

Hepatitis B-core antibody positive donors in liver transplantation and their impact on graft survival: Evidence from the Liver Match cohort study

Mario Angelico^{1,12,*}, Alessandra Nardi², Tania Marianelli^{1,12}, Lucio Caccamo^{3,12}, Renato Romagnoli^{4,12}, Giuseppe Tisone¹, Antonio D. Pinna⁵, Alfonso W. Avolio^{6,12}, Stefano Fagioli^{7,12}, Patrizia Burra^{8,12}, Mario Strazzabosco^{9,10,12}, Alessandro Nanni Costa¹¹,
 For the Liver Match Investigators[†]

¹Liver Unit, Tor Vergata University, Rome, Italy; ²Dept. Mathematics, Tor Vergata University, Rome, Italy; ³Fondazione IRCCS Osp. Maggiore Policlinico, Milan, Italy; ⁴Centro Trapianti di Fegato, Azienda San Giovanni Battista, Turin, Italy; ⁵Centro Trapianti di Fegato, Università di Bologna, Bologna, Italy; ⁶Centro Trapianti di Fegato, Università Cattolica del Sacro Cuore, Rome, Italy; ⁷Centro Trapianti di Fegato, Ospedali Riuniti, Bergamo, Italy; ⁸Unità di Epatologia e Trapianto, Università di Padova, Padua, Italy; ⁹Digestive Disease Section, University of Milan Bicocca, Milan, Italy; ¹⁰Yale University Liver Center, New Haven, USA; ¹¹Centro Nazionale Trapianti, Rome, Italy; ¹²Italian Association for the Study of the Liver (AISF), Rome, Italy

Background & Aims: The appropriate allocation of grafts from HBcAb positive donors in liver transplantation is crucial, yet a consensus is still lacking.

Methods: We evaluated this issue within Liver Match, a prospective observational Italian study. Data from 1437 consecutive, first transplants performed in 2007–2009 using grafts from deceased heart beating donors were analyzed (median follow-up: 1040 days). Of these, 219 (15.2%) were HBcAb positive. Sixty-six HBcAb positive grafts were allocated to HBsAg positive and 153 to HBsAg negative recipients.

Results: 329 graft losses occurred (22.9%): 66 (30.1%) among 219 recipients of HBcAb positive grafts, and 263 (21.6%) among 1218 recipients of HBcAb negative grafts. Graft survival was lower in recipients of HBcAb positive compared to HBcAb negative donors, with unadjusted 3-year graft survival of 0.69 (s.e. 0.032) and 0.77 (0.013), respectively (log-rank, $p = 0.0047$). After stratifying for recipient HBsAg status, this difference was only observed among HBsAg negative recipients (log rank, $p = 0.0007$), 3-year graft

survival being excellent (0.88, s.e. 0.020) among HBsAg positive recipients, regardless of the HBcAb donor status (log rank, $p = 0.4478$). Graft loss due to *de novo* HBV hepatitis occurred only in one patient. At Cox regression, hazard ratios for graft loss were: MELD (1.30 per 10 units, $p = 0.0002$), donor HBcAb positivity (1.56, $p = 0.0015$), recipient HBsAg positivity (0.43, $p < 0.0001$), portal vein thrombosis (1.99, $p = 0.0156$), and DRI (1.41 per unit, $p = 0.0325$).

Conclusions: HBcAb positive donor grafts have better outcomes when transplanted into HBsAg positive than HBsAg negative recipients. These findings suggest that donor HBcAb positivity requires more stringent allocation strategies.

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Introduction

The scarcity of organs available for liver transplantation (LT) has promoted the use of expanded criteria donor (ECD) liver grafts [1,2]. The risk associated with the use of ECD organs is currently estimated through the Donor Risk Index (DRI) [3], a United States-validated predictive model based on several donor-related variables.

In many countries, a relevant proportion of donors have been previously exposed to hepatitis B virus (HBV) infection, but the potential risk associated with their use is not accounted for by the DRI or the Eurotransplant DRI [4], a recent European validation of DRI. Despite the absence of serological markers of active HBV infection, livers harvested from donors positive for hepatitis B core antibodies (HBcAb), may retain covalently closed circular DNA and pre-genomic RNA in hepatocyte nuclei, and may reactivate HBV infection [5]. The prevalence of HBcAb positive donors varies worldwide, reflecting the burden of HBV infection over the

Keywords: Donor-recipient matching; HBcAb positive donors; *De novo* HBV infection; Donor Risk Index.

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* Corresponding author. Address: Hepatology and Liver Transplantation Unit, Dept. of Experimental Medicine and Surgery, Tor Vergata University, Via Montpellier 1, 00133 Rome, Italy. Tel.: +39 0672596801; fax: +39 0672596711. E-mail address: angelico@med.uniroma2.it (M. Angelico).

[†] For the composition of the Liver Match study group see Appendix.

Abbreviations: LT, liver transplantation; ECD, extended criteria donors; DRI, Donor Risk Index; HBV, hepatitis B virus; HBcAb, hepatitis B core antibody; HBsAg, hepatitis B surface antigen; HbIg, hepatitis B immunoglobulins; HBsAb, hepatitis B surface antibody; UNOS, United Network for Organ Sharing; HR, hazard ratio; US, United States; AISF, Italian Association for the Study of the Liver; CNT, Italian National Transplant Center; BMI, body mass index; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; MELD, Model for End-Stage Liver Disease; NUC, nucleos(t)ide analogues; ELTR, European Liver Transplant Registry.



