ORIGINAL ARTICLE

Inferior graft survival of hepatitis B core positive grafts is not influenced by post-transplant hepatitis B infection in liver recipients—A 35-year single-center experience

Andreas Brandl[1,2], Philipp Stolzlechner[1], Stephan Eschertzhuber[3], Felix Aigner[1,3], Sascha Weiss[1,2], Wolfgang Vogel[5], Alexander Krannich[6], Sabrina Neururer[7], Johann Pratschke[1,4,†], Ivo Graziadei[5,8] & Robert Öllinger[1,4]

1 Department of Visceral-, Transplant-, and Thoracic Surgery, Medical University, Innsbruck, Austria
2 Department of General, Visceral, Vascular and Thoracic Surgery, Charité Campus Mitte, Berlin, Germany
3 Department of Anaesthesia and Intensive Care Medicine, Medical University, Innsbruck, Austria
4 Department of General, Visceral and Transplant Surgery, Charité Campus Virchow-Klinikum, Berlin, Germany
5 Department of Internal Medicine II, Gastroenterology & Hepatology, Medical University, Innsbruck, Austria
6 Department of Biostatistics, Coordination Center for Clinical Trials, Charité Universitätsmedizin Berlin, Berlin, Germany
7 Department of Medical Statistics, Medical University, Innsbruck, Austria
8 Department of Internal Medicine, District Hospital Hall, Innsbruck, Austria

Correspondence
Johann Pratschke MD, PHD, Center of Operative Medicine, Department of Visceral, Transplant and Thoracic Surgery, Innsbruck Medical University, Anichstraße 35, 6020 Innsbruck, Austria.
Tel.: +49 30 450 552 001;
fax: +49 30 450 552 900;
e-mail: johann.pratschke@charite.de

† Present address: Klinik für Allgemein-, Visceral- und Transplantationschirurgie, Charité - Universitätsmedizin Berlin, Campus Virchow-Klinikum, Augustenburger Platz 1 13353, Berlin, Germany
Ivo Graziadei and Robert Öllinger contributed equally to this manuscript.

SUMMARY

Nonoptimal liver grafts, and among them organs from anti-HBc+ donors, are increasingly used for liver transplantation. In this retrospective study including 1065 adult liver transplantations performed between 1977 and 2012, we analyzed long-term patient and graft survival and occurrence of HBV infection. A total of 52 (5.1%) patients received an anti-HBc+ graft. The 10-year graft and patient survival of these recipients were 50.9% and 59.0% compared to 72.0% and 76.5% ($P = 0.001$; $P = 0.004$) of patients receiving anti-HBc- grafts, respectively. Cox regression model showed that high urgency allocation ($P = 0.003$), recipient age ($P = 0.027$), anti-HCV+ recipients ($P = 0.005$), and anti-HBc+ organs ($P = 0.048$) are associated with decreased graft survival. Thirteen of 52 (25.0%) patients receiving anti-HBc+ grafts developed post-transplant HBV infection within a mean of 2.8 years. In this study, antiviral prophylaxis did not have significant impact on HBV infection, but long-term survival ($P = 0.008$). Development of post-transplant HBV infection did not affect adjusted 10-year graft survival (100% vs. 100%; $P = 1$). Anti-HBc+ liver grafts can be transplanted with reasonable but inferior long-term patient and graft survival. The inferior graft survival is not, however, related with post-transplant HBV infection as long as early diagnosis and treatment take place.

Transplant International 2016; 29: 471–482

Key words
anti-HBc+ grafts, antiviral prophylaxis, Lamivudine resistance, post-transplant HBV infection

Received: 20 January 2015; Revision requested: 11 February 2015; Accepted: 23 December 2015; Published online: 1 February 2016

doi:10.1111/tri.12741

Introduction

Pushing the boundaries by means of accepting nonoptimal livers from extended criteria donors (ECD) for transplantation offers the possibility to expand the available pool of donors so more patients on the waiting list can benefit from a life-saving liver transplant (LT). During the last decades, some donor-related factors have been associated with reduced graft and patient survival following LT. These factors include prolonged cold ischemic time, high donor age, elevated donor serum sodium (>155 mmol/l), donor hepatosteatosis, elevated transaminases, elevated bilirubin, prolonged downtime, donation after cardiac death, and grafts from donors with either hepatitis B (HBV) or C (HCV) infection [1–5].

The number of grafts used from donors with antibodies against the HBV core antigen (anti-HBc), but hepatitis B surface antigen (HBsAg) negative, that is, "anti-HBc-positive donors" [6], is rising [7–9]. Still, concerns on the usage of these grafts remain. First, due to HBV infection the graft may be chronically damaged [10]. Secondly, there is a risk of HBV transfection; anti-HBc-positive liver donors frequently have occult HBV infection, that is, persistent liver and/or serum HBV DNA without serologic evidence of active HBV infection. Indeed, several studies in HBsAg-negative subjects have shown that there is often the detection in the liver of covalently closed circular DNA (cccDNA) and pregenomic RNA, a marker of ongoing viral replication [11,12], which may significantly increase with the use of post-LT immunosuppression, in particular with corticosteroids [13]. The liver grafts from anti-HBc-positive donors are currently the main source of *de novo* HBV infection after LT [14,15], which is generally defined by the development of positive HBsAg and/or detectable serum or liver HBV DNA in previously HBV-naive recipients. However, the literature documenting the risk of *de novo* HBV infection and the effects on long-term graft and patient survival is scarce and conflicting.

The lack of definite data explains the wide variation in current clinical practice. In a survey carried out in 2001, almost half of liver transplant physicians reported that they did not use anti-HBc-positive donors in HBV-naive recipients [16]. In a more recent international survey, the responders documented using prophylaxis with nucleos(t)ide analogue (mostly Lamivudine, but also Entecavir and Adefovir) in the majority of LT recipients of anti-HBc-positive grafts, and 61% also used hepatitis B immunoglobulin (HBIg) [17]. Recent studies have shown that the use of high genetic barrier nucleos(t)ide analogues, that is, Entecavir or Tenofovir + HBIg is superior compared to Lamivudine + HBIg in the prophylaxis of hepatitis B recurrence after LT [9].

The aim of our study was to analyze long-term outcomes of liver grafts from anti-HBc-positive donors, and incidence and risk factors of post-transplant HBV infection. Secondary endpoint was to investigate whether antiviral prophylaxis and/or anti-HBs titer have an influence on post-transplant HBV infection.

Patients and methods

This retrospective analysis of prospectively collected data includes all consecutive LTs of adult (≥18 years) recipients performed at our center between April 1977 and March 2012 (n = 1065). The following parameters were included in the analysis: donor sex, age, BMI, cytomegalovirus (CMV); anhepatic time, cold ischemic period (CIP), warm ischemic period 2 (WIP2), preservation solution; recipient model for end-stage liver disease (MELD), Child Pugh Score, sex, age, BMI, CMV, HCC, acute liver failure, re-transplantation, HBV infection, Lamivudine resistance, anti-HBs, HBsAg, anti-HBc, and antiviral prophylaxis. Graft loss was defined as re-transplantation or death of the patient.

