Prediction of occult hepatitis B virus infection in liver transplant donors through hepatitis B virus blood markers.

PREDICTION OF OCCULT HEPATITIS B VIRUS INFECTION IN LIVER TRANSPLANT DONORS THROUGH HEPATITIS B VIRUS BLOOD MARKERS

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ABSTRACT

Background: Occult HBV infection, also called OBI, is defined as presence of HBV-DNA in the liver of HBsAg-negative individuals having detectable or undetectable HBV-DNA in blood. However, in deceased donors, results of tissue analysis cannot be obtained prior to allocation for liver transplantation.

Aims: In deceased donors, we investigated OBI prevalence and predictability using blood markers of HBV exposure/infection.

Methods: In 50 consecutive HBsAg-negative/HBcAb-positive and 20 age-matched HBsAg-negative/HBcAb-negative donors, a nested-PCR assay was employed in liver biopsies for OBI diagnosis according to Taormina criteria. All donors were characterized for plasma HBV-DNA and serum HBsAb/HBeAb.

Results: In liver tissue, OBI prevalence was 60% (30/50) in HBcAb-positive donors and nil (0/20) in HBcAb-negative ones ($p<0.0001$). All HBcAb-positive donors with a detectable HBV-DNA in plasma (n=5) or HBsAb>1,000 mIU/mL (n=5) eventually showed OBI, making 10 out of 30 OBI-positive donors in which the condition could have been ascertained prior to transplantation. In the remaining 40 HBcAb-positive donors, HBeAb-positivity and/or HBsAb≥58 mIU/mL signified a 62% OBI probability, while HBeAb-negativity and HBsAb<58 mIU/mL indicated a 29% probability.

Conclusions: In deceased donors, combining HBcAb with other blood markers of HBV exposure/infection allows to predict one third of OBI cases with certainty and speed. These findings might help refine the allocation of livers from HBcAb-positive donors.

KEY WORDS

OBI, plasma HBV-DNA, hepatitis B virus serology, anti-hepatitis B virus core antigen antibody positive donor

LIST OF ABBREVIATIONS:

HBcAb: anti-hepatitis B core antigen antibody

HBeAb: anti-hepatitis B e antigen antibody

HBeAg: hepatitis B e antigen

HBsAb: anti-hepatitis B surface antigen antibody

HBsAg: hepatitis B surface antigen

HBV: hepatitis B virus

HCV: hepatitis C virus

HIV: human immunodeficiency virus

LT: liver transplantation

MELD: Model for End-stage Liver Disease

NAT: nucleic acid testing

OBI: occult hepatitis B virus infection

PCR: Polymerase Chain Reaction

ROC: receiver operating characteristic

INTRODUCTION

Occult hepatitis B virus infection (OBI) is defined as the presence of hepatitis B virus (HBV) DNA in the liver of subjects negative for hepatitis B surface antigen (HBsAg), with or without serological markers of previous viral exposure[1],[2],[3]. In OBI, the lack of circulating HBsAg may be due to rearrangements in the HBV genome in the liver which interfere with gene expression or lead to the production of an antigenically modified S protein[4],[5],[6]. The molecular basis of OBI is related to the long-lasting persistence in the nuclei of hepatocytes of the viral covalently-closed-circular DNA[7]. Almost all OBI cases are infected with a replication-competent HBV kept strongly suppressed by host immune-surveillance and epigenetic factors[8]. As a consequence of the viral suppression, the amount of circulating HBV-DNA is generally undetectable or very low (<200 IU/mL)[8]. Although OBI status is significantly associated with HBV serum markers[3] – especially with anti-hepatitis B core antigen antibody (HBcAb)[9] – the analysis of liver DNA extracts represents the gold standard for OBI evaluation[8].

OBI-positivity in liver grafts may be responsible for *de novo* hepatitis B after liver transplantation (LT), elicited by the state of immunosuppression in the recipients[10]. Yet, a timely recognition of OBI in the liver graft in the context of deceased donor LT is not feasible due to both the technical constraints of biomolecular analyses and the strict timeframe inherent to the transplantation process. For this reason, donor HBcAb-positivity has generally been used as a surrogate marker of OBI in studies focusing on LT outcomes. In the absence of prophylaxis after LT, the overall risk of HBV disease transmission with HBcAb-positive liver grafts ranges from 10% to 15% in HBcAb-positive recipients to 48-58% in naïve ones[11],[12],[13]. In addition, in a recent large Italian prospective cohort study, livers from HBcAb-positive donors (which accounted for 16% of the donor pool) showed a worse outcome when transplanted into HBsAg-negative recipients, a phenomenon unrelated to HBV reactivation[14].

