USE OF HBSAG QUANTIFICATION TO GUIDE HBIG PROPHYLAXIS AFTER LIVER TRANSPLANTATION

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Disclosure: PDS has received speaker's fees from Biotest Italia and Grifols.
Received: 27.01.14 **Accepted:** 19.03.14
Citation: EMJ Hepatol. 2014;1:77-85.

ABSTRACT

Hepatitis B surface antigen (HBsAg) quantification has recently been introduced to guide treatment in chronic hepatitis B virus (HBV) patients. No information is currently available on use of HBsAg levels to guide HBV immune globulin (HBIG) administration after liver transplantation (LT). We performed a retrospective analysis of a prospectively collected database. Patients were included if: adults (≥18 years); recipients of a primary liver graft; HBsAg-positive and HBV DNA-negative at transplantation; hepatitis C and/or HIV-negative; not transplanted for fulminant hepatic failure; on nucleoside analogues. All patients were administered 30,000 IU HBIG, perioperatively, and hepatitis B surface antibody (HBsAb) was tested at day 7, 14, 28, and monthly thereafter. A further 30,000 HBIG were administered if HBsAb <100 mIU/mL and/or HBsAg >100 IU/mL on day 7. The primary endpoint was the efficacy of HBIG as a percentage of patients achieving HBsAg <100 IU/mL and HBsAb ≥100 mIU/mL at day 7. Secondary endpoints were performance of HBsAg levels in predicting HBsAg loss at day 7, HBV recurrence, graft, and patient survival at last follow-up. 41 LT recipients - transplanted between January 2011 and June 30, 2013 - were included (median age 54 years; male 78%). Hepatocellular carcinoma was present in 24 (58.5%) and hepatitis delta in 19 patients (46.4%); 7 (17.1%) patients did not achieve efficacy at day 7 and were boosted with additional 30,000 HBIG. A pre-transplant HBsAg level ≥1,000 IU/mL was associated with 60-fold odds for failure at day 7 (p=0.0002). At a median follow-up of 14 months after LT, graft and patient survival were 100% and no case of HBV recurrence had been observed. Based on our results, we advocate the use of HBsAg titre to guide HBIG prophylaxis after LT.

Keywords: Liver transplantation, hepatitis B virus, HBsAg, hepatitis B immune globulin, quantification.

INTRODUCTION

Hepatitis B virus (HBV)-related liver disease is one of the major indications of liver transplantation (LT) worldwide[1] and the leading indication in Asia.[2,3] When used in combination with oral nucleos(t)ide analogues (NA), hepatitis B immunoglobulin (HBIG) allows prevention of reinfection of the liver graft with HBV recurrence rates at <10% of recipients.[4,5] Although not entirely elucidated, the mechanisms accounting for the efficacy of HBIG prophylaxis are a reduced production of hepatitis B surface antigen (HBsAg) and a decreased rate of escape mutations in the presurface/surface gene and polymerase regions.[3] Due to recent introduction of more potent NA and to costs associated with use of HBIG,[6] there has been impetus to explore reduced-dose and/or short-term HBIG schedules, while maintaining low HBV recurrence rates.[3,7-11] As a result, several prevention strategies have been reported in the literature in the past decade, producing a shift in current practice

from high-dose intravenous (i.v.) HBIG - administered indefinitely from the intraoperative phase[8,9] - to life-long, low-dose intramuscular (i.m.) HBIG - without intraoperative administration[3] - to tailored HBIG schedules based on recipients' recurrence risk,[9,10] or selective and planned HBIG withdrawal.[10,11]

Quantification of HBsAg is increasingly used to determine the treatment response in patients with chronic hepatitis B (CHB).[12] Previous evidence suggests that in hepatitis B envelope antigen (HBeAg)-negative patients, HBV DNA <2,000 IU/mL and HBsAg <1,000 IU/mL can predict inactive carrier status, low risk of hepatocellular carcinoma (HCC), and the probability of HBsAg clearance.[12] When used in combination with HBV DNA, HBsAg decline can be used to predict response to interferon therapy, and a level <100 IU/mL during 6 months may be a marker of sustained response after treatment cessation.[13] There are limited data about the clinical implications of HBsAg quantification for LT recipients undergoing post-transplant prophylaxis with HBIG and on use of pre-transplant HBsAg titres to predict HBsAg clearance after transplantation. We investigated the clinical correlation between pre-transplant HBsAg levels and the probability of HBsAg loss in adult recipients of a liver graft-administered HBV prophylaxis with a combination regimen of NA and HBIG.