Post-transplant hepatitis B was defined as a detectable serum HBsAg and/or viral load (HBV DNA) with histological and immunohistochemical confirmation of HBV by liver biopsy. The cutoff level for anti-HBs positivity in recipients was defined as 100 IU/l [18].

Concerning antiviral prophylaxis, anti-HBc-negative recipients were divided into five different groups based on the use of a nucleos(t)ide analogue reverse transcriptase inhibitor (nRTI), HBIg, or combinations. Group 1 received no nRTI and no HBIg, group 2 HBIg alone, group 3 Lamivudine alone, group 4 Lamivudine and HBIg, and group 5 Tenofovir and HBIg. HBIg was administered 500 IU/day daily starting with 1000 IU in the anhepatic phase until anti-HBs serum levels were >500 IU/l. The target levels from day 8 to 90 were >250 IU/l and after day 90 >100 IU/l. Lamivudine was administered daily at 150 mg, and Tenofovir daily at 245 mg orally.

Immunosuppression post-transplantation followed standard protocols and consisted of corticosteroids and either tacrolimus or cyclosporine, with or without either azathioprine or mycophenolate mofetil (MMF). When indicated, induction therapy with anti-IL2R antagonists, antithymocyte globulin (ATG), or Alemtuzumab was carried out.

Transplant International 2016; 29: 471–482

Patients were followed up at regular intervals. Routine monitoring included liver biochemistry and testing for HBsAg and HBV DNA. HBsAg was measured through standard assay, and Artus (GmbH, Hamburg, Germany) real-time polymerase chain reaction (PCR) was used to detect HBV DNA; Lamivudine resistance was evaluated through "Lamivudine resistance, InnoLipa HBV Dr V2", Innogenetics (Gent, Belgium).

All statistical analyses were performed using either SPSS 20.0 (International Business Machines Corporation, Armonk, NY, USA) or PRISM 6.0 (GraphPad Software, Inc., La Jolla, CA, USA). For cumulative incidence analysis, free software solution R 3.2.2 with the package "cmprsk" was used. Data were provided as mean and standard deviation or median and interquartile range (IQR), and dichotomous variables represented as percentages. To check for normal distribution, the Kolmogorov–Smirnov test was used. For group comparisons, either the Mann–Whitney *U*-test, Student's-*t* test, or the chi-square tests were performed. Kaplan–Meier survival curves were carried out using the log-rank test to curve comparison. An univariate Cox proportional hazards model of the predictor variables was created, and those found to be significant at $P < 0.20$ were selected for the multivariate analysis. The final multivariate model was obtained with a backward selection method. The number of missing data for this model was 832 (77.8%). All statistical tests were two-sided, and *P*-values < 0.05 were considered statistically significant. In order to further analyze the development of HBV infection post-transplant, we calculated a time to event analysis for HBV infection, considering patient death as competing risk and performed additionally landmark analyses. Therefore, at a landmark time of 1 year after transplantation, the HBV status was assessed and used to divide the patients into two groups. Based on this information, the patient, graft, and adjusted graft survival were statistically analyzed.

Results

Transplant characteristics

In total, 1167 LTs were performed during the observation period including 35 (3.0%) living donor procedures. A total of 59 (5.1%) patients received liver grafts from anti-HBc-positive donors. Excluding pediatric recipients ($n = 5$) and donors with active hepatitis B ($n = 2$), that is, HBsAg positivity at the time of organ procurement, 1065 in total and 52 recipients of anti-HBc+ donors remained for analysis. Donor, recipients, and transplantation-related characteristics are shown in Table 1. Univariate analysis between anti-HBc+ and anti-HBc− recipients revealed significant differences in recipients' anti-HBc status ($P = 0.008$) and donor age ($P = 0.001$). Etiologies of liver disease in these 52 recipients are shown in Table 2.

Recipient and graft survival

The median follow-up was 6.3 years. Five- and 10-year survival rates of patients who received grafts from anti-HBc+ donors were 71.3% and 59.0% compared to 82.5% and 76.5% (log-rank $P = 0.004$) in recipients receiving grafts from anti-HBc− donors, respectively (Fig. 1). Five- and 10-year graft survival were 61.7% and 50.9% for anti-HBc+ donors compared to 79.0% and 72.0% for the anti-HBc− group (log-rank $P = 0.001$), respectively (Fig. 2). The analysis of five- and 10-year patient and graft survival rates for anti-HBc+ liver grafts in HBV+ compared to HBV- recipients demonstrated no significant difference ($P = 0.445$; $P = 0.246$) (Figs 3 and 4). Cox regression analysis revealed high urgency allocation associated with decreased patient ($P = 0.046$; HR 2.93; 1.02–8.44) and graft survival ($P = 0.003$; HR 4.23; 1.62–11.05). Recipient age showed significant impact on reducing patient ($P = 0.043$; HR 1.44; 1.00 – 1.07) and graft survival ($P = 0.027$; HR 1.40; 1.00–1.06). Anti-HCV positivity was a strong indicator for worse patient ($P = 0.015$; HR 2.38; 1.18–4.78) and graft survival ($P = 0.005$; HR 2.54; 1.33–4.84). Organs from anti-HBc+ donors ($P = 0.048$; HR 2.17; 1.01–4.68) were identified as a significant predictive factor of lower graft survival (Table 3, Table 4).

Early graft losses (<1 month), not hepatitis B infection related, were higher in the anti-HBc+ group (9.6%) compared to the control group (4.9%); however, it did not reach statistical significance ($P = 0.189$). Early graft losses in the group of patients receiving grafts from anti-HBc-positive donors were due to graft rupture ($n = 1$), caval thrombosis ($n = 1$), and arterial complications ($n = 3$), and all were re-transplanted within 31 (median 12.8, range 1–31) days. Re-LT was performed in early graft losses ($n = 5$), and also due to arterial anastomosis stenosis ($n = 1$). Nineteen of 52 (36.5%) recipients died in the observation period because of infectious disease such as pneumonia, for example, Candida and sepsis ($n = 10$), *de novo* malignancies ($n = 2$), liver failure ($n = 1$), cardiac attack ($n = 1$), Non-Hodgkin's lymphoma ($n = 1$), aortic rupture ($n = 1$), and unknown reasons ($n = 3$).

Table 1. donor, recipient, and transplantation demographics from anti-HBc+ and anti-HBc− grafts in LT.