The relationship between OBI and HBcAb-positivity in liver donors has never been investigated and, above all, no study has addressed the feasibility of donor OBI prediction by HBV blood markers in the setting of LT. The aims of this study were to investigate: 1) OBI prevalence in deceased donors used for LT, with a particular focus on HBcAb-positive donors; 2) the possibility to predict OBI graft status by means of HBV exposure/infection blood markers that can be determined in the donor prior to graft allocation.

METHODS

From November 2010 to August 2013, 52 consecutive HBsAg-negative HBcAb-positive deceased heart-beating donors were prospectively enrolled for OBI testing at the Liver Transplant Center of the University of Turin. Two livers were not used because of evidence of cirrhosis at harvesting and were excluded. So, the study group consisted of 50 HBcAb-positive donors whose livers were eventually transplanted. In the same period, 20 HBsAg-negative HBcAb-negative donors, matched for age to HBcAb-positive ones, were selected as control group. For each of the 70 donors, 5 ml of serum, 10 ml of plasma and a liver needle biopsy (harvested before cross-clamping and collected in RNA-later solution) were obtained. All specimens sent to the laboratory were stored at -80°C until processing.

The allocation policy of grafts from HBcAb-positive donors which was followed in our Center was: first option to HBsAg-positive candidates; second option to HBsAg-negative candidates without hepatitis C virus (HCV) infection; third option to HCV-RNA positive patients[15],[16]. Combined long-term prophylaxis with nucleos(t)ide analogues and high doses of intravenous anti-hepatitis B surface antigen immunoglobulins was employed in HBsAg-positive recipients, while HBsAg-negative recipients of a HBcAb-positive graft received lamivudine and low doses of intravenous anti-hepatitis B surface antigen immunoglobulins.

Serum markers of HBV exposure/infection.

Donor serum samples were tested for the whole panel of HBV markers [HBsAg; hepatitis B e antigen (HBeAg); anti-hepatitis B surface antigen antibody (HBsAb); HBcAb; anti-hepatitis B e antigen antibody (HBeAb); **Table 1**] by the ARCHITECT® chemiluminescent microparticle immunoassays (Abbott Laboratories, Abbott Park, IL). Qualitative results (positive/negative) were assessed using Index S/CO (Sample/Cut Off relative light units), while quantitative results were expressed as milli-International Units per milliliter (mIU/mL). In particular, for HBsAb titers, the sensitivity threshold was 10 mIU/mL and the upper limit of quantification was 1,000 mIU/mL.

Detection and quantification of plasma HBV-DNA

HBV-DNA was detected and quantified in the donor plasma by a fully automated high-sensitivity system, the COBAS® AmpliPrep-COBAS® TaqMan® HBV test ver 2.0 (Roche Molecular Systems, Inc., Branchburg, NJ)[17]. The analysis was performed on 650 µl of human plasma. After HBV-DNA extraction a real-time polymerase chain reaction (PCR) test was performed by the COBAS® TaqMan®analyser with a multiplex TaqMan® assay. The results were expressed as International Units per milliliter (IU/mL). The concentration of HBV-DNA that can be detected with a positivity rate greater than 95% is 9 IU/mL for plasma (which is the lower limit of detection), while the lower limit of quantification is 20 IU/mL.

Detection of intrahepatic HBV-DNA and OBI definition

Frozen liver biopsies were disrupted in 500 µl of lysing buffer by a rotor-stator homogenizer and incubated

overnight with proteinase K (20 mg/mL) at 37°C. After extraction with phenol/chloroform, nucleic acids were precipitated in isopropanol and incubated overnight at -80°C. Precipitated DNA was washed twice with 70% cold ethanol, dried and resuspended in 50 µl sterile water. Concentration and quality were assessed with a spectrophotometer (NanoDrop ND 1000, NanoDrop Technologies, Wilmington, DE).

