Minimization of anti-hepatitis B surface antigen immunoglobulins for prophylaxis of hepatitis B viral recurrence in the first month after liver transplantation: the meaning of HBsAg quantitative level at the time of transplant

MINIMIZATION OF ANTI-HEPATITIS B SURFACE ANTIGEN IMMUNOGLOBULINS FOR PROPHYLAXIS OF HEPATITIS B VIRAL RECURRENCE IN THE FIRST MONTH AFTER LIVER TRANSPLANTATION: THE MEANING OF HBsAg QUANTITATIVE LEVEL AT THE TIME OF TRANSPLANT

Francesco Tandoi*, francesco.tandoi@unito.it, Tel. +39-3493193412, Fax +39-(0)-1169695923

Elisabetta Nada*, elisabetta.nada@alice.it,

Fabrizia Pittaluga†, fpittaluga@cittadellasalute.to.it,

Fabrizio Fop*, fabrizio.fop@gmail.com,

Francesco Lupo*, flupo@cittadellasalute.to.it,

Renato Romagnoli*, renato.romagnoli@unito.it,

Mauro Salizzoni*, mauro.salizzoni@unito.it

*Liver Transplant Center, General Surgery Unit

†Microbiology and Virology Unit

A.O. Città della Salute e della Scienza - Molinette Hospital – University of Turin, Italy

ABSTRACT

Background: Hepatitis B Virus (HBV) recurrence after liver transplantation (LT) has practically disappeared with a prophylaxis combining anti-Hepatitis B surface antigen Immunoglobulins (HBIg) and antiviral drugs. Recently, cost-saving requirements pushed us to move from a fixed schedule of 50,000 IU intravenous HBIg in the first month after LT to an "on demand" administration guided by close monitoring of HBsAg and HBsAb with a serological target of HBsAg negative and HBsAb >300 mIU/mL. In this context, we investigated the meaning of HBsAg quantitative determination at LT in predicting the need of HBIg in the first month after LT.

Methods: From 02/2012 to 07/2013, we performed 35 LTs in HBsAg-positive patients, 18 of whom with Hepatitis Delta-coinfection (Delta-Positive). Anti-HBV prophylaxis was based on nucleos(t)ide analogues from day 1 post-LT and intravenous HBIg (10,000 IU intraoperatively and, in the following days, 5,000-2,500 IU pulses to reach and maintain the serological target).

Results: The HBsAg quantitative level at LT was significantly higher in Delta-Positive recipients. Complete negativization of HBsAg and HBsAb serum level >300 mIU/mL were achieved on day 3 in Delta-Positive and on day 2 in Delta-Negative. A positive linear correlation between HBsAg quantitative level at LT and HBIg administered in the first month after LT was observed (rho=0.788), with a total of 32,500 IU HBIg used in Delta-Positive and 22,000 IU in Delta-Negative recipients (p=0.0016). Compared to the old schedule, we saved a median of 14,750 IU in Delta-Positive and 28,000 IU in Delta-Negative. No HBV recurrence was observed in a median follow-up of 10.5 months.

Conclusions: Delta-Positive patients need higher doses of HBIg to reach the serological target after LT because they have greater HBsAg quantitative levels at LT. In future studies, pre-LT HBsAg quantitative determination will be helpful to predict the actual need of HBIg early after LT.

INTRODUCTION The objective of liver transplantation (LT) in patients with Hepatitis B Virus (HBV) infection is nowadays a close-to-zero HBV hepatitis recurrence rate[1]. Indeed, the combination of intravenous anti-Hepatitis B surface antigen immunoglobulins (HBIg) and antiviral drugs significantly improved outcomes after LT, with an overall risk of HBV hepatitis recurrence after LT lower than 10% in the long term. However, it is a very expensive strategy if fixed schedules[2] and large HBIg doses are used. Recently, stringent cost-saving requirements have emerged[3]. A big part of the expenditures for anti-HBV prophylaxis is concentrated in the first month after LT, when high HBIg doses are needed to reach early HBV surface antigen (HBsAg) negativization and obtain a highly protective HBV surface antibody (HBsAb) titer. Today, extremely sensitive HBV-DNA real-time PCR serum testing and a new HBsAg test for its quantitative determination in serum are available in clinical practice[4]. In this study we investigated the meaning of HBsAg quantitative levels at the time of LT in the setting of a new protocol aiming to minimize the use of HBIg in the first month after LT.