Factor	Anti-HBc neg. (n = 1013)	Nov	Anti-HBc pos. (n = 52)	Nov	P-value
Donor					
Female [%]	40.4 (409/1013)	1013	50.0 (26/52)	52	0.193
BMI [kg/m^2]	24.6 ± 3.38	987	24.7 ± 2.73	52	0.774
Age [years]	40.8 ± 16.44	1013	52.4 ± 14.21	52	<0.001
Recipient					
Female [%]	28.4 (288/1013)	1013	32.7 (17/52)	52	0.530
BMI [kg/m^2]	24.5 ± 3.80	973	25.0 ± 3.90	52	0.421
Age [years]	53.8 ± 10.65	1013	56.2 ± 9.70	52	0.131
MELD	16.6 ± 7.75	343	19.6 ± 8.57	25	0.069
Child A [%]	19.9 (68/342)	342	28.0 (7/25)	25	
Child B [%]	56.1 (192/342)	342	48.0 (12/25)	25	0.632
Child C [%]	24.0 (82/342)	342	24.0 (6/25)	25	
Acute liver failure [%]	7.8 (27/344)	344	11.5 (6/52)	52	0.708
HCC [%]	11.1 (112/1013)	1013	15.4 (8/52)	52	0.365
Re-transplantation [%]	12.9 (131/1013)	1013	17.3 (9/52)	52	0.397
Anti-HBc+ [%]	24.7 (85/344)	344	43.2 (19/44)	44	0.012
HBsAg+ [%]	7.3 (23/344)	344	12.0 (6/52)	52	0.248
Anti-HCV [%]	22.7 (78/344)	344	19.2 (10/52)	52	0.600
Transplantation					
Anhepatic time [min]	53.1 ± 15.07	343	52.1 ± 11.60	47	0.958
HTK [%]	61.0 (210/344)	344	40.0 (10/25)	25	0.059
CIP [min]	495.9 ± 167.2	830	543.7 ± 186.5	47	0.125
WIP 2 [min]	51.4 ± 19.00	796	51.2 ± 14.53	47	0.857

Nov = number of value; continuous data are shown as mean and standard deviation.

Table 2. etiology of liver disease of patients receiving anti-HBc+ liver grafts.

Acute liver failure	6
Autoimmune	1
Cryptogenic cirrhosis	3
Cryptogenic cirrhosis + HCC	2
Alcoholic liver disease	13
Alcoholic liver disease + HCC	4
Hemochromatosis	2
HBV	6
HCV	7
HCV + HCC	2
PBC	1
Polycystic liver disease	3
PSC	2

Antiviral prophylaxis

Regarding the statistical analysis of HBV infection according to prophylactic treatment regimens and state of vaccination, seven patients were excluded due to early graft loss and re-transplantation within 31 days (n = 5) or "lost to follow-up" (n = 2). The comparison of different antiviral prophylaxis on HBV infection post-transplantation did not show any significant difference (P = 0.151); however, there is a higher benefit tendency when combined prophylaxis with Tenofovir + HBIg (0%; 0/3) or Lamivudine + HBIg (15.8%; 3/19) is used compared to Lamivudine monotherapy (33.3%; 1/3), HBIg monotherapy (66.6%; 2/3), or no prophylaxis at all (38.9%; 7/18) (Fig. 5).

Shifting the focus on survival, the patients were divided according to the two eras of antiviral prophylaxis whether nRTI was applied or not. This showed significant worse 5-year patient (50.8% vs. 84.3%; log-rank P = 0.008) and 5-year graft (50.8% vs. 80.3%; log-rank P = 0.018) survival for the no nRTI group compared to the nRTI group (Figs 6 and 7).

Risk factors for HBV infection

Univariate analysis of potential risk factors for HBV infection showed anhepatic time (P = 0.010) and recipient anti-HBc positivity (P = 0.031) as significant risk factors for HBV infection with death as competing event (Table 5).

Transplant International 2016; 29: 471–482

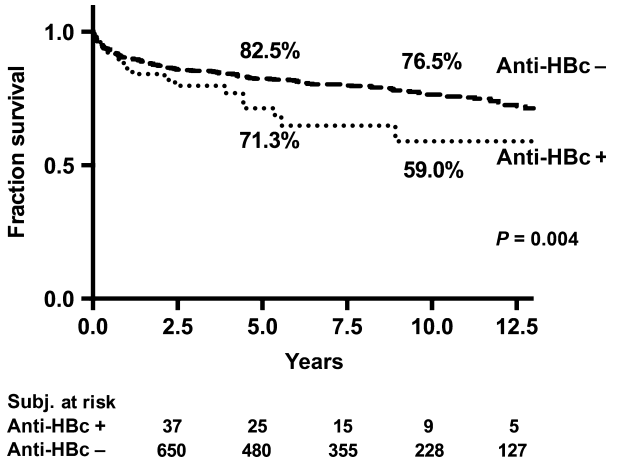

Figure 1 Five- and 10-year patient survival after LT comparing recipients with anti-HBc+ and anti-HBc− liver grafts, curve comparison (log-rank test).

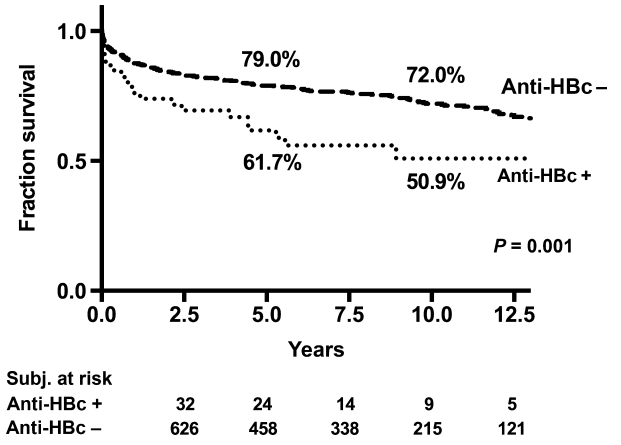

Figure 2 Five- and 10-year graft survival after LT comparing recipients with anti-HBc+ and anti-HBc− liver grafts, curve comparison (log-rank test).

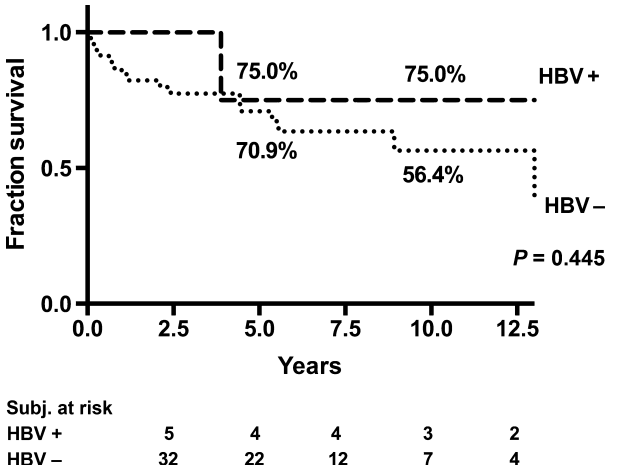

Figure 3 Five- and 10-year patient survival after LT comparing HBV+ and HBV− recipients at the time of transplantation, curve comparison (log-rank test).

