc negative grafts. However, such practice is certainly debatable, especially in areas with a low prevalence of anti-HBc positivity and HBV-infected recipients. The low but definite risk of *de novo* HBV infection should be taken into consideration, and the need for lifelong prophylaxis which would otherwise not be required should also be explained. Nonetheless, as the current study showed excellent perioperative and long-term outcomes using anti-HBc positive grafts, HBsAg naïve recipients should be given the opportunity to receive such grafts with informed consent in areas of low prevalence.

Previous studies showed inferior graft survival with anti-HBc positive organs, but graft loss was likely caused by technical issues and not related to *de novo* HBV infection.[10,11] It was also hypothesized that anti-HBc status might reflect suboptimal graft quality through unexplained mechanisms.[10] Our study has shown the contrary. One important point to note is that donor demographics were very different in our study. Previous studies consisted of almost exclusively deceased donors whilst live donors constituted 534/964 (55.4%) in our cohort. Candidates with evidence of pre-existing liver disease such as fibrosis or steatosis >10% would be ineligible for live donation, therefore live donors represent young and healthy individuals with excellent quality grafts. For deceased donors, although selection was less stringent, contrast imaging would be performed if donor liver function was abnormal and graft biopsy would be taken at procurement if there was any concern about graft quality. Compliance to treatment is another major factor for the excellent outcomes. All patients received education from nurse specialists, and their drug compliance would be checked and reinforced at each clinic visit. Antiviral agents are generally well tolerated, with minimal side effects and drug-drug interactions, therefore compliance is usually not an issue.

HBV DNA was detected in 6/158(3.8%) anti-HBc positive donors in whom pre-transplant samples were available. The level of viremia was extremely low. None of their respective recipients developed *de novo* HBV infection. The presence of circulating HBV DNA is likely an incidental event rather than a clinically significant finding.[29]

The strength of the current study include a large cohort size, with the current study representing the largest single center experience with anti-HBc positive grafts using a standardized protocol of HBV prophylaxis, along with a long (median 7.8 years) follow-up time. There are several limitations of the current study. Firstly, this was a retrospective study of a single center. Secondly, HBV prophylaxis changed from lamivudine to entecavir in 2011, therefore we were unable to evaluate the risk of lamivudine resistance. Thirdly, this study was conducted in an area with high prevalence of anti-HBc positivity, and the

importance of using such grafts may be of less concern to regions with lower seroprevalence.

In conclusion, excellent outcomes with anti-HBc positive grafts for LT can be achieved with the use of oral antiviral alone, with excellent long-term survival, irrespective of recipient HBV status, graft type, MELD and without increase in HCC recurrence.

Financial support

The authors received no financial support to produce this manuscript.

Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

TCL Wong and JYY Fung were responsible for concept and design of study, data collection, drafting of manuscript, statistical analysis and final review of papers. TYS Cui, AHK Lam, JWC Dai, ACY Chan, TT Cheung, KSH Chok, KKC Ng and CM Lo were responsible for study design, data collection, statistical analysis and critical review of manuscript.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2019.03.003.

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