OBI was investigated as previously described[18]. Briefly, extracted liver DNAs were analysed for the presence of HBV genomes by four parallel nested-PCRs to detect HBV S, Core, Pol and X sequences (sensitivity threshold at 5 IU/mL). PCR primers were complementary to highly conserved nucleotide sequences of HBV genome. Two rounds of amplification, 35 cycles each, were performed using HotStartTaq Polymerase (Qiagen, Germany). Appropriate negative and positive controls were included in each PCR experiment. To check for false negatives a parallel PCR for the β-globin gene was performed. Samples positive for at least two HBV targets were scored as OBI-positive according to Taormina expert meeting statements[8].

Intrahepatic HBV-DNA sequencing

Intrahepatic HBV-DNA samples from a selected number of liver donors were amplified with HBV primers flanking the S gene. Because of the low yield of the first-round PCR (sense primer HBV2816, 5'-GGGTCACCATATTCTTGGG-3'; anti-sense primer HBV704, 5'-CGAACCACTGAACAAATGGC-3'), a second-round PCR was performed (sense primer HBV2823, 5'-TCACCATATTCTTGGGAACAAGA-3'; anti-sense primer HBV704). DNA sequencing was performed by Sanger sequencing method by BMR Genomics service (BMR Genomics, Padua, Italy). The sequence chromatograms were visualized using computer software (ChromasPro v.1.41, Technelysium Pty Ltd, Tewantin, Australia) and the deduced amino-acid sequences were aligned using ClustalW2 software (http://www.ebi.ac.uk/Tools/msa/clustalw2/).

Statistical analysis

Collected donor and recipient data were entered into the SPSS 18.0 software (SPSS Inc., Chicago, IL): categorical variables were analyzed with χ^2 test, quantitative variables with non-parametric Mann-Whitney test. In HBcAb-positive donors with undetectable plasma HBV-DNA and serum HBsAb <1,000 mIU/mL, a receiver operating characteristic (ROC) curve was drawn to set the cut-off of HBsAb level for the best prediction of OBI. The level of significance was set at *p* value <0.05.

RESULTS

The 50 HBsAg-negative HBcAb-positive donors and the 20 HBsAg-negative HBcAb-negative ones were similar regarding donor and recipient characteristics except for, as expected, the prevalence rate of markers of HBV exposure/infection and the different etiology of liver disease in the recipient (**Table 1**). Thirty-five (70%) HBcAb-positive donors were allocated to HBsAg-positive recipients and 13 (26%) were used, as second option, in HBsAg-negative/anti-HCV-negative ones. Only 2 (4%) HBcAb-positive grafts were transplanted into anti-HCV-positive candidates, in one case because of serum HCV-RNA negativity of the recipient, in the other case because of donor anti-HCV-positivity. After LT, none of the recipients with a follow-up > 90 days developed clinical, biochemical or serological signs of recurrent or de novo hepatitis B (median follow-up 606 days, range 109-1121).

Biomolecular diagnostics identified OBI in liver biopsies of 60% (30/50) HBcAb-positive donors and of 0% (0/20) HBcAb-negative ones ($p<0.0001$) (**Table 1**). In the latter, no positivity on tissue was ever found for the four genes (S, Core, Pol and X) of HBV genome.

Five HBcAb-positive donors had a detectable HBV-DNA in plasma, four at an extremely low level (<20 IU/mL) and the fifth one at 36 IU/mL (**Table S-1**). HBsAb titers were above the sensitivity threshold of 10 mIU/mL in four of them, while a positivity for all the four genes (S, Core, Pol and X) of HBV genome was found in the liver biopsies of all these five donors, which were classified as OBI-positive (**Table S-1**). All sequenced HBV genomes were genotype D and alignment of the amino-acid sequences showed few polymorphism but no mutation in the a-determinant (or nearby it) able to abolish the two loop structure. In the 50 HBcAb positive donors, OBI could be predicted by a detectable plasma HBV-DNA result with a sensitivity of 17%, a specificity of 100%, a positive predictive value of 100% and an accuracy of 50%.

Out of the other 45 HBcAb-positive donors with an undetectable HBV-DNA in plasma, five were found to have a serum HBsAb titer >1,000 mIU/mL; all those five were eventually classified as OBI-positive (with three showing a positivity for all the four genes of HBV in liver biopsies, **Table S-1**). In the 45 cases with an undetectable plasma HBV-DNA result, OBI could be predicted by a serum HBsAb >1,000 mIU/mL with a sensitivity of 20%, a specificity of 100%, a positive predictive value of 100% and an accuracy of 56%. Adding the five cases with a detectable plasma HBV-DNA to the five cases with serum HBsAb >1,000 mIU/mL, the diagnosis of OBI could have been ascertained prior to LT using only HBV blood markers in 10 (20%) HBcAb-positive donors, accounting for one third (10/30) of the OBI cases overall (**Figure 1**).