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last decades [6]. In the Mediterranean area, higher rates are found among subjects older than 50 years, currently representing the majority of donors [7].

HBcAb positive grafts retain the capability to transmit HBV infection when transplanted to hepatitis B surface antigen (HBsAg) negative LT recipients [8–11]. Indeed, *de novo* HBV infections after LT mainly occur in recipients of HBcAb positive donors [12]. Several LT programs did not transplant HBcAb positive grafts into recipients without evidence of previous HBV exposure, with the consequent loss of potentially successful organs [13]. The risk of HBV transmission from HBcAb positive donors primarily depends on recipients' HBV immune status and the adoption of effective prophylactic strategies. To prevent HBV transmission, most centers have used hepatitis B immunoglobulins (HBIG), given indefinitely following LT, with or without oral antivirals [14]. This expensive practice has been the focus of four recent systematic reviews [15–18]. All suggested that grafts from HBcAb positive donors can be used safely when allocated to HBsAg positive recipients, or to HBcAb/hepatitis B surface antibody (HBsAg) positive recipients receiving appropriate prophylaxis. All agreed that the risk of *de novo* hepatitis is highest for unprotected HBV naïve recipients. Yet, one paper [15] argued that HBsAb positive recipients may not need prophylaxis at all, and another [16] that published studies do not support the view that HBIG and lamivudine combination may be better than lamivudine mono-prophylaxis. Notably, all systematic reviews emphasized the limitations precluding formal recommendation and the need for larger studies with extended follow-up. A similar conclusion was reached by a survey on HBV prevention after HBcAb positive liver donation in North American, European and Asian-Pacific transplant programs, underlying the need to establish an international registry of outcomes using different prophylactic strategies [14].

Few attempts to estimate the actual risk of graft loss associated with the use of grafts from HBcAb positive donors have been made. A multicenter survey before the advent of lamivudine showed that the use of these organs was associated with a decreased 4-year survival [10], though this result was not confirmed in single-center European series [10,19]. The largest study performed so far [20] analyzed the 1994–2006 United Network for Organ Sharing (UNOS) database and found that recipients of HBcAb positive donor grafts had significantly higher unadjusted graft loss (hazard ratio [HR]: 1.35) compared to recipients of HBcAb negative donors. However, this significance disappeared after adjusting for a few covariates.

In the present study, we analyzed the Italian Liver Match prospective database on LT to investigate the impact of HBcAb positive donor graft allocation on graft survival, with special attention to donor-recipient matching.

Materials and methods

Liver Match is a prospective observational cohort study underway in Italy, promoted by the Italian Association for the Study of the Liver (AISF) and the Italian National Transplant Center (CNT). The study protocol was approved by ethical committees of participating centres in the early 2007. Patients were entered in the study from June 1, 2007 to May 31, 2009. Data were entered in the web-based CNT electronic network by trained data managers and adjusted for errors or incompleteness by the coordinating team. Missing data and inconsistencies were reconciled through specific queries. The study was overseen by a Steering Committee.

Study population, data collection and quality assessment

All consecutive LTs performed during the recruitment period at 20 out of 21 Italian Transplant Centers (roughly 90% of the adult LT activity in the country) were recruited. Two patients lost at follow-up after discharge, pediatric LTs, re-transplantations, and LTs due to fulminant hepatic failure were excluded. LTs performed using HBsAg positive and HCV positive donors were also excluded. Thus, the study cohort included 1437 elective adult first LTs (including 48 combined liver-kidney transplants) performed using grafts from deceased heart beating donors, 219 (15.2%) of whom were HBcAb positive.

Data were recorded prospectively and included a variety of donor, recipient and operative and perioperative data, already described in detail [7]. For the purpose of this study, relevant donor data were: age, gender, height, cause of death, DRI, weight, body mass index (BMI), serum biochemistry, history of insulin-dependent diabetes mellitus, serum markers of previous or current exposure to HBV and hepatitis C virus (HCV). Recipient data relevant to this study were: age, gender, height, weight, BMI, etiology of liver disease, hepatocellular carcinoma (HCC), disease severity based on the Model for End Stage Liver Disease (MELD) scores, history of insulin-dependent diabetes mellitus, markers of previous or current exposure to HBV, HCV, and portal vein thrombosis. Operative and perioperative data relevant to this study included the use of split or partial livers and cold ischemia time.

Follow-up data were collected electronically, every 3–6 months during the first year and then yearly. The main outcome measure was graft survival time. Causes of graft loss were recorded according to the European Liver Transplant Registry (ELTR) criteria. Recurrent or *de novo* HBV infection was defined as HBsAg and/or HBV DNA serum positivity at any time after LT. At time of data analysis, the median follow-up was of 1035 days.

Database completeness and overall consistency were verified through quarterly investigator meetings and frequent web meetings among study investigators. This close monitoring allowed minimization of inconsistencies and missing data.