MATERIALS AND METHODS

Patients

This was a retrospective analysis of a prospectively collected database on adult (≥18 years) LT recipients transplanted at our institution for HBV-related disease. In January 2011 we implemented a quantitative measurement of HBsAg level for patients on the LT waiting list and transplant patients during their follow-up period. Patients were included in the current analysis if: 1) male or female recipients of a primary, whole-size, ABO-compatible liver graft from a deceased donor; 2) ≥18 years at transplantation; 3) HBsAg-positive (±hepatitis delta [HDV]); 4) HBV DNA-negative; 5) on NA therapy for ≥30 days before surgery. Patients were excluded from analysis if: 1) transplanted for HBV-related fulminant hepatic failure; 2) co-infected with hepatitis C virus and/or HIV; 3) enrolled in concurrent clinical trials on post-transplant HBV prophylaxis or immunosuppressants; 4) the liver was transplanted in combination with other organs; 5) deceased donors were HBsAg-positive.

Treatment Schedule

Patients underwent administration of 30,000 HBIG i.v. (NeoHepatect™, Biotest, Dreiech, Germany) over 5 days, starting from the day of transplant (6,000 daily) and in combination with NA. HBsAg titres were measured at transplantation (baseline) and at day 7, 14, 28, and monthly thereafter. Titres of the antibody to HBsAg (HBsAb) and HBV DNA were obtained at transplantation and at day 7, 14, 28, and monthly thereafter. If patients failed to achieve HBsAg titres <100 IU/mL and/or HBsAb ≥100 mIU/mL at day 7, a further 30,000 IU HBIG were administered i.v. over 5 days (6,000 daily). Once HBsAg was <100 IU/mL and HBsAb was ≥100 mIU/mL, patients were switched to i.m. (Igantibe™, Grifols, Barcelona, Spain) or subcutaneous (s.c.) (Zutectra™, Biotest, Dreieich, Germany) HBIG within 14 days of last i.v. administration, and dosing was adjusted as per HBsAb ≥100 mIU/mL through the entire follow-up period. With regard to NA, patients on lamivudine (LAM) were switched to entecavir (ETV, Baraclude™, Bristol-Myers-Squibb Italy, Rome, Italy) 0.5 mg/day starting from transplantation, while patients on ETV or tenofovir disoproxil (TDF, VireadTM, Gilead Italy, Milan, Italy) were kept on their pre-transplant treatment. Patients on combination of LAM and adefovir dipivoxil pre-transplantation (Hepsera™, Gilead Italy, Milan, Italy) were switched to TDF after surgery.

Immunosuppression

Patients were administered quadruple immunosuppression with 20 mg basiliximab i.v. (Simulect™, Novartis Italy, Origgio [VA]) at transplantation and on day 4, in association with tacrolimus (TAC, Prograf™, Astellas Pharma SpA, Assago [MI], Italy), steroids, and mycophenolate mofetil (MMF). TAC was initiated within 5 days after surgery according to renal function, and trough levels were 6-10 ng/mL for the first year and 3-8 ng/mL thereafter. MMF was initiated immediately after surgery at 1 g/day and maintained for 4 months, unless otherwise indicated by renal function. Steroids were started intraoperatively at 10 mg/kg and tapered within 3 months after surgery. Introduction of everolimus (EVR, target range 3-8 ng/mL) (Certican™ Novartis Italy, Origgio [VA]) starting at month 1 was evaluated on an individual basis when TAC minimisation was sought (3-5 ng/mL), such as in the presence of TAC-related adverse effects (i.e. renal function deterioration, neurotoxicity, diabetes mellitus, and cardiovascular

complications) or in patients with HCC and unfavourable prognosticators on explant histology (microvascular invasion, perineural infiltration, low grading).

