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ORIGINAL ARTICLE

Use of anti-HBc positive allografts in adult liver transplantation: toward a safer way to expand the donor pool

Dino Donataccio,[1] Francine Roggen,[1] Chantal De Reyck,[1] Catherine Verbaandert,[2] Monique Bodeus[3] and Jan Lerut[1]

1 Liver Transplant Unit, Cliniques Universitaires Saint-Luc, Université catholique de Louvain, Brussels, Belgium
2 Department of Pathology, Cliniques Universitaires Saint-Luc, Université catholique de Louvain, Brussels, Belgium
3 Unit Virology, Cliniques Universitaires Saint-Luc, Université catholique de Louvain, Brussels, Belgium

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Correspondence
Prof. Jan P. Lerut MD, PhD, FACS, Liver Transplant Unit – Starzl Room, Cliniques Universitaires St-Luc, Université catholique de Louvain, Av. Hippocrates 10, 1200 Brussels, Belgium. Tel.: +32 2 764 5306; fax: +32 2 764 9039; e-mail: lerut@chir.ucl.ac.be

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Summary

The use of livers from anti-hepatitis B core (HBc) positive donors can alleviate donor shortage. Nineteen of 367 (6%) adults receiving anti-HBc positive allografts [three were hepatitis B antigen (HBsAg) negative, hepatitis B antibody (HBsAb) positive; four were HBsAg positive and 12 were not exposed to hepatitis B viral (HBV) infection] were retrospectively reviewed. In HBsAg negative recipients, immunoprophylaxis (IP) was guided by viral serology and immunohistochemistry (IH) of day 0 and day 7 liver biopsies. If IH was negative, IP was stopped. None of three HBsAg negative, HBsAb positive recipients infected; one (replicating) of four HBsAg positive recipients reinfected and seven of eight (87.5%) HBsAg, HBsAb negative recipients, who did not receive long-term IP, infected after a median time of 2 years (range 1–5); one patient died of liver failure. Four HBsAg, HBsAb negative recipients, receiving life-long IP, remained infection free. Anti-HBc positive donor livers must be directed selectively first to HBsAg positive recipients, next to recipients having HBV antibodies and finally to HBV-naive recipients. Identification of both donor and recipient risk factors for HBV infection before transplantation allows indiscriminate use of antiviral prophylaxis. The necessity for IP therapy should be guided by HBV-DNA testing of donor liver tissue and serum. IH of early liver biopsies is an unreliable marker for predicting antiviral treatment requirements.

Introduction

The gap between the number of available liver donors and the number of potential recipients is continuously widening. Use of liver grafts originating from hepatitis B antigen (HBsAg) negative but anti-hepatitis B core (HBc) positive donors represents a means to expand the donor pool [1,2]. However, transmission of HBV infection, especially to HBV-naive patients, has been reported to be present in 17–94% of recipients [3–11].

The successful use of specific anti-HBs immunoglobulins (HBIg), with or without lamivudine, in liver transplantation (LT) for HBsAg positive, including replicating, liver recipients, has changed the approach to the acceptability of these grafts, even in HBV-naive liver recipients [12–18].

The aim of this study was to evaluate outcome of such grafts in adult LT, the value of early post-transplant HBV immunohistochemistry (IH) of the donor liver as a tool to guide prophylactic antiviral therapy and to review the recent literature in order to define the most valuable and economically justified use of such grafts.

Materials and methods

Between February 1992 and March 2004, 22 of 367 (6%) adults (>15 years old) received a graft originating from

HBsAg negative anti-HBc positive donor. All organs were allocated by the Eurotransplant International Foundation. The actual Eurotransplant allocation policy does not imply a more precise determination of the HBV status of the anti-HBc positive donor. Three patients receiving anti-HBc positive livers during the study period were excluded from the analysis as they died within 3 months of LT without any sign of HBV allograft infection.

The median age of the 19 patients was 46 years (range 20–64). Diagnoses were: alcoholic cirrhosis (four patients), HBsAg positive B cirrhosis (six patients), anti-HCV positive cirrhosis (two patients), fulminant hepatitis (three patients), secondary biliary cirrhosis (two patients), cryptogenic cirrhosis (one patient), and hemangio-endothelioma (one patient).

All sera of the recipients had complete pre- and post-transplant testing for HBsAg and anti-HBc by microparticle enzyme immunoassays (Axsym; Abbott Diagnostics, Wiesbaden-Delkenheim, Germany). For logistical and financial (there is no reimbursement for testing for these indications in Belgium) reasons, HBV-DNA was not measured in any of the donor serum or liver tissue samples.