Statistical analysis

After completion of the verification process, the database was transferred to the biostatistical board at the Department of Mathematics, Tor Vergata University, whose members were blinded with respect to the identity of Transplant Centers and individual patients. DRI was computed assuming that local and regional US sharings were equivalent to regional and national sharings in Italy, respectively [3]. Since several continuous covariates showed a skew distribution, which significantly departed from the normal density, median, first and third quartiles were used as summary statistics; categorical variables were described by absolute and relative frequencies. Associations between categorical variables were evaluated by Chi-square test; Fisher exact test was preferred in case of sparse tables. Continuous covariates were compared by *t*-test or Wilcoxon rank-sum test, when a significant departure from normality was detected.

As a preliminary step for the multivariate analysis, the marginal effect of donor and recipient characteristics was evaluated by stratified Kaplan Meier survival curves. The log-rank test was used for comparison. Multivariate analysis was based on the Cox model [21] and all recorded variables were initially included. Predictors of graft loss were identified by a non-automate backward selection, taking correlation structure among covariates and clinical interpretation of their effects into account. Plots of martingale residuals vs. covariate values were used to detect non-linear effects of continuous variables [22]. Possible time dependent effects were evaluated by plots and test statistics based on Schoenfeld residuals [23,24]. Once detected, the time dependent effect was modelled extending the Cox model, with the introduction of cubic spline functions [25].

Results

Previous exposure to HBV infection and organ allocation

The general characteristics of donors and recipients have been described elsewhere [7]. The study cohort included 219 HBcAb positive and 1218 HBcAb negative donors, whose features are summarized in Table 1A. HBcAb positive donors were significantly older than HBcAb negative donors ($p < 0.0001$), had a lower height ($p = 0.0509$), and a higher BMI ($p = 0.0171$). They also

Table 1. Main characteristics of donor and recipients as related to HBcAb donor status. (A) Donors characteristics; (B) recipients characteristics.

A

Donor features	HBcAb positive donors (n = 219)		HBcAb negative donors (n = 1218)		p value
	Median or n	Q1-Q3 or %	Median or n	Q1-Q3 or %	
Age (yr)	60	52-71	54	38-67	<0.0001
Male sex	124	56.62	678	55.67	0.7931
Cause of death					0.1266
Trauma	45	20.55	330	27.09	
Anoxia	9	4.11	57	4.68	
Cerebral haemorrhage	141	64.38	682	55.99	
Other	24	10.96	149	12.24	
Height (cm)	168	160-175	170	162-175	0.0509
BMI	25.7	23.4-27.8	24.9	22.9-27.3	0.0171
Sodium	149	143-155	148	142-154	0.0981
Split liver*	2	0.91	80	6.57	0.0009
Cold ischemia time (h)	7.1	5.8-8.3	7.3	6.0-8.8	0.0959
DRI	1.65	1.41-1.87	1.55	1.24-1.82	0.0004

B

Recipient features	HBcAb positive donors (n = 219)		HBcAb negative donors (n = 1218)		p value
	Median or n	Q1-Q3 or %	Median or n	Q1-Q3 or %	
Age (yr)	55	49-61	54	47-60	0.0375
Male sex	175	79.91	950	78.00	0.5275
Portal vein thrombosis	6	2.74	29	2.38	0.7512
BMI	25.1	23.2-27.7	25.0	22.9-27.6	0.4523
Serum creatinine	1.00	0.80-1.20	1.00	0.80-1.10	0.4656
Serum bilirubin	2.50	1.20-5.30	2.59	1.30-5.70	0.3602
INR	1.40	1.20-1.70	1.40	1.20-1.70	0.5675
MELD LT	15	10-21	15	11-21	0.6915
HCC	101	46.12	546	44.83	0.7237
HCV positive	110	50.23	558	45.81	0.2278
HBsAg positive	66	30.14	253	20.77	0.0021
Alcoholic cirrhosis	26	11.87	216	17.73	0.0328
Diabetes	42	19.53	174	15.06	0.0987

*According to the current recommendations of the Italian National Transplant Center, HBcAb positive donor grafts are not split and allocated to elective pediatric recipients.

underwent fewer split procedures ($p = 0.0009$), consistent with the current recommendation in Italy to avoid HBcAb positive grafts allocation to pediatric recipients. The DRI was significantly higher in HBcAb positive than in HBcAb negative donors (median DRI: 1.65 vs. 1.55, $p = 0.0004$). As shown in Table 1B, recipients of HBcAb positive donor grafts were slightly older than recipients of HBcAb negative grafts.

The Liver Match cohort comprised 319 (22.2%) HBsAg positive and 1118 HBsAg negative recipients. Briefly, HBsAg positive LT recipients received grafts from older donors compared to HBsAg negative recipients (median age 57 vs. 55 years, respectively, $p = 0.0052$), with a higher DRI (median: 1.61 vs. 1.56, $p = 0.0170$) and a higher BMI (Table 2A). HBsAg positive recipients had a significantly lower MELD score ($p < 0.0001$), a higher prevalence of HCC and a lower prevalence of HCV co-infection ($p < 0.0001$) compared to HBsAg negative recipients (Table 2B).