METHODS From February 2012 to July 2013, 172 first LTs were performed at the Liver Transplant Center of the University of Turin from deceased heart-beating donors in adult recipients. Among them, 39 (23%) transplants were carried out in HBV positive cirrhotic patients; 4 patients, with a history of HBV infection, were excluded because of HBsAg negative at the time of LT. The remaining 35 HBsAg positive patients were divided into two groups, Delta-Positive (n=18) and Delta-Negative (n=17), on the basis of the positivity for the Hepatitis Delta Virus coinfection. The following up-to-date laboratory techniques for serological tests were employed in this study: HBV-DNA serum level quantitation by real-time PCR (COBAS Amplicor-COBAS TaqMan – Roche – Switzerland), with a sensitivity threshold at 20 IU/mL; HBsAg quantitative serum level determination by chemiluminescent microparticle immunoassay (ARCHITECT HBsAg – Abbott – Illinois, US) with a sensitivity threshold at 0.05 IU/mL and HBsAb by chemiluminescent microparticle immunoassay (ARCHITECT HBsAb – Abbott – Illinois, US) with a sensitivity threshold at 10 mIU/mL. The clinical variables collected and analyzed were: donor features (age, sex, cause of death, Donor Risk Index, HBV core antibody [HBcAb]-positivity), recipient features (age, sex, biochemical Model for End-stage Liver Disease [MELD] score at listing and at LT, waiting list time, hepatocellular carcinoma presence, HBsAb, HBcAb, HBeAg and HBeAb positivity, HBV-DNA

level at listing and at LT, HBsAg quantitative level at listing and at LT), post-LT features in the first month (HBsAg quantitative level, HBsAb titer and HBIg administered; HBV-DNA level on day 7 and 30). Serum HBsAg quantitative level and HBsAb titer were determined daily from day 0 up to day 8 after LT, then every 3 days until day 30. The new protocol of anti-HBV prophylaxis used in these 35 HBsAg positive recipients was based on: 1) nucleos(t)ide analogues, starting from day 1 post-LT (the antiviral drug already used pre-LT or lamivudine in new onset); 2) HBIg, starting with 10,000 IU during the LT operation (5,000 IU in anhepatic phase, 5,000 IU at the end of surgery) and subsequently 5,000 IU/day until HBsAg negativization and achievement of an HBsAb protective level greater than 300 mIU/mL (*serological target*). Thereafter, pulses of 2,500 IU of HBIg were administered only if HBsAb titer was less than 300 mIU/mL. For reference, before 02/2012, a fixed schedule of 50,000 IU of HBIg was administered in the first month post-LT in our Center: 10,000 IU in anhepatic phase, 38,000IU in the next 7 days, and 2,000 IU on day 21, without close monitoring of HBsAb and HBsAg. Categorical variables were analyzed with χ^2 test, quantitative variables with non-parametric Mann-Whitney test; the correlation between variables was tested with Spearman test. A receiver operating characteristic (ROC) curve was drawn to set the cut-off of HBsAg quantitative level for the best prediction of HBIg need in the first month after LT. The level of significance was placed at *p*-value <0.05.

RESULTS All the recipients were HBsAg positive, HBsAb negative, HBcAb positive and HBeAg negative both at listing and at LT. All the donors were HBsAg negative and half of them were HBcAb positive. Table 1 summarizes the characteristics of the study population, stratified for the presence or absence of Hepatitis Delta Virus coinfection. No significant differences were observed except for the higher prevalence of hepatocellular carcinoma in Delta-Negative recipients (82% vs 50%, p=0.044). The median quantitative level of HBsAg at LT was 2,865.20 IU/mL (range 0.47–10,355.35) in Delta-Positive recipients and 399.01 IU/mL (range 1.30–6,577.19) in Delta-Negative (p=0.13). The serological target of HBsAg negative and HBsAb > 300 mIU/mL was reached on day 3 (range day 1-day 8) in Delta-Positive recipients and on day 2 (range day 1-day 4) in Delta-Negative ones (p=0.058). The median cumulative dose of HBIg administered between day 0 and day 30 in the 35 study patients was 28,500 IU (range 9,500-51,500); it was significantly higher in Delta-Positive subjects than