Liver Histology

At donor's surgery, liver histology was obtained if clinically indicated. Post-transplantation, liver biopsy was performed in cases of suspicion of acute cellular rejection, HBV recurrence, or whenever clinically indicated.

Enrolment Period and Setting

Patient enrolment into the present treatment schedule started in January 2011. Current analysis includes patients transplanted until 30 June 2013. Setting: academic hospital. In Italy, the compulsory National Health System (NHS) covers for post-transplant prophylaxis with HBIG and pre and post-transplant treatment with NA.

Variables

The variables included in the current analysis were: 1) patients' demographics (gender, ethnicity, age at transplantation, body weight, height, body mass index); 2) donors' demographics (gender, ethnicity, age, body weight, height, body mass index, liver histology if available); 3) clinical (with focus on HDV co-infection and presence of HCC); 4) transplant data (date, model for end-stage liver disease [MELD] at transplant); 5) immunosuppression (drugs, doses, blood levels, and duration of treatment); 6) non-immunosuppressive treatment (with focus on HBIG dosing); 7) laboratory tests (liver function tests, serum creatinine, HBsAg (IU/mL), HBsAb (mIU/mL), and HBV DNA qPCR (IU/mL); 8) histology when clinically indicated.

Laboratory

HBsAg quantification and HBsAb titres were obtained with a chemiluminescence immunoassay (Architect™, Abbott, Chicago, Illinois, USA) with an inferior sensitivity threshold of 0.05 IU/mL for HBsAg and 10 mIU/mL for HBsAb. For the purposes of the current analysis, HBsAb values >1,000 mIU/mL were capped at 1,000.

Endpoints

The primary endpoint of the proposed study was efficacy of HBIG as the percentage of patients achieving HBsAg <100 IU/mL and HBsAb ≥100

Table 1: Demographic and clinical characteristics of the overall population (#41 patients).

Variable	
Age (years, median: IQR)	54, 9
Male (n, %)	32, 78
Ethnicity (Caucasian n, %)	41, 100
NA (LAM n, %)	20, 48.8
Duration of NA, months (median, IQR)	15, 12
HDV (n, %)	19, 46.4
HCC (n, %)	24, 58.5
MELD (median, IQR)	18.5, 8
Baseline HBsAg titre, IU/mL (median, IQR)	345.6, 419.8
Donor age, (years, median: IQR)	73, 11
Donor HBcAb (positive n, %)	11, 26.8
Brain dead donors (n, %)	41, 100
Whole-size graft (n, %)	41, 100
Time from listing to transplantation, months (median, IQR)	4, 12

HBsAg: hepatitis B surface antigen; HCC: hepatocellular carcinoma; HDV: hepatitis delta; IQR: interquartile range; LAM: lamivudine; MELD: model for end-stage liver disease; NA: nucleos(t)ide analogues; HBcAb: antibody to hepatitis B core antigen.

mIU/mL at day 7. The secondary endpoints were: evaluation of the performance of a pre-transplant HBsAg titre ≥1,000 IU/mL for prediction of the risk of failure at day 7;[13] identification of the pre-transplant HBsAg cut-off value for prediction of the risk of failure at day 7; incidence of HBV recurrence, defined as HBsAg and/or HBV DNA positivity after negativity or consistent liver histology; graft and patient survival at last follow-up. Graft survival was censored at the time of re-listing at the transplant centre(s) or at latest follow-up. Patient survival was censored at time of death or latest follow-up. HBV recurrence was censored at the time of HBV DNA and/or HBsAg positivity, histology, or latest follow-up.