The group of HBsAg negative LT recipients comprised 347 HBsAb positive patients of whom 220 were HBcAb positive and 127 were HBcAb negative, 198 HBcAb positive/HBsAb negative patients, and 563 HBsAb and HBcAb negative patients (10 HBsAb data were missing).

As shown in Fig. 1, 66 (30.1%) grafts from HBcAb positive donors were allocated to HBsAg positive recipients. Of the remaining 153 grafts, 65 (42.5%) were transplanted to recipients who were negative for both HBsAb and HBcAb, 39 (25.5%) to recipients who were HBcAb positive only, 11 (7.2%) to recipients HBsAb positive-only, and 38 (24.8%) to recipients positive for both HBsAb and HBcAb.

Graft survival as related to HBcAb positive donor allocation

A total of 329 graft losses occurred: 66 (30.1%) among 219 LTs performed using grafts from HBcAb positive donors and 263 (21.6%) among 1218 performed using HBcAb negative donor grafts. The estimated graft survival probability was significantly worse in recipients of HBcAb positive compared to recipients of HBcAb negative donor grafts (log rank $p = 0.0047$). However, after data stratification in relation to HBsAg recipient positivity, it appeared (Fig. 2A) that graft survival did not differ between HBsAg carriers who received grafts from HBcAb positive or negative donors (log rank, $p = 0.4478$): HBsAg positive recipients showed a 3-year graft survival as high as 0.88 (s.e. 0.020),

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Table 2. Main characteristics of donor and recipients as related to HBsAg recipient status. (A) Donors characteristics; (B) recipients characteristics.

A

Donor features	HBsAg positive recipients (n = 319)		HBsAg negative recipients (n = 1118)		p value
	Median or n	Q1-Q3 or %	Median or n	Q1-Q3 or %	
Age (yr)	57	45-71	55	39-67	0.0052
Male sex	183	57.37	619	55.37	0.5258
Cause of death					0.0149
Trauma	67	21.00	308	27.55	
Anoxia	10	3.13	56	5.01	
Cerebral haemorrhage	193	60.50	630	56.35	
Other	49	15.37	124	11.09	
Height (cm)	170	163-175	170	160-175	0.9489
BMI	25.7	23.4-27.7	24.8	22.9-27.4	0.0384
Sodium	148	142-155	148	142-154	0.5708
Split liver	15	4.70	67	5.99	0.3807
Cold ischemia time (h)	7.0	6.0-8.7	7.3	6.0-8.8	0.1264
DRI	1.61	1.34-1.87	1.56	1.25-1.82	0.0170

B

Recipient features	HBsAg positive recipients (n = 319)		HBsAg negative recipients (n = 1118)		p value
	Median or n	Q1-Q3 or %	Median or n	Q1-Q3 or %	
Age (yr)	55	48-60	54	47-60	0.4912
Male sex	278	87.15	847	75.76	<0.0001
Portal vein thrombosis	9	2.82	26	2.33	0.6799
BMI	25.4	23.6-27.3	24.9	22.7-27.7	0.1103
Serum creatinine at LT	0.90	0.80-1.10	1.00	0.80-1.20	0.0787
Serum bilirubin at LT	2.00	1.20-4.80	2.70	1.30-6.00	0.0017
INR at LT	1.40	1.20-1.70	1.40	1.20-1.70	0.1902
MELD at LT	13	9-19	15	11-21	<0.0001
HCC	190	59.56	457	40.88	<0.0001
HCV positive	54	16.93	614	54.92	<0.0001
Alcoholic cirrhosis	1	0.31	241	21.56	<0.0001
Diabetes (70 missing)	29	9.39	187	17.62	0.0005

regardless of the donor HBcAb status. In sharp contrast, grafts from HBcAb positive donors showed a significantly lower survival (log rank, $p = 0.0007$) than those from HBcAb negative donors, when they were transplanted to HBsAg negative recipients (Fig. 2B). These grafts had a higher DRI (median: 1.65 vs. 1.54, $p = 0.0011$) and greater prevalence of HCV infection (64.7% vs. 53.4%, $p = 0.0088$) compared to recipients of HBcAb negative grafts, while MELD values were similar in the two groups.

We next explored the outcome of HBcAb positive/negative grafts in relation to the HBcAb/HBsAb status of HBsAg negative recipients. While the HBcAb/HBsAb status of the recipient had no impact on graft survival among LT performed using HBcAb negative grafts (Supplementary Fig. 1A), there was a trend towards a lower graft survival among all groups of patients who received HBcAb positive grafts (Supplementary Fig. 1B). The poorest outcome was observed in recipients positive for HBcAb only and the best in HBsAb positive recipients.

The impact of HBcAb donor positivity on graft survival was further investigated in relation to HCV infection, present in more than one half of HBsAg negative recipients. As shown in Fig. 3,

survival of grafts from HBcAb positive donors was worse in both HCV-negative and HCV-positive recipients (A, $p = 0.0562$ and B, $p = 0.0073$, respectively).