In the ROC curve drawn in the remaining 40 HBcAb-positive donors with an undetectable plasma HBV-DNA result and serum HBsAb <1,000 mIU/mL, the best compromise between sensitivity and specificity for OBI prediction was found at a HBsAb titer of 58 mIU/mL (area under the curve=0.59). Among them, a 62% (16/26) prevalence of OBI was present in donors with HBeAb-positivity and/or HBsAb ≥58 mIU/mL, while only 4 of the 14 (29%) HBeAb-negative and HBsAb <58 mIU/ml donors were positive for OBI (**Figure 1**). Of these four donors, 2 were HBcAb-positive and HBsAb positive with a titer of 11 mIU/mL and 18 mIU/mL respectively, while 2 were HBcAb-positive alone (**Table S-1**). Then, in the 40 cases with an undetectable

plasma HBV-DNA and a serum HBsAb <1,000 mIU/mL, OBI could be predicted by the presence of HBeAb-positivity and/or HBsAb ≥58 mIU/mL with a sensitivity of 80%, a specificity of 50%, a positive predictive value of 62% and an accuracy of 65%.

DISCUSSION

The first study finding is that OBI prevalence in a consecutive cohort of HBsAg-negative HBcAb-positive liver donors was 60%, while it was nil in a selected group of HBcAb-negative ones. In a research on individuals without hepatic disease, Raimondo et al[19] reported the same prevalence of OBI in HBcAb-positive subjects, but a 7.3% in seronegative ones. We acknowledge that our study, mainly focusing on HBcAb-positive donors, was downsized for the recognition of seronegative OBI. However, since the prevalence of HBcAb-positivity is higher in elderly donors[20],[21], we matched for age the HBcAb-negative group with the HBcAb-positive one in order to balance baseline conditions. Despite this, we encountered no OBI case in HBcAb-negative donors, supporting the concept that the occurrence of seronegative OBI might be a rare occurrence in the setting of LT.

The second, most relevant, study finding is that OBI can be detected with certainty in 10% of HBcAb-positive donors through high-sensitivity plasma HBV-DNA testing. Various mechanism, both host- and viral-related, have been proposed to explain HBV inhibition and OBI status induction, but the reasons for persistence of low levels of HBV-DNA in the absence of detectable HBsAg remain partially undefined[22]. HBV mutations in surface gene interfering with antibody recognition of HBsAg (false OBI) or deletions in pre-S region leading to an impaired HBsAg synthesis have been described[23],[24]. In our study, however, we sequenced the surface coding gene and found no mutations capable of modifying the antigenicity of the S protein, thus indicating that host factors play a major role in suppressing the viral activities[25]. Recently, Bes[26] observed a potent HBV-specific T-cell response to HBV antigens in blood donation candidates with OBI, even when anti-HBs levels were undetectable; that response had the capability to suppress viral replication to low viral loads and to reduce HBsAg expression to undetectable levels.

At variance with circulating HBV genomes, which are a direct proof of OBI, extremely elevated titers of serum anti-HBsAg antibodies provide only an indirect evidence. Nevertheless, a mechanism of chronic antigenic booster exerted by a virus actively replicating within the liver can be suggested to explain why all our 5 (10%) HBcAb-positive donors with a HBsAb titer >1,000 mIU/mL were eventually classified as OBI-positive.

From a practical point of view, in the current era of safety and quality concerns in LT, nucleic acid testing (the so-called NAT)[27] is already employed frequently to minimize the risk of donor-to-recipient viral transmission (not only HBV, but also HCV and human immunodeficiency virus, HIV), especially when the donor presents specific risk factors. The technique of HBV-DNA detection in plasma is rapid, reliable and feasible prior to graft allocation, as it is HBsAb titering. So, these tests could be proposed for the routine typing of HBcAb-positive donors, in whom the presence of a detectable plasma HBV-DNA result or a serum HBsAb titer >1,000 mIU/mL signify a 100% specificity and positive predictive value for OBI detection. In HBcAb-positive donors, such implementation would allow the recognition of OBI in 20% of them and, more notably, would result in the quick identification of as much as one third of the OBI-positive grafts that are currently employed for LT.