Data Collection and Management

The study was approved by our Internal Review Board (IRB) and was carried out in compliance with the principles set forth in the Helsinki Declaration and in the Italian Medicinal Agency (Agenzia Italiana del Farmaco [AIFA]) code set for observational drug studies in human populations (www.aifa.gov.it). Data were imputed into an electronic database at our institution. Data anonymity and management were in agreement with the Italian data protection code law.

Statistical Analysis

According to type and level of distribution, data are reported as medians, interquartile ranges (IQR), percentiles, means, standard deviations (SD), ranges, extremes, and frequencies, as appropriate. The chi-square and the Fisher's tests were used for categorical variables, while the t-test or the Mann-Whitney tests were used for variables with continuous distribution according to their level of variance. A receiver operating characteristic (ROC) analysis was carried out to select the pre-transplant HBsAg cut-off value for prediction of HBsAg loss at day 7. Graft and patient survivals were obtained with the Kaplan-Meier curves. The level of statistical significance was set at 5% and the confidence interval at 95%.

RESULTS

Pre-Transplant

Out of 51 HBsAg-positive patients transplanted between January 2011 and June 30 2013, a total of 41 matched the eligibility criteria and were included in the current analysis (Table 1, Figure 1). The median (IQR) age was 54 years (9) (range 30-66), and 32 patients (78%) were male. Ethnicity was Caucasian in all patients.