HBV prophylaxis and graft survival

HBV prophylaxis was employed at most centers. It was compulsory in HBsAg positive recipients, being in all cases a combination of HBIg and nucleos(t)ide analogues (NUC) (usually lamivudine). These patients maintained HBV prophylaxis indefinitely, although HBIg doses were gradually reduced and eventually suspended, according to center-specific protocols. Among the 153 HBsAg negative recipients of HBcAb positive donor grafts, 19 received no prophylaxis, 132 received a prophylactic protocol according to center policy (87 HBIg + NUC, 38 NUC alone and 7 HBIg alone), and no data were available in 12 LTs. The type of prophylaxis was partially influenced by the recipient HBV profile, as in general, combined prophylaxis with HBIg and NUC was used less frequently in HBsAb positive recipients (Table 5). Graft loss was apparently unrelated to the type of HBV prophylaxis or the

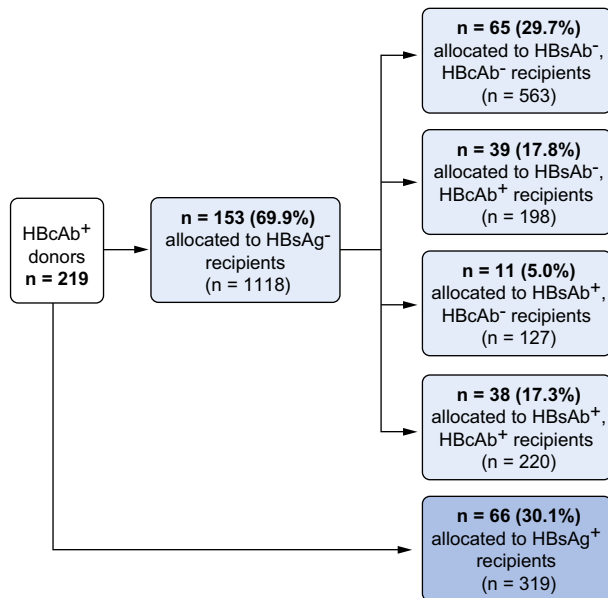


Fig. 1. Flow diagram showing the allocation of HBcAb positive donor grafts in the whole cohort.

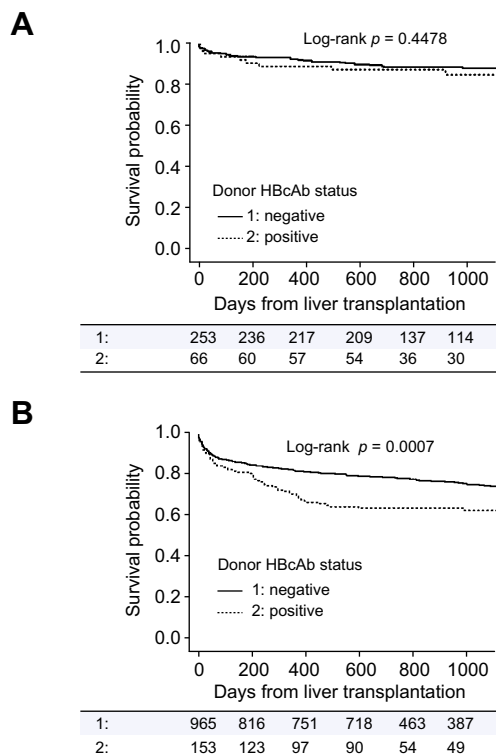


Fig. 2. Estimated graft survival probability stratified by donor HBcAb in HBsAg positive and negative transplant recipients. (A) HBsAg positive recipients; (B) HBsAg negative recipients. Continuous line depicts HBcAb negative donor grafts and dashed line depicts HBcAb positive donor grafts.

absence of prophylaxis. Unfortunately, no information was recorded on the dose and duration of HBIG and NUC.

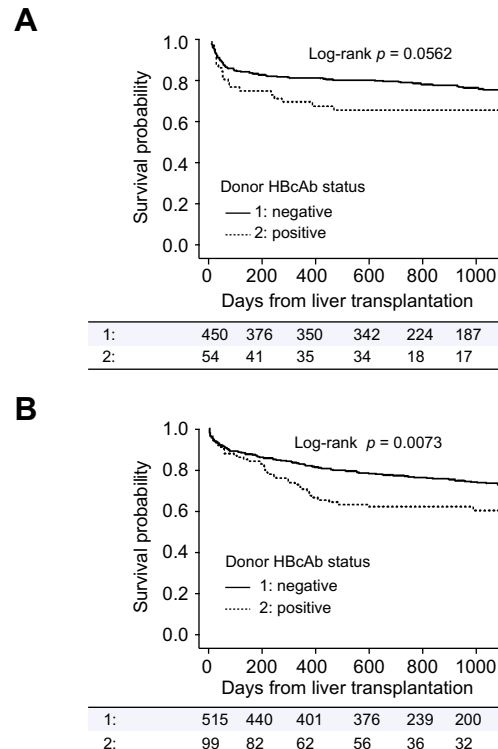


Fig. 3. Estimated graft survival probability stratified by donor HBcAb status in HCV negative and HCV positive transplant recipients who were negative for serum HBsAg. (A) HCV negative recipients; (B) HCV positive recipients. Continuous line depicts HBcAb negative donor grafts and dashed line depicts HBcAb positive donor grafts.