As for the remaining 80% HBcAb-positive donors, the serological prediction of OBI remains elusive. Only a presumptive indication can be provided by the combination of other serum markers of HBV exposure (such as HBeAb or HBsAb at lower titers, allowing a 50% specificity and a 62% positive predictive value), but considerable uncertainties persist in the diagnosis of OBI in those liver donors before LT.

Potential clinical implication of our findings is that a timely and certain diagnosis of OBI in the liver donor could help guide graft allocation. In a prospective observational study, Angelico et al[14] showed that the outcome of HBcAb-positive grafts is worse when they are transplanted into HBsAg-negative candidates: the effect on survival was not due to HBV reactivation (which accounted for only 1.5% of graft losses in an era of extensive use of anti-HBV prophylaxis), rather recurrent HCV disease was the most frequent cause of graft loss (19.7%). These findings are totally in line with our retrospective experience regarding the outcome of LT using livers from HBcAb positive donors[15],[16], and explain our current allocation policy tending to avoid the transplantation of those organs in HCV-infected candidates. In immunocompetent hosts, a viral interaction within the liver of HBV and HCV has already been hypothesized to be responsible for a worse course of liver disease[9],[28], but a consensus is still lacking on the negative impact of donor OBI on fibrosis progression after LT in HCV-positive recipients[29]. In any case, in our opinion, it is sensible to enforce allocation of certainly OBI-positive grafts (based on serum HBcAb and plasma HBV-DNA or serum HBsAb results) to HBsAg-positive patients. In fact, those subjects already require a combined anti-HBV prophylaxis according to current guidelines[11]. Furthermore, they suffer from no detrimental effect on survival related to that type of donor[14] and are anyhow exposed to a non-negligible rate of graft reinfection, even in the absence of clinically apparent manifestations[30],[31].

In conclusion, in deceased liver donors, combining HBcAb with other blood markers of HBV exposure/infection allows to predict one third of OBI cases with certainty and speed. These notions might help refine the allocation process of liver grafts harvested from HBcAb-positive donors.

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FIGURE LEGEND

Figure 1: Diagnostic flow diagram for prediction of occult hepatitis B virus infection in hepatitis B surface antigen negative and anti-hepatitis B core antigen antibody positive donors using hepatitis B virus blood markers in the setting of liver transplantation

HBcAb=anti-hepatitis B core antigen antibody; **HBeAb**=anti-hepatitis B e antigen antibody; **HBsAb**=anti-hepatitis B surface antigen antibody; **HBV**=hepatitis B virus; **OBI**=occult hepatitis B virus infection.

Figure(s)