After LT, HBV serology was performed weekly up to 1 month and then at 2, 3, 6 and 12 months, yearly and when clinically indicated. All patients had a routine liver biopsy at day 0 (reperfusion biopsy), day 7, at 6 and 12 months and when clinically indicated.

Liver biopsies were fixed in 10% buffered formaldehyde, processed for routine paraffin sections, and stained with hematoxylin–eosin and blue trichromic stain. Immunohistochemical detection was performed, using anti-HBcAg (1/100; Novocastra, Newcastle upon Tyne, UK) and anti-HBsAg (1/1500; Biomeda, Foster City, CA, USA) rabbit antibodies. Antibodies were detected by a classical streptavidin-biotin-peroxidase system and 3,3′-diaminobenzidine revelation.

Hepatitis B viral allograft prophylaxis consisted of HBIg (Hepacaf®; CAF Red Cross Belgium, Brussels, Belgium). Initially, 10 000 units of HBIg were administrated intravenously during the anhepatic phase and afterwards for the first 7–10 days post-LT (short-term course) in order to achieve protective levels of >250 mIU/ml. Prolongation of immunoprophylactic (IP) therapy was based on recipient HBsAg positivity at the time of LT, on the evolution of the immunoglobulin clearance rate and on the results of the IH examination on day 0 and day 7 biopsies in all (other) recipients. This strategy was chosen because the precise determination of the HBV-donor status, in the absence of HBV-DNA serum and liver tissue determination, was not available at transplantation.

Diagnosis of allograft HBV *de novo* infection or reinfection was based on elevation of liver tests and positive HBsAg viral serology, with or without HBeAg or HBV-DNA, confirmed by histologic examination, including IH, of all liver tissue samples.

In cases of *de novo* allograft infection, patients were treated with lamivudine (150 mg/day, Zeffix®; Glaxo-Smith-Kline, Genval Belgium) and in cases of viral mutation adefovir (dose titrated to renal function, Hepsera®; Gilead, Foster City, CA, USA) was introduced.

All patients were followed for at least 1 year or until death.

Results

The detailed results of this patient series are summarized in Table 1.

None of three HbsAg negative, hepatitis B antibody (HBsAb) positive patients developed *de novo* infection. They all received a short-term course of HBIg.

None of three, nonreplicating, HBsAg positive recipients reinfected their allograft under long-term IP guided by regular determination of anti-HBs titers. One replicating HBsAg positive patient reinfected his allograft 4 months after LT; viral mutation emerged after 4 years of lamivudine therapy. He is doing well 81 months post-LT using a combination of lamivudine and adefovir therapy. HBsAg remains positive, HBV-DNA levels are low (0.9 mEq/ml) but liver tests are normal.

Seven of the first eight (87.5%) HBsAg, HBsAb negative recipients had *de novo* allograft infection after short-term IP. In all eight cases, IP was discontinued based on the negative IH of day 0 and day 7 liver biopsies. Allograft infection occurred after a mean of 27 months (range 12–60). All seven patients were treated using lamivudine. One patient died 54 months post-LT, while waiting for re-LT, of rapidly progressing *de novo* HBV cholestatic hepatitis.

Two other *de novo* infected patients developed a severe cholestatic hepatitis: one patient is doing well 25 months post-LT and one patient died while waiting for re-LT because of biliary sepsis caused by ischemic type biliary tract lesions.

None of the other four HBsAg negative HBV recipients have infected their graft under long-term (indefinite) IP; three of them were naive patients and one was anti-HBc positive.

Discussion

Because of the growing gap between patients who receive a transplant and the number of patients on the waiting list, there is an ever increasing need to expand the liver donor pool. One way to do so is to use grafts from donors who are HBsAg negative but who are positive for antibodies to hepatitis core antigen. HBcAb is the earliest

Table 1. HBV prophylaxis type and duration in relation to recurrent and *de novo* HBV infection after transplantation of anti-HBcore positive liver allografts.

Recipient	Pre-LT HBV status	IP	IP duration	Reinfection	*De novo* infection	Delay LT infection (months)	HBV recurrent	*De novo* infection	R	Post-LT (months)	Cause death	Outcome HBsAg
HBsAg+												
HBcAb+	R	HBIg	LL	+		4			LAM + adefovir	A 81		+
	NR	HBIg	LL	–			1/3 (33.3%)			A 27		–
	NR	HBIg	LL	–						A 16		–
HBcAb–	NR	HBIg + LAM	LL	–			0/1 (0%)			A 29		–
							1/4 (25%)					
HBsAg–												
HBsAb–		None	–		+	17			LAM	A 146		NA
HBcAb–		None	–		+	60			LAM	A 133		+
		None	–		+	40			LAM	D 54	HBV	+
		HBIg	10 days		+	30			LAM	A 62		–
		HBIg	10 days		+	12			LAM	A 60		+
		HBIg	10 days		+	12		7/11 (63.6%)	LAM	D 36	Bil. inf.	+
		None	–		–					A 49		–
		HBIg + LAM	LL		–					A 34		–
		HBIg	10 days		+	15			LAM	A 25		–
		HBIg	LL		–					A 16		–
		HBIg	LL		–					A 12		–
HBcAb+		HBIg	LL		–			0/1 (0%) 7/12 (58.3%)		A 11		–
HBsAg–												
HBsAb+		HBIg	7 days		–					A 129		–
		None	–		–			0/3 (0%)		A 127		–
		HBIg	10 days		–					A 18		–