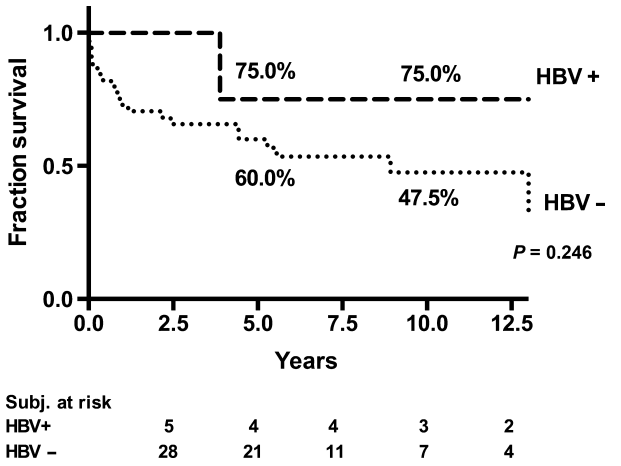

Figure 4 Five- and 10-year graft survival after LT comparing HBV+ and HBV− recipients at the time of transplantation, curve comparison (log-rank test).

Effect of anti-HBs on HBV infection and survival

Recipients with sufficient anti-HBs titer (>100 IU/l) at day of transplantation developed no HBV infection (0%; 0/7) compared to recipients with anti-HBs titer (<100 IU/l) (37.5%; 9/24) ($P = 0.076$) (Fig. 8). Ten-year patient (68.6%) and graft (51.4%) survival in recipients with anti-HBs level >100 IU/l compared to 10-year patient (63.0%) and graft (50.9%) survival in recipients with anti-HBs level <100 IU/l (log-rank $P = 0.612$, $P = 0.663$) showed no significant difference.

Effect of HBV infection on survival

The cumulative incidence of HBV infection after 1 year (10.3%) is lower compared to patient death without earlier infection or graft loss (15.2%), but becomes higher after 3 years (22.5% vs. 17.7%) (Fig. 9). Landmark analysis of 10-year adjusted (censored by death) graft survival of the recipients showed no significant difference in HBV-infected recipients (100%) compared to recipients with no HBV infection (100%, $P = 1$). This analysis showed no difference ($P = 0.126$) in 10-year patient survival between both groups (Fig. 10).

Therapy of HBV infection post-transplantation

All recipients with HBV infection received post-transplant first-line treatment with Famciclovir, Lamivudine, or Adefovir. In case of recurrence, therapy was switched

Table 3. Cox regression model for patient's death.

Equation variable	Hazard ratio	95% Confidence interval	*P*-value
Year of transplantation	1.10	0.90–1.33	0.353
Donor			
Age [per decade]	1.07	0.88–1.36	0.523
BMI [per kg/m^2]	1.07	0.98–1.16	0.122
Anti-HBc pos.	1.83	0.70–4.78	0.215
Recipient			
Age [per decade]	1.44	0.94–1.94	0.043
BMI [per kg/m^2]	0.97	0.88–1.05	0.528
HCC	1.40	0.56–3.53	0.472
Anti-HCV pos.	2.38	1.18–4.78	0.015
MELD	1.00	0.95–1.04	0.836
CIP [per hour]	1.06	0.93–1.14	0.416
WIP 2 [per min]	1.01	0.98–1.03	0.577
High urgency allocation	2.93	1.02–8.44	0.046

Table 4. Cox regression model for graft loss.

Equation variable	Hazard ratio	95% confidence interval	*P*-value
Year of transplantation	1.08	0.92–1.26	0.357
Donor			
Age [per decade]	1.11	0.90–1.36	0.299
BMI [per kg/m^2]	1.02	0.94–1.11	0.642
Anti-HBc pos.	2.17	1.01–4.68	0.048
Recipient			
Age [per decade]	1.40	0.98–1.82	0.027
BMI [per kg/m^2]	0.96	0.89–1.04	0.341
HCC	1.21	0.49–3.01	0.681
Anti-HCV pos.	2.54	1.33–4.84	0.005
MELD	1.01	0.97–1.05	0.539
CIP [per hour]	1.00	0.92–1.11	0.748
WIP 2 [per min]	1.01	0.99–1.03	0.353
High urgency allocation	4.23	1.62–11.05	0.003

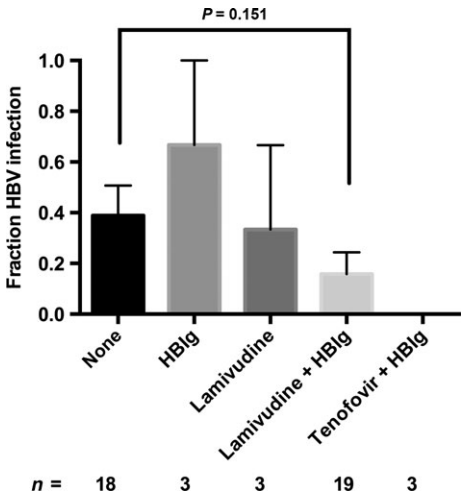

Figure 5 influence of prophylactic treatment on HBV infection (mean; SEM) after LT of anti-HBc+ grafts.

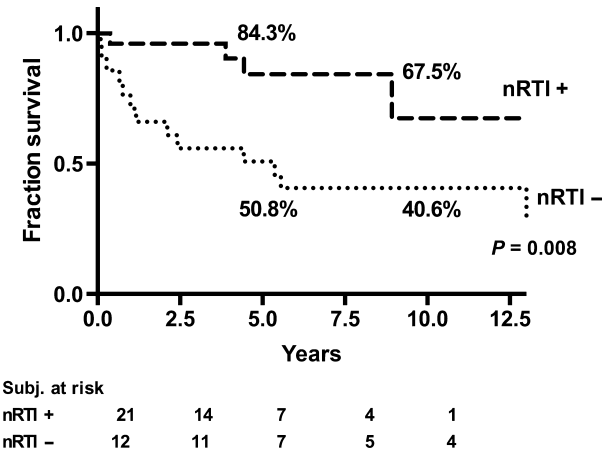

Figure 6 Five- and 10-year patient survival after LT comparing recipients with and without prophylactic treatment with nRTI, curve comparison (log-rank test).

to Tenofovir. Seven of twelve patients (58.3%) developed Lamivudine resistance during therapy after a mean of 5.1 years (range 2.3–12.0 years).

Outcome of post-transplant HBV infection

Eight of 27 anti-HBc− recipients developed post-transplant HBV infection post-LT after 2.1 years (range: 0.8–4.1). Recipients can be differently grouped based on their antiviral prophylaxis (Table 6). The first group (n = 3) did not receive any prophylaxis at all. After 1.6 years (0.9–2.2), post-transplant HBV infection occurred. The second group (n = 2) received antiviral prophylaxis with HBIg monotherapy. HBV infection first appeared after 0.9 years (0.8–1.0). The third group (n = 3) was prophylactically treated with Lamivudine and HBIg and developed HBV infection after 3.3 years (2.3–4.1) due to Lamivudine resistance in all three cases.