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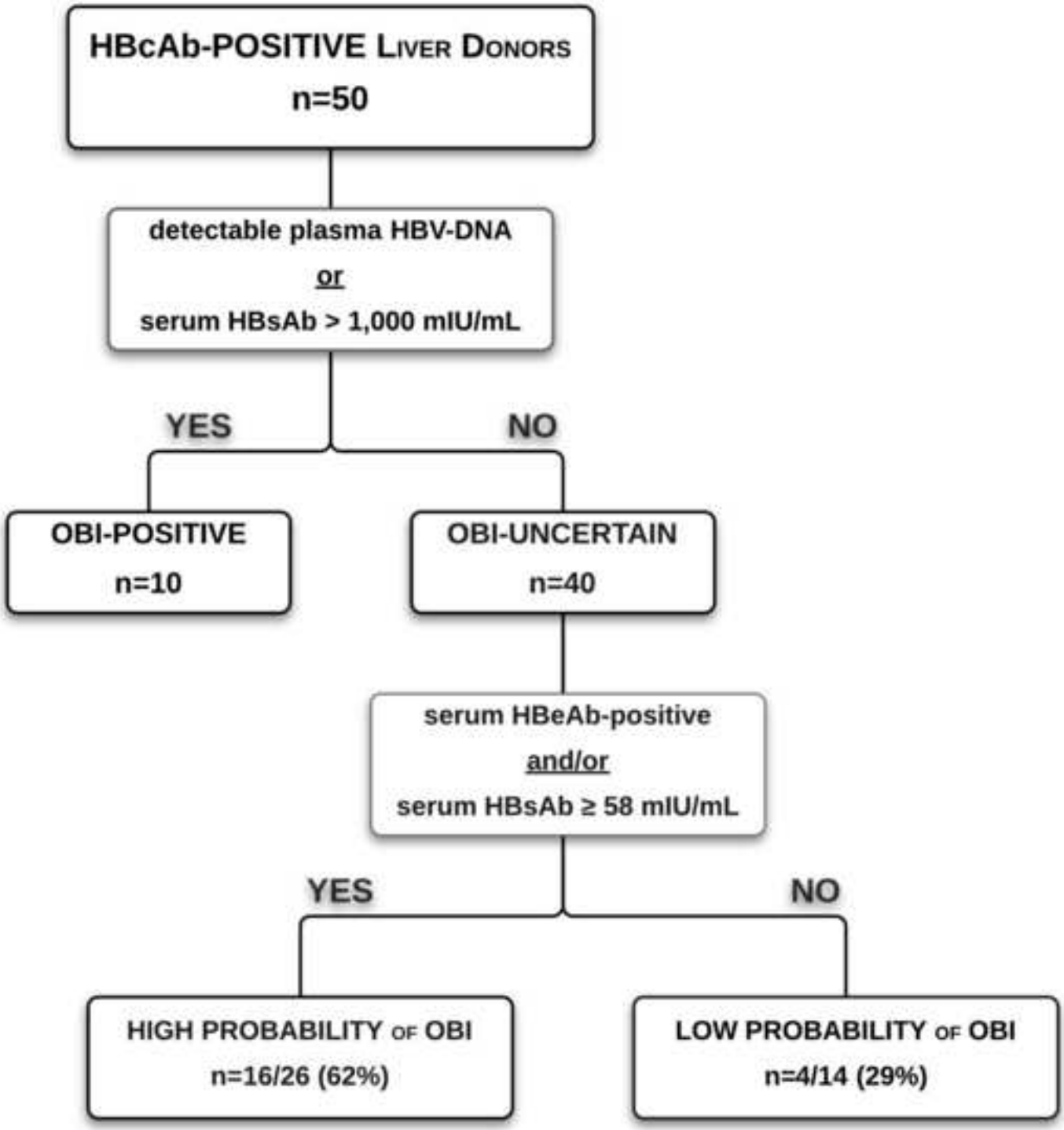

Figure(s)

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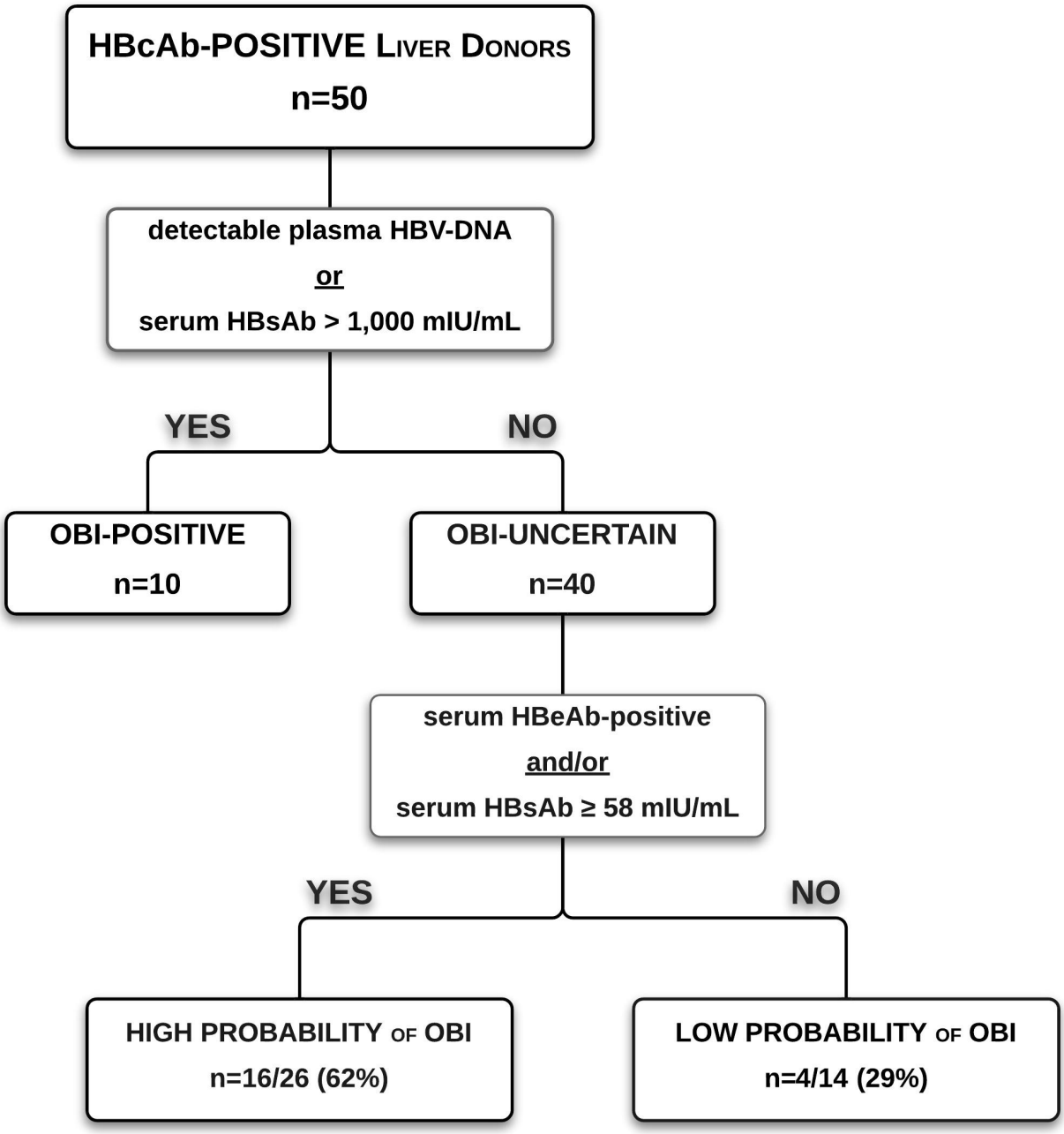