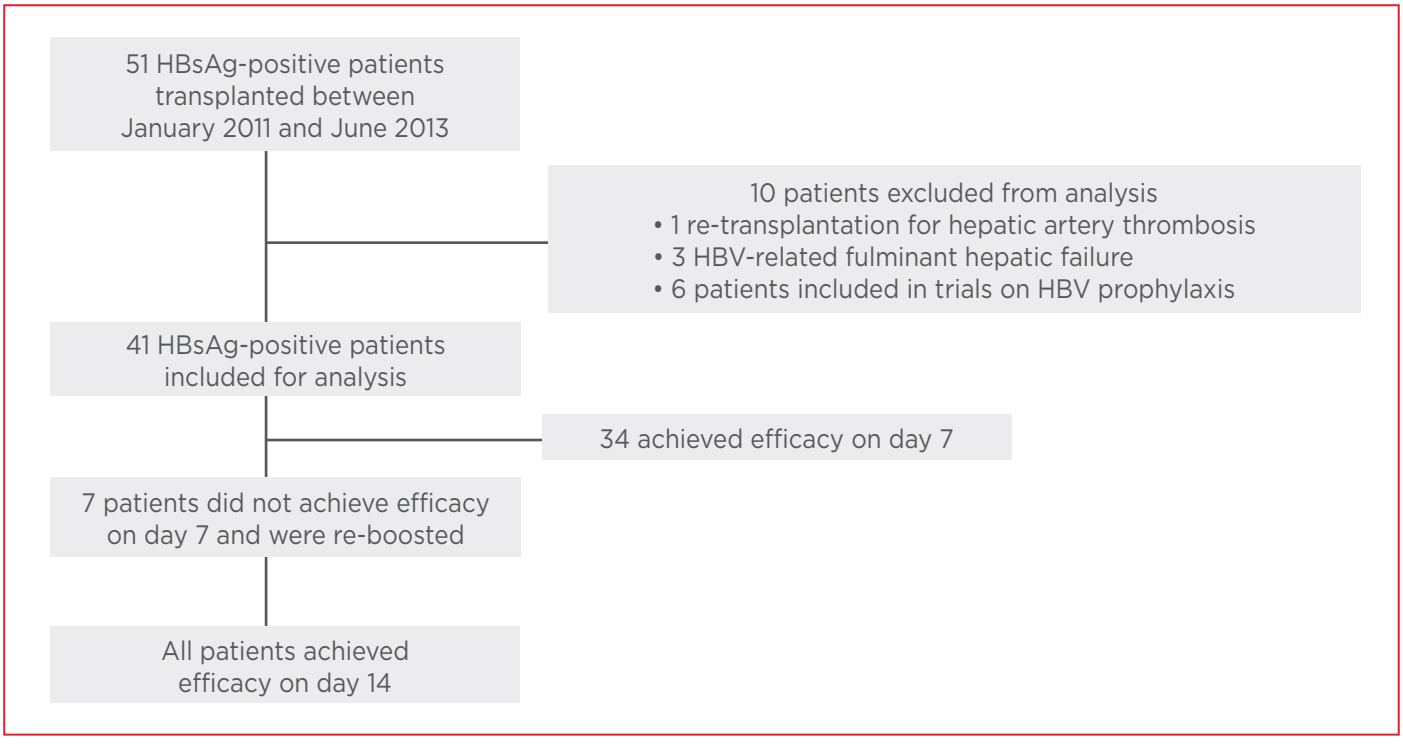

Figure 1: Patients' disposition algorithm.
HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus.

HCC was present in 24 (58.5%) and HDV infection in 19 (46.4%). Pre-transplantation, 20 (48.8%) patients were on LAM, 18 (43.9%) on ETV, 2 (4.9%) on LAM + ADF, and 1 (2.4%) on TDF. The median (IQR) duration of pre-transplant treatment with NA was 15 (12) months (range 3-43) in the overall population, whilst it was significantly longer (p<0.0001) for patients on LAM (median [IQR], 22.5 (16.2) months) versus those on ETV (median [IQR], 11.4 (6) months) (*data not shown*). Four (5.9%) patients had been administered interferon at any time point pre-transplantation. The median (IQR) time from wait listing to transplantation was 4 (12) months (range 1-14 months) and the median (IQR) MELD score at transplantation was 18.5 (8) (range 11-22). All donors were brain dead and graft was whole-size in all cases. The median (IQR) donor age was 73 (11) years (range 43-89) (Table 1).

Post-Transplant

On day 7 post-transplantation, the current schedule achieved efficacy in 34 (82.9%) patients (Table 2, Figure 1); 7 patients (17.1%) required additional administration of 30,000 IU HBIG, and all achieved efficacy on day 14 (Table 2, Figure 1). The HBsAg titre decreased from a median (IQR) of 345.6 (419.8) IU/mL (range 54.6-16,523) at transplantation to a median (IQR) of 0 (0) IU/mL (range 0-3,673.2) on day 7 (p=0.017), and the 7-day median (IQR) HBsAb level was 1,000 (342.2) mIU/mL (range 6.5-1,000). On day 14, HBsAg was lost in all patients and the median (IQR) HBsAb titre was 806.7 (345.7) mIU/mL (range 432.8-1,000) (p=0.45 between day 7 and 14). At a median (IQR) follow-up of 14 (6) months after LT (range 7-35) graft and patient survival were 100% and no cases of HBV recurrence had been observed.

Table 3 illustrates the univariate comparison between efficacy patients (#34) and failures (#7). Patients not achieving efficacy at day 7 had higher pre-transplant HBsAg levels than efficacy patients (median (IQR) 3,352.05 (2885.4) versus 345.2 (243.5) IU/mL; p<0.0001). An HBsAg titre ≥1,000 IU/mL was associated with 62.2 (95% CI 6.0-1993.2) odds for failure at day 7 (p=0.0002). The ROC curve analysis revealed that the cut-off value for prediction of HBsAg clearance at day 7 was 876.5 IU/mL (area under ROC curve =0.98) and was associated with 100% sensitivity (95% CI, 54.1-100%), 94.3% specificity (95% CI, 80.8-99.3%), 75% positive predictive, and 100% negative predictive values (Figure 2).

Table 2: Results.