Discussion

Organ shortage has led us to use nonoptimal livers for transplantation. Many centers reject anti-HBc+ livers for transplantation due to concerns related with either poor organ quality [19,20] or HBV transmission [16,21]. We investigated the outcome of the use of anti-HBc+ liver grafts at our department during the last 35 years with respect to patient and graft survival, HBV

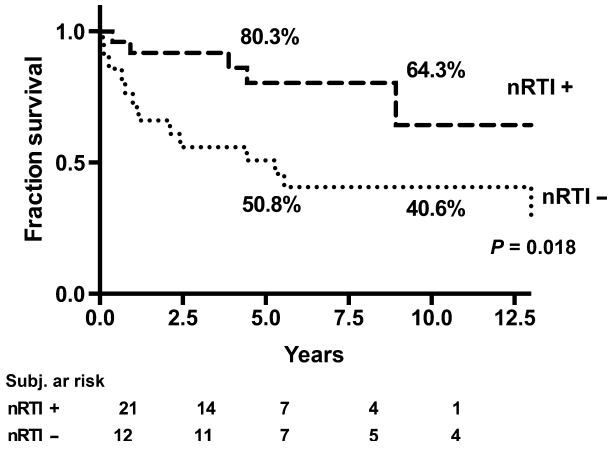

Figure 7 Five- and 10-year graft survival after LT comparing recipients with and without prophylactic treatment with nRTI, curve comparison (log-rank test).

Table 5. univariate analysis of potential risk factors in patients receiving anti-HBc+ liver grafts for HBV infection with death as competing event.

Factor	Hazard ratio (HR)	95% CI for HR	*P*-value
Donor			
Female [%]	0.51	0.16–1.58	0.244
BMI [kg/m^2]	0.96	0.75–1.21	0.710
Age [decades]	1.24	0.84–1.83	0.278
Recipient			
Female [%]	0.50	0.17–1.51	0.218
BMI [kg/m^2]	1.04	0.88–1.22	0.657
Age [decades]	1.02	0.49–2.12	0.959
MELD	1.05	0.97–1.13	0.253
Child A [%] (*reference*)	–	–	–
Child B [%]	0.32	0.03–3.33	0.342
Child C [%]	1.96	0.37–10.4	0.429
Anti-HBc+ [%]	0.20	0.05–0.87	0.031
HBsAg+ [%]	na	–	–
CMV match [%]	2.79	0.62–12.5	0.180
High urgency allocation [%]	na	–	–
Transplantation			
No prophylaxis	1.67	0.60–4.8	0.342
Anhepatic time [min]	1.13	1.03–1.23	0.010
HTK [%]	1.30	0.21–8.04	0.774
CIP [hours]	1.03	0.87–1.21	0.736
WIP 2 [min]	1.00	0.95–1.05	0.993

infection, and prophylaxis/anti-HBs status. So far, this represents the largest single-center study of long-term follow-up with a median of 6.3 years focused on anti-HBc+ grafts suitability for LT.

The use of anti-HBc+ liver grafts in HBV-naive patients applying an antiviral prophylaxis is generally accepted [22,23] with comparable patient and graft

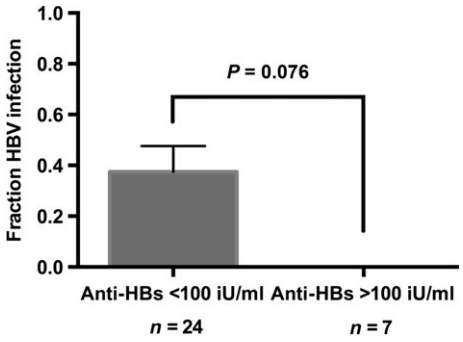

Figure 8 HBV infection post-transplant (mean; SEM) comparing recipients with anti-HBs titer above and below 100 IU/l at day of transplantation.

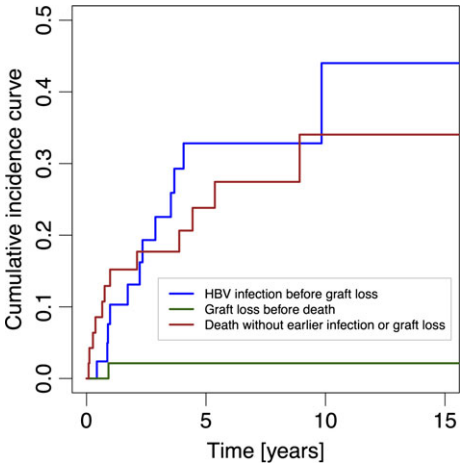

Figure 9 cumulative incidence of HBV infection before graft loss, of graft loss before death and of death (without earlier infection or graft loss).

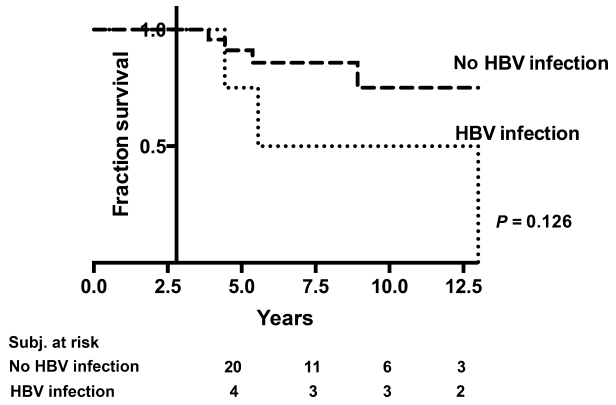

Figure 10 Ten-year patient survival after LT regarding development of HBV infection (landmark 2.8 years), curve comparison (log-rank test).

survival [7,8,24]. In our retrospective analysis, in which 18 (38.3%) patients did not receive any antiviral prophylaxis (mainly due to historical reasons), we show

Table 6. outcome of *de novo* HBV infection after LT.

Patient no.	Age at TX	Disease	HBsAg	Anti-HBs	Anti-HBc	Prophylaxis	Year of TX	HBV infection [years post-TX]	Treatment	HBsAg neg. [years post-Tx]	Present viral load[years post-Tx]	Death [years post-Tx]	Cause of death
I	56	Alcoholic cirrhosis	Neg.	<2.0	Neg.	No	2005	1,73	Lamivudine	2,32	n.d. 3,0	5,56	Liver failure
II	50	Alcoholic cirrhosis	Neg.	<2.0	Neg.	No	2007	2,22	None	–	NN	4,43	Suicide
III	62	Alcoholic cirrhosis	Neg.	<2.0	Neg.	No	2001	0,90	Lamivudine +Famciclovir	2,30	n.d. 7,52	–	–
IV	29	Alcoholic cirrhosis	Neg.	94,2	Neg.	HBIg	2003	0,87	Lamivudine +Adefovir, Tenofovir	–	6900 IU/l 1,16	1,17	Sepsis, pneumonia, recurrence of alcohol disease
V	52	Alcoholic cirrhosis	Neg.	NN	Neg.	HBIg	2000	0,99	None	–	>2 000 000 IU/l 1,53	2,42	*De novo* Npl. bronchii
VI	71	HCC in alcoholic cirrhosis	Neg.	<2.0	Neg.	Lamivudine +HBIg	2005	4,06	Tenofovir	4,98	n.d. 4,98	–	–
VII	68	Acute on chronic alcoholic cirrhosis	Neg.	48,7	Neg.	Lamivudine +HBIg	2009	2,34	Tenofovir	2,84	n.d. 2,58	–	–
VIII	65	Polycystic liver disease	Neg.	NN	Neg.	Lamivudine +HBIg	2003	3,53	Tenofovir	–	30 IU/l 6,97	–	–