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Table(s)

TABLE 1: Comparison of donor and recipient features in the 70 donors tested for blood markers of hepatitis B virus exposure/infection and for occult hepatitis B virus infection in the liver .

	HBcAb-Positive Donors n=50	HBcAb-Negative Donors n=20	*p value*
DONOR FEATURES			
Age (years)	67 (57-76)	73 (64-81)	*0.1*
Gender			
Male	29 (58%)	9 (45%)	*0.42*
Cause of brain death			
Cerebrovascular	41 (82%)	16 (80%)	
Trauma	6 (12%)	3 (15%)	*0.93*
Anoxia	3 (6%)	1 (5%)	
Donor Risk Index	1.91 (1.72-2.19)	2.05 (1.83-2.20)	*0.4*
Macrovesicular steatosis (%)	0 (0-10)	0 (0-5)	*0.66*
Microvesicular steatosis (%)	5 (0-20)	0 (0-10)	*0.29*
Fibrosis stage (Ishak score)	0 (0-0)	0 (0-0)	*0.79*
HBV blood markers			
Serum HBsAg positive	0	0	
Serum HBeAg positive	0	0	
Serum HBsAb >10 mIU/mL	41 (82%)	3 (15%)	***<0.0001***
Serum HBeAb positive	18 (36%)	0	***0.0019***
Detectable HBV-DNA in plasma	5 (10%)	0	0.14
OBI in liver biopsy	30 (60%)	0	***<0.0001***
RECIPIENT FEATURES			
Age (years)	57 (51-61)	58 (54-62)	*0.084*
Gender			
Male	41 (82%)	18 (90%)	*0.49*
Liver Disease Etiology			
Hepatitis B virus	35 (70%)	2 (10%)	***<0.0001***
Hepatitis C virus	2 (4%)	6 (30%)	

Alcohol	7 (14%)	5 (25%)	
Other	6 (12%)	7 (35%)	
Hepatocellular Carcinoma prevalence	19 (38%)	10 (50%)	0.42
MELD at transplantation	15 (11-18)	16 (12-21)	0.31

Quantitative variables are expressed as median (interquartile range); categorical variables as number (prevalence, %).

HBcAb=anti-hepatitis B core antigen antibody; **HBeAb**=anti-hepatitis B e antigen antibody; **HBeAg**=hepatitis B e antigen; **HBsAb**=anti-hepatitis B surface antigen antibody; **HBsAg**=hepatitis B surface antigen; **HBV**=hepatitis B virus; **MELD**=Model for End-stage Liver Disease; **OBI**=occult hepatitis B virus infection.

Supplementary Material (for online publication)

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TABLE S-1: Panel showing the results of hepatitis B virus blood markers, of four nested-polymerase chain reaction assays in liver biopsies and of occult hepatitis B virus infection graft status in the 50 anti-hepatitis B core antigen antibody positive donors.