in Delta-Negative ones, respectively 32,250 IU (range 12,500–51,500) and 22,000 IU (range 9,500–38,000) (p=0.0016). In Delta-Positive group, only one patient needed 1,500 IU of HBIg more than the old schedule to obtain the serological target. HBV-DNA in serum was negative on day 7 and on day 30 in all patients. A positive linear correlation between HBsAg quantitative levels at LT and the amount of HBIg administered in the first month after LT was observed (rho=0.788; p<0.0001) (**Figure 1**). The best compromise between sensitivity and specificity able to predict a consumption of HBIg from day 0 to day 30 above the median dose of 28,500 IU was found at an HBsAg quantitative level of 530 mIU/mL; at that level, the area under the curve in the ROC curve was equal to 0.882. In the first month after LT, the median amount of HBIg saved in reference to the old schedule was 21,500 IU (-1,500-40,500) in the overall population; the median saving was nearly double in Delta-Negative subjects (28,000 IU, range 12,000–40,500) compared to Delta-Positive ones (14,750 IU, range -1,500–37,500) (p=0.0019). Assuming a cost of € 0.578 per 1 IU of HBIg, € 12,400 was saved per patient (€ 16,200 if Delta-Negative, € 8,500 if Delta-Positive). That figure was largely superior to the overall costs ascribable to the new policy of close monitoring of serological HBsAg and HBsAb levels (cost = € 19.5/day, making less than € 300 for fifteen determinations in the first month after LT). During the follow-up, none of the patients died, while 4 patients (11%) had an early re-transplantation (two due to hepatic artery thrombosis, two for primary non-function). After the first month post LT, anti-HBV prophylaxis was continued with nucleos(t)ide analogues and HBIg doses were titered to maintain HBsAb serum levels greater than 150 mIU/mL. No HBV hepatitis recurrence nor HBV viral reactivation was hitherto observed in a median follow-up of 315 days (range 92-598).

DISCUSSION The new protocol of HBIg minimization, which was prospectively introduced in our Center from February 2012, showed to be safe since it was capable of ensuring the recipient's full protection against HBV reactivation after LT. Moreover, it was also cost-effective because it enabled the optimization of the HBIg dose to the actual patient's need and allowed considerable savings in HBIg use. In comparison with Delta-Negative subjects, Delta-Positive ones have much more circulating HBsAg and, therefore, need larger doses of HBIg and more days to reach the serological target of HBsAg negativization and HBsAb > 300 mIU/mL. In the future, pre

LT HBsAg quantitative determination will be helpful to predict and optimize the dose of HBIg administered in the early phases after LT, independently from the presence of Hepatitis Delta Virus coinfection.

ACKNOWLEDGMENT The Authors thank Miss Elizabeth Clarke for her kind assistance in revising the manuscript.

REFERENCES

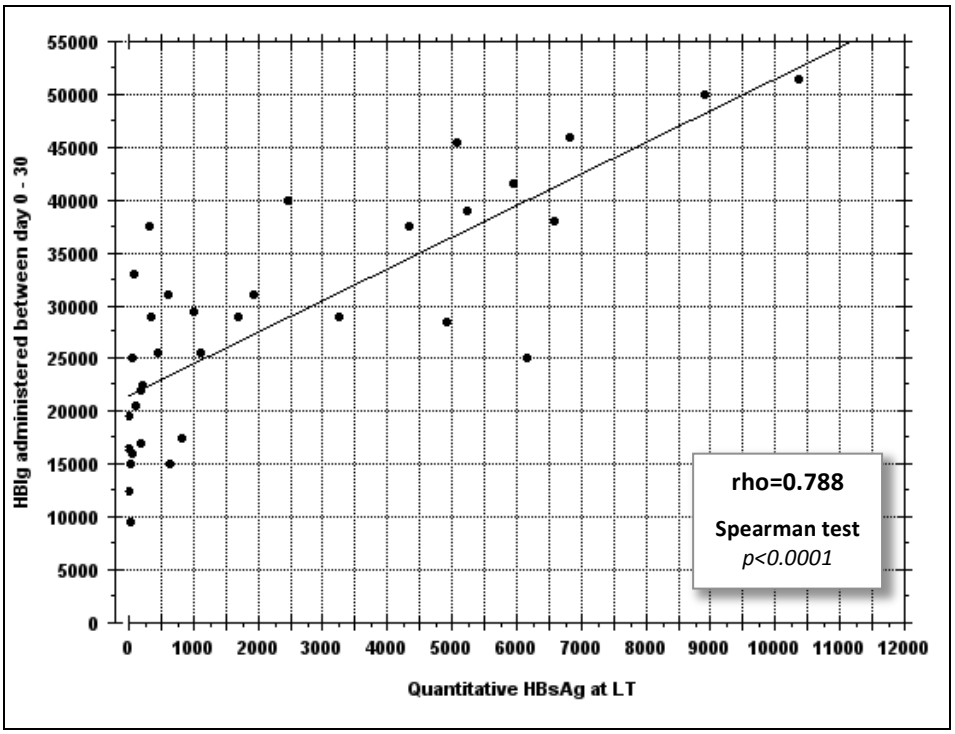
1. Burra P, Germani G, Adam R, et al. Liver transplantation for HBV-related cirrhosis in Europe: an ELTR study on evolution and outcomes. J Hepatol. 2013 Feb;58(2):287–96.
2. Terrault NA, Zhou S, Combs C, et al. Prophylaxis in liver transplant recipients using a fixed dosing schedule of hepatitis B immunoglobulin. Hepatology 1996 Dec;24(6):1327–33.
3. Dan YY, Wai CT, Yeoh KG, Lim SG. Prophylactic strategies for hepatitis B patients undergoing liver transplant: a cost-effectiveness analysis. Liver Transplant 2006 May;12(5):736–46.
4. Popp C, Krams D, Beckert C, et al. HBsAg blood screening and diagnosis: performance evaluation of the ARCHITECT HBsAg qualitative and ARCHITECT HBsAg qualitative confirmatory assays. Diagn Microbiol Infect Dis. 2011 Aug;70(4):479–85.