Variable	Overall (#41)	Efficacy (#34)	Failures (#7)	p*
HBsAg titre at day 7, IU/mL (median, IQR)	0 (0)	0 (0)	2,178 (916)	<0.0001
HBsAg titre at day 14, IU/mL (median, IQR)	0 (0)	0 (0)	0 (0)	-
HBsAb titre at Day 7, mIU/mL (median, IQR)	1,000 (342.2)	1,000 (231.2)	12.9 (14)	<0.0001
HBsAb titre at Day 14, mIU/mL (median, IQR)	806.7 (345.7)	786.4 (345.7)	837.7 (434.7)	0.85
HBV recurrence** (%)	0 (0)	0 (0)	0 (0)	-
Graft survival** (%)	100	100	100	-
Patient survival** (%)	100	100	100	-

HBsAb: antibody to hepatitis b surface antigen; HBsAg: hepatitis B surface antigen; IQR: interquartile range; HBV: hepatitis B virus.
*p is between efficacy and failure groups.
**median (IQR) follow-up is 14 (6) months (range 7-35).

Table 3: Univariate comparison between efficacy patients (#34) and failures (#7).

Variable	Efficacy (#34)	Failures (#7)	p
Age, years (median, IQR)	54 (13)	53 (7)	0.65
Males (n, %)	25, 73.5	5, 71.4	0.65
NA (LAM n, %)	19, 55.9	3, 42.8	0.56
Duration of NA, months (median, IQR)	15 (13)	17 (19)	0.61
HDV (n, %)	15, 57.1	4, 44.1	0.68
HCC (n, %)	21, 61.7	3, 42.8	0.42
MELD (median, IQR)	22 (8)	19.5 (8)	0.88
Donor age, years (median, IQR)	73 (12)	70.5 (10)	0.91
Donor HBcAb (positive n, %)	9, 26.5	2, 28.6	0.88
HBsAg titre, IU/mL (median, IQR)	345.2 (243.5)	3,352.05 (2,885.4)	<0.0001
HBsAg titre ≥1,000 (n, %)	1, 3.0	5, 71.4	0.0002

HBsAg: hepatitis B surface antigen; HCC: hepatocellular carcinoma; HDV: hepatitis delta; IQR: interquartile range; MELD: model for end-stage liver disease; NA: nucleos(t)ide analogues; HBcAb: antibody to hepatitis B core antigen.