Causes of graft loss

The causes of graft loss did not differ significantly between recipients of HBcAb positive and HBcAb negative grafts: in general, infections and post-operative complications were more frequent in the early phase, while disease recurrences thereafter. However, there was a trend toward a higher frequency of graft loss due to recurrent diseases and to development of *de novo* tumors among recipients of HBcAb positive grafts. Recurrent HCV-related disease was the single most frequent cause of graft loss, accounting for 16.3% and 19.7% in recipients of HBcAb negative and positive grafts, while recurrent HCC accounted for 8.4% and 7.6%, respectively. Graft loss due to *de novo* HBV hepatitis occurred in one recipient of HBcAb positive graft (1.5%), though HBV reactivation was reported in 7 recipients of HBcAb positive donor grafts experiencing graft loss (Supplementary Table 2).

Predictors of graft loss at multivariate analysis

All variables were initially included in the multivariate analysis. Table 3 shows those identified as significant predictors of graft loss at Cox regression in the whole population. These included MELD at LT (HR = 1.30 per 10 units), donor HBcAb positivity (HR = 1.56), recipient HBsAg positive status (HR = 0.43), portal vein thrombosis (HR = 1.99), and DRI (HR = 1.41 per unit).

The inclusion of HCV status and presence of HCC did not add any significant contribution to the final model. Please note that the same significant predictors of graft loss were identified when Cox regression analysis was restricted to HBsAg negative

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Table 3. Hazard ratios for graft loss: Results from the final Cox's model (n = 1421).

Variable	Hazard Ratio	95% CI		p value
MELD at LT	1.300 (per 10 units)	1.134	1.491	0.0002
Recipient HBsAg status (positive vs. negative)	0.426	0.304	0.597	<0.0001
Portal vein thrombosis (yes vs. no)	1.988	1.139	3.496	0.0156
Donor HBcAb status (positive vs. negative)	1.555	1.184	2.042	0.0015
DRI	1.406 (per unit)	1.029	1.921	0.0325

Table 4. Three-year estimated survival for different allocations of HBcAb positive donor grafts.

MELD at LT	DRI	Portal vein thrombosis	Recipient HBsAg status	Estimated survival probability at 3 yr	95% CI
15	1	no	negative	0.70	(0.62, 0.79)
15	1	no	positive	0.86	(0.81, 0.92)
20	1	no	negative	0.67	(0.59, 0.76)
20	1	no	positive	0.84	(0.78, 0.91)
20	2	no	negative	0.57	(0.48, 0.66)
20	2	no	positive	0.78	(0.71, 0.86)
25	1	no	negative	0.63	(0.54, 0.73)
25	1	no	positive	0.82	(0.75, 0.90)
25	2	no	negative	0.52	(0.43, 0.63)
25	2	no	positive	0.76	(0.68, 0.85)
25	2	yes	negative	0.28	(0.12, 0.61)
25	2	yes	positive	0.58	(0.40, 0.84)
35	1	no	negative	0.55	(0.44, 0.69)
35	1	no	positive	0.77	(0.68, 0.88)
35	2	yes	negative	0.19	(0.06, 0.56)
35	2	yes	positive	0.49	(0.29, 0.82)

Table 5. Type of immunoprophylaxis used as related to the HBsAb and HBcAb status of HBsAg negative recipients of HBcAb positive donor grafts.

Type of prophylaxis	Recipient HBV immunological status				Total LT
	HBsAb+ HBcAb+	HBsAb+ HBcAb-	HBsAb- HBcAb+	HBsAb- HBcAb-	
None	4 (9.8%)	0	8 (8.3%)	7 (9.9%)	19
NUC only	7 (17.1%)	4 (36.3%)	12 (12.5%)	14 (19.7%)	37
HBIG only	2 (4.8%)	3 (27.3%)	2 (2.1%)	0	7
HBIG and NUC	22 (53.7%)	4 (36.4%)	66 (68.7%)	48 (67.6%)	140
Unknown	6 (14.6%)	0	8 (8.3%)	2 (2.8%)	16
Total LT recipients	41 (18.7%)	11 (5.0%)	96 (43.8%)	71 (32.4%)	219 (100%)

HBIG, hyperimmune hepatitis B immunoglobulins; NUC, nucleos(t)ide analogues.

recipients (Supplementary Table 1). Notably, the interaction between donor HBcAb and recipient HBsAg status was not significant after adjusting for other covariates, indicating that there is still an increased, albeit reduced risk associated with the use of HBcAb donors in HBsAg positive recipients. The estimated HR associated with HBcAb donor positivity was 1.59 in HBsAg negative and 1.36 in HBsAg positive recipients, respectively. This increased risk was compensated only in part by the more favourable profile of HBsAg positive recipients.

As shown in Fig. 4, the estimated hazard ratio of graft loss for HBcAb positive vs. HBcAb negative donors was not constant over

time, reaching the maximum around one year after LT and decreasing thereafter.

For a user friendly illustration of the impact of HBcAb positive donor graft allocation, the 3-year survival probability was estimated from the final Cox model in different clinical scenarios (Table 4). For any given combination of the predictors of graft loss, the allocation of HBcAb positive donor grafts to HBsAg positive recipients was associated with an approximate gain of 20% in 3-year graft survival. On the other hand, the allocation of these donors to HBsAg negative recipients with high MELDs and/or additional risk factors resulted in poor outcomes.