Donor N.	HBV-DNA (IU/mL)	HBsAb (mIU/mL)	HBcAb	HBeAb	HBV S	HBV Core	HBV Pol	HBV X	OBI graft status
25	Detectable, 36	13	Positive	Negative	+	+	+	+	Positive
9	Detectable, <20	159	Positive	Positive	+	+	+	+	Positive
13	Detectable, <20	215	Positive	Positive	+	+	+	+	Positive
18	Detectable, <20	<10	Positive	Negative	+	+	+	+	Positive
38	Detectable, <20	751	Positive	Positive	+	+	+	+	Positive
7	Undetectable	>1000	Positive	Positive	+	+	+	+	Positive
10	Undetectable	>1000	Positive	Negative	+	+	+	+	Positive
40	Undetectable	>1000	Positive	Positive	+	+	+	+	Positive
44	Undetectable	>1000	Positive	Positive	+	+	-	+	Positive
50	Undetectable	>1000	Positive	Positive	+	-	-	+	Positive
11	Undetectable	<10	Positive	Positive	+	+	+	+	Positive
12	Undetectable	300	Positive	Positive	+	+	+	+	Positive
14	Undetectable	79	Positive	Positive	+	+	+	+	Positive
28	Undetectable	99	Positive	Negative	+	+	+	+	Positive
30	Undetectable	62	Positive	Negative	+	+	+	+	Positive
31	Undetectable	96	Positive	Positive	+	+	+	+	Positive
32	Undetectable	67	Positive	Negative	+	+	+	+	Positive
37	Undetectable	22	Positive	Positive	+	+	+	+	Positive
39	Undetectable	60	Positive	Negative	+	+	+	+	Positive
41	Undetectable	18	Positive	Negative	+	+	+	+	Positive
45	Undetectable	<10	Positive	Negative	+	+	+	+	Positive
48	Undetectable	42	Positive	Positive	+	+	+	+	Positive
20	Undetectable	11	Positive	Negative	+	+	-	+	Positive
43	Undetectable	284	Positive	Negative	+	-	+	+	Positive
8	Undetectable	101	Positive	Positive	+	+	-	-	Positive
21	Undetectable	228	Positive	Negative	+	+	-	-	Positive
19	Undetectable	636	Positive	Negative	+	-	+	-	Positive
24	Undetectable	<10	Positive	Negative	+	-	+	-	Positive
3	Undetectable	77	Positive	Negative	-	+	+	-	Positive
33	Undetectable	328	Positive	Negative	-	-	+	+	Positive
1	Undetectable	82	Positive	Negative	+	-	-	-	Negative
36	Undetectable	636	Positive	Positive	+	-	-	-	Negative
6	Undetectable	448	Positive	Negative	-	-	+	-	Negative
23	Undetectable	<10	Positive	Negative	-	-	+	-	Negative
4	Undetectable	11	Positive	Negative	-	-	-	+	Negative
15	Undetectable	46	Positive	Positive	-	-	-	+	Negative
2	Undetectable	77	Positive	Positive	-	-	-	-	Negative
5	Undetectable	19	Positive	Negative	-	-	-	-	Negative
16	Undetectable	64	Positive	Negative	-	-	-	-	Negative
17	Undetectable	<10	Positive	Negative	-	-	-	-	Negative
22	Undetectable	<10	Positive	Negative	-	-	-	-	Negative
26	Undetectable	138	Positive	Negative	-	-	-	-	Negative

27	Undetectable	<10	Positive	Negative	-	-	-	-	Negative
29	Undetectable	643	Positive	Negative	-	-	-	-	Negative
34	Undetectable	56	Positive	Positive	-	-	-	-	Negative
35	Undetectable	16	Positive	Negative	-	-	-	-	Negative
42	Undetectable	17	Positive	Negative	-	-	-	-	Negative
46	Undetectable	29	Positive	Negative	-	-	-	-	Negative
47	Undetectable	215	Positive	Negative	-	-	-	-	Negative
49	Undetectable	<10	Positive	Negative	-	-	-	-	Negative

HBcAb=anti-hepatitis B core antigen antibody; **HBeAb**=anti-hepatitis B e antigen antibody; **HBsAb**=anti-hepatitis B surface antigen antibody; **HBV**=hepatitis B virus; **OBI**=occult hepatitis B virus infection.