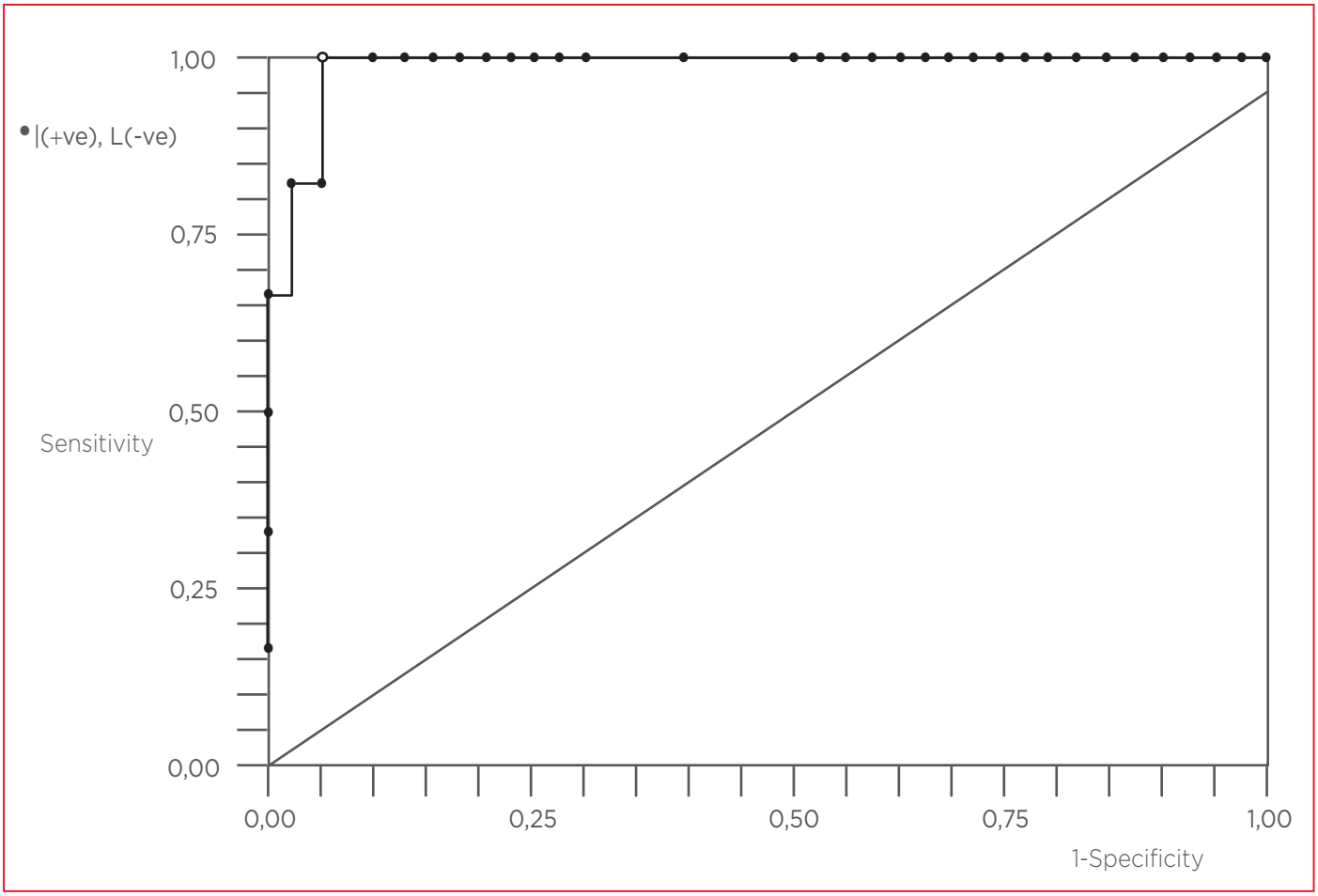

Figure 2: ROC curve analysis for pre-transplant HBsAg levels (IU/mL) in predicting probability of failure at day 7.

HBsAg: hepatitis B surface antigen; ROC: receiver operating characteristics.

DISCUSSION

In the setting of LT for HBsAg-related disease, HBsAg clearance is sought to reduce the risk of disease recurrence and its negative impact on graft and patient survival. To achieve this, the policy most frequently adopted post-transplantation is combination treatment with NA and HBIG.[2,5] Recent experimental data support the evidence that HBsAb is necessary for HBsAg neutralisation and clearance during HBV infection.[14] HBsAb exerts antiviral activities by blocking viral entry into cells, interfering with virion release, accelerating HBV clearance from the circulation, and leading to a decreased rate of escape mutations in the pre-surface gene/surface gene and polymerase regions.[15] Previous *in vitro* and *in vivo* studies have demonstrated that HBIG suppresses functional maturation of cytokines by human blood-derived dendritic cells and inhibits proliferation of peripheral T cells, thus reducing the incidence of rejection in the post-transplant period.[16] After LT, combination prophylaxis with HBIG and NA provides better control of HBV reinfection (<10% up to 3 years after LT)[17-20] versus either HBIG (15-25%)[21,22] or LAM mono-prophylaxis (20-40%),[23,24] and administration of HBIG is usually initiated in the intraoperative, anhepatic phase[8] or immediately after surgery.[3] The recent introduction of novel NA with better resistance profiles and the costs associated with immune globulins are challenging the paradigm of long-term HBIG administration and raising interest in low-dose HBIG and/or HBIG-sparing protocols.[11] Replacement of long-term HBIG with active immunisation has resulted in conflicting results, with some authors reporting achievement of protective HBsAb titres,[25,26] and others only a 7.1% rate (1/14 patients) of seroconversion after vaccination.[27]