significantly worse 10-year patient and graft survival (Figs 1 and 2). Inferior graft survival of anti-HB+ organs is confirmed by Cox regression and multivariate regression analysis. Early graft loss occurred twice as often in recipients with anti-HBc+ grafts, although not reaching statistical significance due to sample size and its associated causes with arterial complications, vena cava thrombosis, and graft rupture. A possible explanation is the significant lower donor age for anti-HBc− liver graft recipients. HCV-positive recipients showed inferior patient and graft survival in Cox regression analysis due to high rate of HCV recurrence [25] leading to cirrhosis within 5 years after LT in 20–30% of patients [26], and hence confirming previously published studies [27–30]. High urgency allocation has a negative impact on both patient and graft survival and could be explained by the poor condition of the recipients and the acceptance of nonoptimal liver grafts in a time pressure situation. Fruehauf *et al.* [31] confirmed this result in a nationwide study in 2011. While anti-HBc+ liver grafts seem to have inferior graft quality [32] due to chronic hepatitis B infection [10], post-transplant HBV infection is not influencing graft survival. It still remains unclear through which mechanism the suboptimal graft quality can be explained. Further histologic examinations of these grafts might be promising. We suspect that the chronic damage of the liver graft might be the leading cause of worse hepatocyte performance. We consider this observation of great importance, given that no long-term studies have shown so far a similar risk in addition to the increased morbidity. Anti-HBc+ liver grafts are carrying the risk of potential development of *de novo* HBV infection in anti-HBc− recipients due to latent virus or low-level replication that can be reactivated in the setting of immunosuppression [11,12,33]. Therefore, it is important to know whether the donor presents an active HBV infection. In routine, both HBsAg and anti-HBc status should be mentioned in the donor report. Nevertheless, a recent study supports the relevance of occult hepatitis B infection in these grafts, which should be considered showing prevalence of 60% [34].

None of the patients receiving an anti-HBc-positive graft who had an anti-HBs titer >100 IU/l at the time of LT developed an HBV infection during the observation period. This finding is similar to previous multicenter studies results [35], although there are studies demonstrating that there is no elimination of the risk of developing HBV in patients receiving anti-HBc+ livers [17,36]. If the center's organ allocation policy allows, anti-HBc− recipients could be chosen, and those should present sufficient anti-HBs levels. In these cases, vaccination of anti-HBc− recipients prior to wait-listing could be useful to prevent post-transplant HBV infection. There is evidence, however, showing that vaccination might be inefficient [37], due to increasing anergy related with liver disease progression.

Overall incidence of post-transplant HBV infection (25.0%) is higher in our series compared to previously reported studies [22,38]. Cholongitas *et al.*, [22] described that *de novo* HBV infection was observed in 3.0% of anti-HBc− recipients (1/33) who received Lamivudine mono-prophylaxis and in 0% of anti-HBc−/anti-HBs+ recipients (0/17) with a median follow-up of 25 months; Skagen *et al.* described a rate of 11% (8/73) and 2% (1/44), respectively. The higher incidence of post-transplant HBV infection may be explained by the high rate (38.3%) (18/47) of recipients, who did not receive antiviral prophylaxis, either because they were transplanted during the 1990s when no antiviral drugs was yet available, or was not applied. The higher incidence of post-transplant HBV infection may in addition be explained by the substantially longer observational period of 56 months compared to previous studies [22,38]. In our study, in the cohort of recipients of anti-HBc+ grafts, Lamivudine resistance occurred in 60% (3/5) of the recipients receiving Lamivudine mono-prophylaxis after a median of 57.6 months (range 4.8–129.6 months). The patient and graft survival rate in our study showed beneficial effect for antiviral prophylaxis including nRTI. In regard to the antiviral prophylaxis, the combination of Lamivudine or Tenofovir with HBIg might be recommended, as it has shown the lowest HBV infection rates, even though no statistical significance was reached due to small case numbers. This observation is in accordance with previous results published to date [7,39]. Nevertheless, further multicenter studies are needed to evaluate the ideal prophylaxis involving Tenofovir instead of Lamivudine with or without the use of HBIg to prevent HBV infection post-LT.

The WIP2 in this study is for both groups similar, so the anhepatic time was mainly prolonged due to technical issues, for example, hemostasis, before the graft transplantation. Ijtsma *et al.* [40] showed lower 1-year graft survival in those recipients with anhepatic time >100 min. An explanation for the deleterious effect of a prolonged anhepatic phase could be, in addition to the acute elimination of hepatic metabolism, the hemodynamic changes associated with the interrupted portal

venous system in the absence of a venovenous bypass. During this phase, cytokines, metabolites, and other toxins accumulate in the splanchnic system. Previous studies addressing the topic of serum cytokine levels during LT have shown the anhepatic phase to mark the start of a sharp increase in the recipient serum interleukin-6 (IL-6) level to over 100 times the levels measured in control patients [41–43]. Experimental studies have indicated deteriorated ischemia/reperfusion injury with an increased transcription rate of cytokines such as IL-6, IL-10, tumor necrosis factor, transforming factor, and macrophage inflammatory protein 1 in liver tissue samples. The recipient serum IL-6 level has also been shown to be a marker for hemodynamic performance during LT [44] or a predictive factor for complications after LT [45,46]. A correlation between up-regulation of cytokines and post-transplant hepatitis B was not shown so far.

Twelve patients who developed post-transplant HBV infection were primarily treated with Lamivudine; however, 58.3% (7/12) developed Lamivudine resistance after a median of 5.1 years. Although Lamivudine resistance has been previously reported to be lower than in our study [47,48], it is also recognized that resistance is increasing along the time on therapy [49]. The late onset observed in our cohort underlines the importance of periodic lifespan surveillance. Ideally, surveillance should be performed periodically (3–6 months) including HBsAg and HBV DNA, as normal liver function tests (LFTs) do not exclude the possibility of HBV reactivation. Regarding HBV infection, Tenofovir treatment showed to be the most effective drug in our analysis; however, due to the fact that Tenofovir was only recently available, the number of patients on therapy was very low ($n = 3$) and within a short observation period. Superiority of Tenofovir has been also described in post-LT cohorts [50] and non-LT cohorts [51]. Once the occurrence of HBV is confirmed and considering that spontaneous resolution is unlikely [17,41,42], the treatment should be initiated to prevent graft damage. Although some centers opt to treat post-transplant HBV infection only when LFTs are elevated [52], we have chosen to treat HBV DNA positivity, achieving an excellent adjusted graft survival after 10 years, with no significant difference to grafts without HBV infection. Changes in immunosuppressive therapy are usually not required, although the discontinuation of steroids, if it is possible without compromising immunosuppression efficacy seems to be reasonable, considering the presence of a glucocorticoid-sensitive enhancer in HBV DNA and its effect on virus [53]. In patients with no prophylaxis or poor compliance, the treatment with Lamivudine was started with a good response in terms of HBV DNA clearance, although the introduction of a second agent was sometimes necessary. To avoid Lamivudine resistance, we recommend the usage of Tenofovir or Entecavir in compliant patients, justified by their antiviral potency, resistance profile and minimal nephrotoxicity.