HBV, hepatitis B viral infection; Ag, antigen; Ab, antibody; LT, liver transplantation; R, replicating viral status; NR, nonreplicating viral status; IP, immunoprophylaxis; HBIg, specific anti-HBs immunoglobulin; LAM, lamivudine; LL, life-long; NA, not available; Bil. inf., biliary infection; A, alive; D, death.

antibody detected following HBV infection; it can persist for the lifetime of a previously infected patient.

Two to fifteen percent of liver donors are anti-HBc positive. Prevalence is related to age. The proportion of positive anti-HBc livers in donors >60 years may rise to 25% (vs. 3.6% in donors <20 years) [19]. This is of importance in LT, because older donors are used more frequently and because there is a high risk of viral disease transmission in liver recipients (in contrast to the almost nonexistent transmission risk in kidney and heart recipients).

Review of the recent literature has clearly shown that HBV infection can be transmitted using anti-HBc donors in 17–94% of cases (Table 2). Viral transmission is explained by the ongoing viral replication, which has been demonstrated in these livers [20]. The high risk of disease transmission and the economic impact related to the life-long need for expensive allograft viral IP, obliges one to optimize the use of such allografts.

The risk of transmission of viral infection is clearly dependent on the recipient anti-HBs and anti-HBc status. Indeed, HBV-naive patients who are both anti-HBs and anti-HBc negative are at greatest risk of infection. This risk should be taken seriously as *de novo* infection can behave aggressively and even lead to fatal cholestatic hepatitis [21,22].

Grafts from HBsAg negative anti-HBc positive donors should, therefore, first be directed to HbsAg positive patients as they will require life-long IP anyway. Secondly, these livers should be directed to anti-HBs positive patients, as these do not seem to require IP. It is not clear if it is necessary to treat anti-HBc positive, anti-HBs negative patients with HBIg. Finally these livers should only be used in HBV-naive patients if they are in a critical clinical condition and if adequate, IP can be guaranteed. Viral prophylaxis using life-long HBIg with or without lamivudine is mandatory. Encouraging short-term follow-up data are available in relation to the protective value of lamivudine monotherapy in anti-HBc positive liver transplants (Table 2).The high incidence of lamivudine resistance after its long-term use, reported to be 45% (range 13–65) after 21–36 months should, however, be kept in mind [23].

The results from this retrospective study show that the use of anti-HBc positive livers in HBV-naive patients using short-term IP based on the IH of day 0 and day 7 liver biopsies and on early post-transplant HBV serology is an inadequate approach. If no other virological testing is available, long-term IP is necessary in order to avoid *de novo* infection.

The selective approach adopted by the Miami group in relation to donor and recipient risk factors for post-transplant HBV infection after use of anti-HBc positive allografts needs much attention [24]. This group analyzed all donor serum samples in duplicate for HBsAg, anti-HBs and anti-HBc; one-fifth of donors had a false positive anti-HBc.

In confirmed anti-HBc positive donors, serum samples and liver biopsies were processed for HBV-DNA by qualitative polymerase chain reaction (PCR) assay (sensitivity 50 copies/ml). HBV-DNA detection by real-time PCR (Lightcycler; Roche Diagnostics, Mannheim, Germany) was obtained in <2 h. After transplantation serologic studies were repeated regularly and immunoperoxydase stains for HBs and HBc antigens were performed in addition to routine histologic examination. If donor and recipient risk factors for post-transplant HBV infection are identified before transplantation, indiscriminate use of antiviral prophylaxis can be avoided. Indeed prophylactic antiviral treatment was discontinued and even safely avoided without risk of allograft infection, if the HBV-DNA status of the donor was negative at the time of transplant; donor serology was an insufficient measure to guide the therapy. Determination of HBV-DNA status is thus mandatory at the time of transplantation to allow safe and efficacious use of anti-HBc positive livers. Complete virological screen can spare patients from unnecessary administration of HBIg. Liver allocation organisms should, therefore, urge recipient centers to (re)perform complete donor HBV testing. The burden of a 24 h a day laboratory logistics in the transplant center will be largely counterbalanced by the sparing of a very costly IP therapy, averaging e.g. in our unit about 6.000 euros/year. When implanting anti-HBc positive liver, the Miami group recommends combined HBIg and lamivudine prophylactic therapy for cases where, in the least, donor or recipient are HBV-DNA positive; lamivudine therapy if donor and recipient are both HBV-DNA negative and no prophylaxis if the recipient is HBsAg negative but anti-HBs positive. When donor HBV-DNA is not available, lamivudine is administered when the recipient is HBsAg and anti-HBs negative.