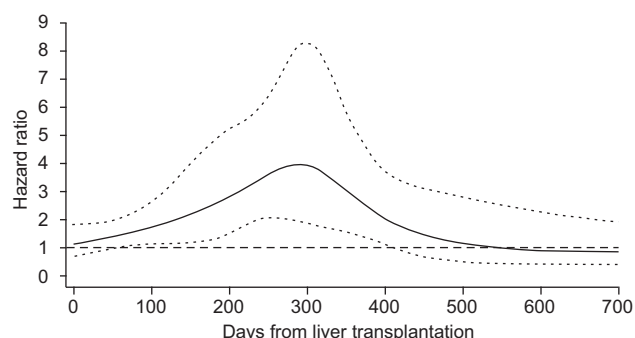


Fig. 4. Cox-derived estimates of the time dependent hazard ratio for graft loss in HBcAb positive vs. HBcAb negative donors in the whole cohort.

Discussion

Although HBcAb positive donor grafts are known to retain the potential to transmit HBV infection after LT [5,8–11], several small-sized studies have shown that their use under defined favourable conditions is associated with a limited number of HBV reactivations and overall satisfactory results [10,15–18]. Because of the disparity between the number of donors and recipients, it is therefore mandatory to use all suitable HBcAb positive donor grafts within appropriate allocation strategies. To date there is no standardized prophylactic regimen for HBsAg negative recipients of HBcAb grafts and many different approaches are used, from no prophylaxis to the use of NUC, HBIG or their combination [13–18,27]. In addition, most studies have looked only at the rate of post-LT HBV reactivation. Only few examined the medium/long-term outcome of HBcAb positive grafts in relation to their allocation and none has used a prospective, evidence-based approach.

The present study was performed within the framework of Liver Match, a prospective observational cohort study including all consecutive LT performed over a 2-year period in Italy, a country with relevant previous exposure of the general population to HBV infection. Our data show that HBsAg negative LT recipients of grafts from HBcAb positive donors had a distinctly lower graft survival compared to those who received their graft from HBcAb negative donors. This difference remained highly significant after adjusting for covariates by Cox regression analysis. Notably, the less favourable outcome of LTs performed with HBcAb positive donor grafts was not observed in the subgroup of HBsAg positive recipients, who indeed achieved excellent results, regardless of the HBcAb status of the donor.

In our study, the characteristics of HBcAb positive donors allocated to HBsAg positive or HBsAg negative recipients were similar, including comparable DRI values. Yet, there were three major recipient-related differences: the MELD score was lower among HBsAg positive than HBsAg negative recipients, a finding attributable to the greater proportion of compensated patients with HCC as primary indication for LT; HBsAg positive recipients had less often concomitant HCV infection compared to HBsAg negative recipients (16% vs. 60%, respectively, $p < 0.0001$); all HBsAg positive LT recipients received intra- and perioperative HBV prophylaxis with HBIG, followed by continued long-term prophylaxis, usually based on dual therapy with HBIG and lamivudine. In contrast, HBsAg negative recipients of HBcAb positive grafts received less stringent prophylaxis, or eventually no prophylaxis at all,

reflecting the current lack of consensus in this setting [15–18]. These peculiar features of HBsAg positive recipients partly counterbalanced the increased risk associated with the use of HBcAb positive donor grafts, as evidenced by the lack of significant interactions in the Cox model. Yet, it should be noted that while increasing MELD was invariably associated with a higher risk of graft loss at Cox regression, HCV infection did not emerge as a significant risk factor in the present series, likely because the current median follow-up is only limited to 3 years.

Reduced survival of HBcAb positive grafts was found years ago within a retrospective multicenter North-American study [9], but this finding was not confirmed in later single-center studies after the introduction of lamivudine prophylaxis [10,19]. Yu *et al.* [20] retrospectively evaluated the UNOS data from 1994 to 2006 showing that HBsAg negative recipients of adult first LT receiving HBcAb positive donor grafts had a significantly worse unadjusted graft survival compared to recipients of HBcAb negative donors (HR for graft loss: 1.35, 95% c.i. 1.21–1.50). However, after adjusting for predictors of post-LT survival, only a non-significant trend was observed (HR: 1.09, 95% c.i. 0.95–1.46). Yet, it should be noted that at least one fourth of the cases from this study were excluded from the multivariate analysis due to missing data, which highlights the weakness of retrospective analyses and the importance of using a prospective observational methodology, given that a randomized study in this setting would be unethical.

A recent retrospective study of the UNOS STAR registry including 958 HBsAg negative recipients of HBcAb positive livers, analyzed the effect of different prophylactic antiviral strategies on patients and graft mortality [26]. This study found a favourable impact of HBIG in reducing patient mortality compared to lamivudine-only and, interestingly, no graft loss was attributable to hepatitis B. In agreement with this finding, we had virtually no evidence that the higher rate of graft loss in HBcAb positive donor graft recipients was due to HBV infection.