One of the limitations of this study is the heterogeneous collective of groups receiving or not receiving antiviral prophylaxis that leads to smaller subgroups of patients and therefore weakens statistical tests. Another weakness is the number of missing data for the multivariate model. Nevertheless, this study reflects the use of anti-HBc+ grafts in clinical practice during the last 35 years and is due to the consideration of the authors valuable to show worse graft survival and underline the importance of patient surveillance.

In summary, based on our results and the evidence published so far, anti-HBc+ liver grafts show worse graft survival after 10 years compared to anti-HBc− grafts. However, HBV infection, if treated, has no deleterious effect on 10-year patient and graft survival. Hence, we suggest to primarily allocate these grafts to patients who are HBsAg+ or anti-HBc+, and in case that no such patient is on the waiting list, to immunized recipients with an anti-HBs titer >100 IU/ml, who also seem to benefit. Even more important, prophylaxis with Lamivudine or Tenofovir should be carried out in any case. Surveillance and compliance are both fundamental for early diagnosis of HBV infection and timely detection of Lamivudine resistance. Adequate treatment of HBV infection leads to reasonable long-term graft and patient survival rates and allows an effective usage of anti-HBC+ liver grafts, considered therefore to be suitable for LT. The mechanism for inferior graft quality still remains unclear, and further studies evaluating the graft quality are, however, necessary.

Authorship

AB: participated in data acquisition, data analysis, interpretation and writing the article. PS: participated in data acquisition and data analysis. SE, FA and SW: participated in data acquisition. WV, AK and SN: participated in data analysis and interpretation. JP: participated in data analysis, interpretation and revised the manuscript. IG and RÖ: participated in research design and supervision and revised the manuscript critically.

Funding

The authors have no financial relationships to disclose.

Conflicts of interest

The authors have no conflict of interests.

REFERENCES

1. Bernat JL, D'Alessandro AM, Port FK, *et al.* Report of a National Conference on Donation after cardiac death. *Am J Transplant* 2006; **6**: 281.
2. Busuttil RW, Tanaka K. The utility of marginal donors in liver transplantation. *Liver Transpl* 2003; **9**: 651.
3. Cuende N, Grande L, Sanjuan F, Cuervas-Mons V. Liver transplant with organs from elderly donors: Spanish experience with more than 300 liver donors over 70 years of age. *Transplantation* 2002; **73**: 1360.
4. Feng S, Goodrich NP, Bragg-Gresham JL, *et al.* Characteristics associated with liver graft failure: the concept of a donor risk index. *Am J Transplant* 2006; **6**: 783.
5. Foley DP, Fernandez LA, Leverson G, *et al.* Donation after cardiac death: the University of Wisconsin experience with liver transplantation. *Ann Surg* 2005; **242**: 724.
6. Nadig SN, Bratton CF, Karp SJ. Marginal donors in liver transplantation: expanding the donor pool. *J Surg Educ* 2007; **64**: 46.
7. Bohorquez HE, Cohen AJ, Girgrah N, *et al.* Liver transplantation in hepatitis B core-negative recipients using livers from hepatitis B core-positive donors: a 13-year experience. *Liver Transpl* 2013; **19**: 611.
8. Marciano S, Gaite LA, Bisignano L, *et al.* Use of liver grafts from anti-hepatitis B core-positive donors: a multicenter study in Argentina. *Transplant Proc* 2013; **45**: 1331.
9. Cholongitas E, Vasiliadis T, Antoniadis N, Goulis I, Papanikolaou V, Akriviadis E. Hepatitis B prophylaxis post liver transplantation with newer nucleos(t)ide analogues after hepatitis B immunoglobulin discontinuation. *Transpl Infect Dis* 2012; **14**: 479.
10. Liaw YF, Chu CM. Hepatitis B virus infection. *Lancet* 2009; **373**: 582.
11. Marusawa H, Uemoto S, Hijikata M, *et al.* Latent hepatitis B virus infection in healthy individuals with antibodies to hepatitis B core antigen. *Hepatology* 2000; **31**: 488.
12. Mason AL, Xu L, Guo L, Kuhns M, Perrillo RP. Molecular basis for persistent hepatitis B virus infection in the liver after clearance of serum hepatitis B surface antigen. *Hepatology* 1998; **27**: 1736.
13. Scullard GH, Smith CI, Merigan TC, Robinson WS, Gregory PB. Effects of immunosuppressive therapy on viral markers in chronic active hepatitis B. *Gastroenterology* 1981; **81**: 987.
14. Jilg W, Sieger E, Zachoval R, Schatzl H. Individuals with antibodies against hepatitis B core antigen as the only serological marker for hepatitis B infection: high percentage of carriers of hepatitis B and C virus. *J Hepatol* 1995; **23**: 14.
15. Rokuhara A, Tanaka E, Yagi S, *et al. De novo* infection of hepatitis B virus in patients with orthotopic liver transplantation: analysis by determining complete sequence of the genome. *J Med Virol* 2000; **62**: 471.
16. Burton JR Jr, Shaw-Stiffel TA. Use of hepatitis B core antibody-positive donors in recipients without evidence of hepatitis B infection: a survey of current practice in the United States. *Liver Transpl* 2003; **9**: 837.
17. Perrillo R. Hepatitis B virus prevention strategies for antibody to hepatitis B core antigen-positive liver donation: a survey of North American, European, and Asian-Pacific transplant programs. *Liver Transpl* 2009; **15**: 223.
18. Takaki A, Yagi T, Yasunaka T, *et al.* Which patients respond best to hepatitis B vaccination after a hepatitis B virus-related liver transplantation? *J Gastroenterol* 2013; **48**: 1373.
19. Ganem D, Prince AM. Hepatitis B virus infection–natural history and clinical consequences. *N Engl J Med* 2004; **350**: 1118.
20. Lai JC, Feng S, Roberts JP. An examination of liver offers to candidates on the liver transplant wait-list. *Gastroenterology* 2012; **143**: 1261.
21. Chang MS, Olsen SK, Pichardo EM, *et al.* Prevention of *de novo* hepatitis B in recipients of core antibody-positive livers with lamivudine and other nucleos(t)ides: a 12-year experience. *Transplantation* 2013; **95**: 960.
22. Cholongitas E, Papatheodoridis GV, Burroughs AK. Liver grafts from anti-hepatitis B core positive donors: a systematic review. *J Hepatol* 2010; **52**: 272.
23. Avolio AW, Halldorson JB, Burra P, Dutkowski P, Agnes S, Clavien PA. Balancing utility and need by means of donor-to-recipient matching: a challenging problem. *Am J Transplant* 2013; **13**: 522.
24. Avolio AWA S, Sallizoni M, Pinna A, *et al.* Use of HBcAb positive liver grafts. Medium term results according to donor-recipient risk factors. *Liver Transpl* 2011; **17**: S202.
25. Coilly A, Roche B, Samuel D. Current management and perspectives for HCV recurrence after liver transplantation. *Liver Int* 2013; **33**(Suppl. 1): 56.
26. Gane EJ. The natural history of recurrent hepatitis C and what influences this. *Liver Transpl* 2008; **14** (Suppl. 2): S36.
27. Forman LM, Lewis JD, Berlin JA, Feldman HI, Lucey MR. The association between hepatitis C infection and survival after orthotopic liver transplantation. *Gastroenterology* 2002; **122**: 889.
28. Neumann UP, Berg T, Bahra M, *et al.* Long-term outcome of liver transplants for chronic hepatitis C: a 10-year follow-up. *Transplantation* 2004; **77**: 226.
29. Berenguer M, Prieto M, San Juan F, *et al.* Contribution of donor age to the recent decrease in patient survival among HCV-infected liver transplant recipients. *Hepatology* 2002; **36**: 202.
30. Ravaioli M, Grazi GL, Dazzi A, *et al.* Survival benefit after liver transplantation: a single European center experience. *Transplantation* 2009; **88**: 826.
31. Fruhauf NR, Fischer-Frohlich CL, Kutschmann M, Schmidtmann I, Kirste G. Joint impact of donor and recipient parameters on the outcome of liver transplantation in Germany. *Transplantation* 2011; **92**: 1378.
32. Angelico M, Nardi A, Marianelli T, *et al.* Hepatitis B-core antibody positive donors in liver transplantation and their impact on graft survival: evidence from the Liver Match cohort study. *J Hepatol* 2013; **58**: 715.
33. Michalak TI, Pasquinelli C, Guilhot S, Chisari FV. Hepatitis B virus persistence after recovery from acute viral hepatitis. *J Clin Invest* 1994; **93**: 230.