In conclusion, anti-HBc positive liver allografts represent a valuable way to expand the allograft pool. The use of such donors should be selective in order to obtain the most justified economic approach. Routine HBV serology and IH of early liver biopsies are both unreliable markers for predicting antiviral treatment requirements. Based on data in recent literature the need for, expensive, IP should be guided by HBV-DNA testing of the donor. *De novo* graft infection can be aggressive, but can be controlled in most cases using nucleoside analogues. The more frequent use of anti-HBc positive donors, related to the major increase in age of liver donors, represents an argument more in favor of obligatory vaccination policy in all naive patients waiting for a (renal or liver) allograft.

Table 2. Recurrent and *de novo* HBV infection in liver recipients of HBcAb positive donors.

						HBsAg+ recipient status					HBsAg− recipient status						HBsAg− recipient status				
References	Center	Year	*n*	FU (months)	HBV-DNA donor and/or recipient	Recurrent infection *n* (%)	Graft protection				HBsAb− *de novo* infection *n* (%)	HBsAb−, HBcAb+ *de novo* infection *n* (%)	Graft protection				HBsAb+ *de novo* infection *n* (%)	Graft protection			
							HBIg	Duration	LAM	Duration			HBIg	Duration	LAM	Duration		HBIg	Duration	LAM	Duration
Prieto *et al.* [19]	Valencia	2001	33*	29 (0.2–58)	ND/NA	–					15/24 (62.5)		NA		NA		0/6	NA		NA	
Yu *et al.* [14]	Los Angeles	2001	15	17 (2–40)	ND	0/6	+		+	LL	0/7		–		+	LL	0/2	–		+	LL
Joya-Vazquez *et al.* [13]	Pittsburgh	2002	14†	40 (x–102)	ND/NA	9/13 (69.2)	+		+	NA	–						–				
Manzarbeitia *et al.* [16]	Philadelphia	2002	35‡	25 (3–65)	ND	1/4 (25)	+		+	NA	4/13 (30.8)		–		–		1/14 (7.1)	–		–	
Nery *et al.* [23]	Miami	2003	62	23 (1–75)	–/NA (34/14)	2/13 (15.4)	–		+	LL	1/7 (14.3)	1/10 (10)	–		+	LL	0/18	–		–	
					+ (14)	0/4	+		+	LL	0/1	0/3	+		+	LL	0/6	+		+	LL
Fàbrega *et al.* [17]	Santander	2003	7	23 (9–36)	–	–					0/6		+		+	7–10 days	0/1	+		+	7–10 days
Loss *et al.* [15]	New Orleans	2003	14	33 (22–51)§	–	–					0/11		+	Anh	+	LL	1/3 (33.3)	+	Anh	+	LL
Montalti *et al.* [25]	Bologna	2004	44	NA	ND	1¶/26 (3.8)	+		+/–	NA	1¶/18 (5.5)		+		+/–	NA	–				
Lee *et al.* [18]	Seoul	2004	18**	40 (13–80)	ND	–					1/2 (50)		+	6 days	–		2/12 (16.6)	+	6 days	–	
UCL	Brussels	2005	19	56 (11–146)	IH donor liver	1/1 (R) (100)	+	LL	–		7/8 (87.5)		–/+	10 days	–		0/2	+	10 days	–	
						0/3 (NR)	+	LL	–		0/3	0/1	+	LL	–		0/1	–		–	

HBV, hepatitis B viral infection; Ag, antigen; Ab, antibody; HBIg, specific anti-HBs immunoglobulins; UCL, Université catholique de Louvain; LT, liver transplantation; FU, follow-up; NA, not available; ND, not done; LL, live-long; IH, immunohistochemistry; R, replicant HBV status at moment of LT; NR, nonreplicant HBV status at moment of LT; LAM, lamivudine; Anh, HBIg during anhepatic phase.

*Three patients not evaluated (two hepatitis B recipients, one patient died within 3 months post-LT).

†One patient lost to follow up.

‡Four patients dying within 3 months post-LT not evaluated.

§Follow up in 13 patients.

¶Untreated patient.

**Four patients dying post-LT not evaluated; living donor liver transplantation.

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