One further strategy to reduce the economic burden associated with HBIG is aiming at lower HBsAb protective titres. While some authors still comply with titres emerging from early studies on HBIG monotherapy (<6 months post-LT =500 mIU/mL; 6-12 months post-LT =251-500 mIU/mL; and >1 year post-LT =150-250 mIU/mL),[22] the majority have lowered the threshold to ≥100 mIU/mL throughout the post-transplant period[3] and a few are suggesting even lower thresholds (≥50 mIU/mL) with favourable results.[5] Several reports have documented the feasibility and efficacy of a low-dose HBIG schedule, which currently seems the strategy most frequently adopted in Asia and Southern Europe.[28-30] Other investigators have attempted to discontinue HBIG after the initial post-transplant period and continue with antiviral monotherapy alone, especially in low-risk patients (usually HBeAg negative with undetectable HBV DNA at transplant), with recurrence rates varying from 0-16%.[11,31-33] However, HBIG withdrawal may be associated with a variable risk for HBsAg re-emergence and HBV recurrence, according to a patient's risk profile and viral characteristics. In one of the series with the longest patient follow-ups, the probability of HBV recurrence 4 years after HBIG discontinuation was 9%,[34] and eventual studies have yielded similar results (6.3% recurrence at a median follow-up of 24 months after HBIG withdrawal).[11]

Limited information is currently available on the factors contributing to HBsAg clearance after LT. To better understand the role of pre-transplant HBsAg titres, we set up the current study using recent evidence on HBsAg quantification in CHB. Introduction of commercial quantitative assays has improved our understanding of the fate of HBsAg in CHB patients, and a combination of HBsAg <1,000 IU/mL and HBV DNA <2,000 IU/mL can identify a 3-year inactive state in genotype D HBeAg-negative carrier populations.[13] In Asian populations, where genotype B and C are dominant, HBsAg levels between 10 and 100 IU/mL can predict loss over time.[35] In low-viraemic carriers, HBsAg levels <1,000 IU/mL have been associated with reduced risk for HBeAg-negative hepatitis, cirrhosis, and HCC.[35] In CHB patients treated with interferon, HBsAg quantification is being introduced to guide response to treatment,[13] whilst HBsAg decline is slow for CHB patients on NA. However, a rapid decline in HBsAg may help to identify patients with higher probability of HBsAg clearance in this latter category.[35]

Our study hypothesised that pre-transplant HBsAg levels might influence the rate and velocity of HBsAg loss after LT with a schedule of fixed-dose HBIG administration. This was in view of tailoring HBIG administration on the individual risk profile and improving cost-effectiveness of HBV prophylaxis. Our preliminary data suggest that patients with pre-transplant HBsAg ≥1,000 IU/mL require higher HBIG doses to achieve HBsAg clearance and protective HBsAb titres (≥100 mIU/mL). These patients presented a 60-fold higher risk for HBsAg persistence at day 7 after a course of

30,000 IU HBIG (p=0.0002) and underwent further boosting with 30,000 IU HBIG, as per the current protocol. However, future studies should focus on tailoring HBIG dosing until HBsAg clearance has been achieved by means of on-demand HBIG schedules guided by daily/weekly HBsAg and/or HBsAb testing. The cut-off value of 876.5 IU/mL we found in the present trial needs to be validated in larger series before being used in clinical practice to guide HBIG dosing in the early post-transplant phase.

CONCLUSION

The major limitation of the current study is that we did not fully investigate all viral and disease characteristics that might determine the fate of HBsAg clearance after LT, such as viral genotype and resistance, previous anti-viral treatments (NA versus INF), liver disease history, and severity. All of these factors should be evaluated and integrated to provide optimal care to HBsAg-positive LT recipients. However, to the best of our knowledge, this is the very first time that HBsAg quantification has been explored in LT population as a marker of HBsAg loss and seroconversion with a fixed-dose HBIG schedule. We advocate larger series to introduce such a marker in clinical practice and guide, among other clinical parameters, HBIG dosing in the early post-transplant course.

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