Table 1: Characteristics of the 35 HBsAg-positive LT recipients, stratified for the presence or absence of Hepatitis Delta Virus coinfection.

	Delta-Positive Recipients	**Delta-Negative Recipients**	
	n=18	**n=17**	***p-value***
Donor			
Age (yrs)	69.1 (24.2-84.8)	63.9 (52.9-84.2)	*p=0.5*
Sex M:F	10:8	11:6	*p=0.7*
Cause of brain death			
Cerebrovascular	11 (61%)	13 (76%)	*p=0.3*
Trauma	6 (33%)	2 (12%)	
Others	1 (6%)	2 (12%)	
Donor Risk Index (DRI)	1.92 (1.28-2.37)	1.87 (1.40-2.43)	*p=0.4*
HBcAb positivity	9 (50%)	8 (47%)	*p=0.99*
Recipient			
Age (yrs)	55.7 (25.8-66.8)	57.5 (45.4-63.4)	*p=0.44*
Sex M:F	14:4	16:1	*p=0.3*
Hepatocellular carcinoma prevalence	9 (50%)	14 (82%)	***p=0.044***
Biochemical MELD score			
at listing	13 (6-26)	10 (6-23)	*p=0.4*
at LT	13 (6-34)	10 (7-32)	*p=0.4*
Waiting list time (days)	46 (8-1270)	48 (1-207)	*p=0.5*
Quantitative HBsAg (IU/mL)			
at listing	6,206.47 (0.55–507,902.00)	2,313.40 (2.83–635,501.00)	*p=0.9*
at LT	2,865.20 (0.47–10,355.35)	399.01 (1.30–6,577.19)	*p=0.13*
HBV-DNA (IU/mL)			
at listing	20 (0–16593)	20 (0–11265)	*p=0.17*
at LT	20 (0–4259)	20 (0–163)	*p=0.8*
HBeAb positivity	15 (83%)	14 (82%)	*p=0.99*
Post-LT			
Day of serological target reached	3 (1–8)	2 (1–4)	*p=0.058*
HBIg used day 0 – day 30 (IU)	32,500 (12,500–51,500)	22,000 (9,500–38,000)	***p=0.0016***
HBIg saved day 0 – day 30 (IU)	14,750 (-1,500–37,500)	28,000 (12,000–40,500)	***p=0.0019***

Quantitative variables are expressed as median (range). Categorical variables are expressed as number (prevalence, %).
LT, liver transplantation. **MELD,** Model for End-stage Liver Disease. **HBV,** Hepatitis B Virus. **HBsAg,** HBV surface antigen. **HBsAb,** HBV surface antibody. **HBIg,** intravenous anti-Hepatitis B surface antigen immunoglobulins.

Figure 1: Correlation between HBsAg quantitative levels at LT and the cumulative dose of HBIg administered between day 0 and day 30 after LT in 35 HBsAg-positive recipients.



LT, liver transplantation**. HBIg,** intravenous anti-Hepatitis B surface antigen immunoglobulins.