Taken together, these data suggest that HBcAb positivity represents a surrogate marker of suboptimal graft quality, acting through still unexplained mechanisms. Notably, the estimated higher hazard ratio of graft loss for HBcAb positive vs. HBcAb negative donors was not constant over time, being negligible in the early post-LT and reaching the maximum around one year. This may reflect subtle unfavourable interactions of HBcAb positivity with other concurrent risk factors, which may undermine recipient's health during the first year after LT. Notably, none of the recorded causes of graft loss statistically differed between HBcAb positive or negative donors, although recurrent diseases and *de novo* non-HCC tumors were slightly more frequent in HBcAb positive donor recipients. It may be relevant to add that, in the non-transplant setting, HBcAb positivity has been occasionally associated with an unfavourable evolution of HCV-related liver disease, though this finding remains controversial [27–28]. In our study the increased risk of graft loss associated with donor HBcAb positivity was found both in HCV positive and negative recipients. While this finding does not exclude the possibility of an interaction of HBcAb positivity with HCV infection, it also suggests the presence of additional unfavourable cofactors.

In our study, virological analyses were not routinely performed, but only prompted by clinical events. Thus a precise estimate of the frequency of HBV reactivation is missing. However, analysis of the causes of graft loss, recorded according to ELTR definitions, showed that clinically evident HBV reactivation was

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negligible. The present results strengthen the concept that HBcAb positive donor grafts should be first offered to patients transplanted for HBV-related liver disease (i.e., HBsAg positive recipients), a subgroup of patients who in any case would receive intra-/perioperative HBIg administration, followed by long-term prophylaxis against HBV recurrence. In our study, there was an approximate 50% greater chance to allocate a HBcAb positive graft to a HBsAg positive recipient, however this preferential allocation was not the most common practice in Italy, in the biennium 2007 to 2009, as two thirds of HBcAb positive grafts were allocated to HBsAg negative recipients, although more than 23% of LT recipients were HBsAg positive. Addressing the reason for this allocation pattern is beyond the purpose of this study. Suffice here to say that the lack of evidence-based recommendations in this setting likely contributed. On the other hand, the excellent outcomes observed in HBsAg positive recipients of HBcAb positive grafts suggest that HBIg given at higher doses and/or with more stringent schedules might be beneficial also to HBsAg negative recipients of these organs, as suggested by the recent analysis of the UNOS STAR series [26].

In summary, a few key points emerge from this study. The use of HBcAb positive donor grafts is associated with excellent results when they are transplanted to HBsAg positive recipients receiving standard prophylaxis against HBV. Therefore, HBcAb positive donor grafts should be used preferentially in the latter context. Conversely, the use of HBcAb positive donor grafts in HBsAg negative recipients requires attention, as it may be associated with suboptimal survival, especially in patients with high MELDs and/or additional risk factors. The presence of HBcAb in HBsAg negative recipients does not seem to be protective and cannot justify the allocation of HBcAb positive donor grafts to these patients. Whether HBsAb recipient positivity is associated with better outcomes remains uncertain. Overall, these findings suggest that the use of HBcAb positive donor grafts requires more stringent allocation strategies than currently adopted.

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Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Appendix A

* Composition of the liver match study group

Liver Match Steering Committee:

M. Angelico, Rome (study coordinator), U. Cillo, S. Fagioli, M. Strazzabosco

AISF/CPT Governing Board: P. Caraceni, P.L. Toniutto

CNT Governing Board: A. Nanni Costa

Participating Liver Transplant Centers and investigators:

Torino (M. Salizzoni, R. Romagnoli, G. Bertolotti, D. Patrono)

Milano, Niguarda Hospital (L. De Carlis, A. Slim, J.M.E. Mangoni)

Milano, Fond. IRCCS Osp. Maggiore Policlinico (G. Rossi, L. Caccamo, B. Antonelli)
Milano, IRCCS Tumori (V. Mazzaferro, E. Regalia, C. Sposito)
Bergamo (M. Colledan, V. Corno, F. Tagliabue, S. Marin)
Padova (U. Cillo, A. Vitale, E. Gringeri)
Verona (M. Donataccio, D. Donataccio)
Udine (U. Baccarani, D. Lorenzin, D. Bitetto)
Genova (U. Valente, M. Gelli, P. Cupo)
Modena (G.E. Gerunda, G. Rompianesi)
Bologna (A.D. Pinna, G.L. Grazi, A. Cucchetti, C. Zanfi)
Ancona (A. Risaliti, M. G. Faraci)
Roma Tor Vergata (G. Tisone, A. Anselmo, I. Lenci, D. Sforza)
Roma Gemelli (S. Agnes, M. Di Mugno, A.W. Avolio)
Roma POIT (G.M. Ettorre, L. Migliore, G. Vennarecci)
Roma Sapienza (P. Berloco, M. Rossi, S. Ginanni-Corradini, A. Molinaro)
Napoli (F. Calise, V. Scuderi, O. Cuomo, C. Migliaccio)
Bari (L. Lupo, G. Notarnicola)
Palermo (B. Gridelli, R. Volpes, S. Li Petri)
Cagliari (F. Zamboni, G. Carbotta, S. Dedola)
Data collection and verification & biostatistics
A. Nardi, T. Marianelli, C. Gavrila, A. Ricci, F. Vespasiano (Rome)

Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jhep.2012.11.025>.

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