34. Tandoi F, Caviglia GP, Pittaluga F, *et al.* Prediction of occult hepatitis B virus infection in liver transplant donors through hepatitis B virus blood markers. *Dig Liver Dis* 2014; **46**: 1020.
35. Yu L, Koepsell T, Manhart L, Ioannou G. Survival after orthotopic liver transplantation: the impact of antibody against hepatitis B core antigen in the donor. *Liver Transpl* 2009; **15**: 1343.
36. Loss GE Jr, Mason AL, Nair S, *et al.* Does lamivudine prophylaxis eradicate persistent HBV DNA from allografts derived from anti-HBc-positive donors? *Liver Transpl* 2003; **9**: 1258.
37. Rosenau J, Hooman N, Hadem J, *et al.* Failure of hepatitis B vaccination with conventional HBsAg vaccine in patients with continuous HBIG prophylaxis after liver transplantation. *Liver Transpl* 2007; **13**: 367.
38. Skagen CL, Jou JH, Said A. Risk of *de novo* hepatitis in liver recipients from hepatitis-B core antibody-positive grafts – a systematic analysis. *Clin Transpl* 2011; **25**: E243.
39. Cholongitas E, Papatheodoridis GV. High genetic barrier nucleos(t)ide analogue(s) for prophylaxis from hepatitis B virus recurrence after liver transplantation: a systematic review. *Am J Transplant* 2013; **13**: 353.
40. Ijtsma AJ, van der Hilst CS, de Boer MT, *et al.* The clinical relevance of the anhepatic phase during liver transplantation. *Liver Transpl* 2009; **15**: 1050.
41. Boros P, Suehiro T, Curtiss S, *et al.* Differential contribution of graft and recipient to perioperative TNF-alpha, IL-1 beta, IL-6 and IL-8 levels and correlation with early graft function in clinical liver transplantation. *Clin Transpl* 1997; **11**: 588.
42. Faybik P, Hetz H, Krenn CG, Baker A, Berlakovich GA, Steltzer H. Perioperative cytokines during orthotopic liver transplantation without venovenous bypass. *Transplant Proc* 2003; **35**: 3019.
43. Santiago F, Bueno P, Olmedo C, *et al.* Time course of intraoperative cytokine levels in liver transplant recipients. *Transplant Proc* 2006; **38**: 2492.
44. Arranz J, Soriano A, Garcia I, *et al.* Effect of proinflammatory cytokines (IL-6, TNF-alpha, IL-1beta) on hemodynamic performance during orthotopic liver transplantation. *Transplant Proc* 2003; **35**: 1884.
45. Hassan L, Bueno P, Ferron-Celma I, *et al.* Early postoperative response of cytokines in liver transplant recipients. *Transplant Proc* 2006; **38**: 2488.
46. Weiss S, Kotsch K, Francuski M, *et al.* Brain death activates donor organs and is associated with a worse I/R injury after liver transplantation. *Am J Transplant* 2007; **7**: 1584.
47. Jain A, Orloff M, Abt P, *et al.* Use of hepatitis B core antibody-positive liver allograft in hepatitis C virus-positive and -negative recipients with use of short course of hepatitis B immunoglobulin and Lamivudine. *Transplant Proc* 2005; **37**: 3187.
48. Yen RD, Bonatti H, Mendez J, Aranda-Michel J, Satyanarayana R, Dickson RC. Case report of lamivudine-resistant hepatitis B virus infection post liver transplantation from a hepatitis B core antibody donor. *Am J Transplant* 2006; **6**(5 Pt 1): 1077.
49. Fabrizi F, Dulai G, Dixit V, Bunnapradist S, Martin P. Lamivudine for the treatment of hepatitis B virus-related liver disease after renal transplantation: meta-analysis of clinical trials. *Transplantation* 2004; **77**: 859.
50. Tanaka T, Renner EL, Selzner N, Therapondos G, Lilly LB. One year of hepatitis B immunoglobulin plus tenofovir therapy is safe and effective in preventing recurrent hepatitis B post-liver transplantation. *Can J Gastroenterol Hepatol* 2014; **28**: 41.
51. Ceylan B, Yardimci C, Fincanci M, *et al.* Comparison of tenofovir and entecavir in patients with chronic HBV infection. *Eur Rev Med Pharmacol Sci* 2013; **17**: 2467.
52. Kim HY, Choi JY, Park CH, *et al.* Adult living donor liver transplantation using hepatitis B core antibody-positive grafts in Korea, a hepatitis B-endemic region. *Gut Liv* 2011; **5**: 363.
53. Tur-Kaspa R, Burk RD, Shaul Y, Shafritz DA. Hepatitis B virus DNA contains a glucocorticoid-responsive element. *Proc Natl Acad Sci USA* 1986; **83**: 1627.