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Hepatitis B virus reactivation after heart transplant: Incidence and clinical impact

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ABSTRACT

Background: Occult hepatitis B infection consists of persistence of HBV genomes in hepatocytes,absence of serum HBsAg, low/undetectable serum HBVDNA. Reactivation of HBV infection may occur during immunosuppression, but few data are available in heart transplant.

Objectives: We followed-up heart recipients with or without markers of previous HBV infection,evaluating prevalence of HBV markers, incidence of HBV reactivation and its virological and clinical features.

Study design: Heart failure patients listed for heart transplant (2007–2013) were screened for current or past HBV infection. Transplanted patients with past HBV infection (anti-HBc + / ± anti-HBs + /HBVDNA −) were followed up as cases, and an equal number of HBV negative patients as controls. Virological reactivation was detected by standard real-time and home-made highly sensitive PCR (surface/core HBVDNA regions). Clinical status and progression were assessed by liver histology, ultrasound or elastography.

Results: 67 patients underwent heart transplant, including 4 (5.9%) HBsAg + subjects. Cases were 11/67 (16.4%). During a median follow-up of 30 months, only one of these 11 patients presented viral reactivation (HBVDNA 209 IU/mL) at month 22, and started antiviral treatment. Four other recipients showed virological events of uncertain significance (sensitive PCR-only intermittently positive). Clinical signs of liver disease were observed in only one case at the last follow-up. A nonsignificant difference in survival was observed between cases and all other heart recipients without prior HBV contact (death rate 5/11 *vs* 15/52, respectively; $p = 0.097$).

Conclusions: HBV genotypic reactivation in HBsAg − /anti-HBc + /HBVDNA − heart recipients is uncommon. Virological events of uncertain significance occur more frequently; their clinical impact seems to be negligible.

1. Background

Hepatitis B virus (HBV) infection remains a major public health problem with about 248 million people chronically infected [1]. Sexual and parenteral transmission occurs not only from HBV surface antigen (HBsAg) positive subjects, but also from HBsAg-negative donors [2,3] with the so called "occult hepatitis B infection (OBI)". OBI consists in the long-term persistence of viral genomes (covalently closed circular DNA and/or messenger RNA) in hepatocytes and peripheral blood mononuclear cells (PBMCs), very low (< 200 IU/mL) or detectable but non-measurable serum HBVDNA, and no serum HBsAg or biochemical evidence of hepatic damage [4]. Patients in this condition can be further classified as seropositive (anti-HBc + and/or anti-HBs +) or seronegative (anti-HBc − and anti-HBs −).

In the organ transplant setting, OBI relevance spans from potential HBV transmission to non-immune recipients to acute reactivation of the infection and consequent development of HBV-related liver disease, leading to liver cirrhosis and hepatocellular carcinoma [5–15]. HBV reactivation can have a wide range of clinical presentations, from asymptomatic viremia to fulminant hepatic failure [16] and has mostly been studied in subjects undergoing cytotoxic chemotherapy or immunosuppressive treatment for hematological malignancies and autoimmune disorders [17]. Non hepatic solid organ transplant recipients are also at risk of HBV reactivation (HBVr). Most data were generated among kidney recipients [18], where HBVr increased mortality [19], and was associated with detectable viral load [20], older age and use of

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T-cell-depleting strategies [19]. In this setting, antiviral prophylaxis and/or treatment were effective in controlling HBV reactivation and/or disease, improving patient outcomes [21]. In contrast, few data regarding incidence and management of HBVr after heart transplant exist, translating into absence of specific evidence-based recommendations in this setting [22,23]. In recipients with markers of past HBV infection (anti-HBc+ and/or anti-HBs+/HBVDNA−), there is a low (~5%) risk of HBVr; it usually occurs during the first year after transplant and it is due to loss of protective anti-HBs followed by a rise in HBVDNA and then seroreversion to a positive HBsAg state [24]. Given the low overall risk of reactivation, current guidelines do not recommend routine antiviral prophylaxis in HBsAg−/anti-HBs+ non-hepatic solid organ transplant candidates, although this could be considered for patients at high risk of reactivation (anti-HBc+ alone or intense immunosuppression) [22,24]. All HBsAg−/anti-HBc+ patients should be tested for HBVDNA before and after starting immunosuppressive treatment and, if it is positive or HBVr occurs, they should be treated similarly as HBsAg positive patients with nucleos(t)ide analogues [22,25,26]. The optimal frequency of monitoring or the HBVDNA threshold at which antiviral therapy should be initiated remain unclear.

2. Objectives

In this study we analysed the baseline prevalence of HBsAg−/anti-HBc+/± anti-HBs+ in a cohort of heart transplant recipients and donors, evaluated the ensuing incidence of HBVr, and particularly its virological and clinical features.

3. Study design

This is an observational, single center cohort study on chronic heart failure patients who underwent screening for heart transplant at the Monaldi Hospital in Naples, Italy, from 2007 to 2013. Under written informed consent, these patients underwent a baseline virological screening for current or past HBV infection at the time of wait listing. Heart transplant recipients with serologic evidence of previous and clinically resolved hepatitis B infection (HBsAg−/anti-HBc+/± anti-HBs+; HBV cases) were prospectively followed up simultaneously with a comparable group of recipients without any marker of HBV infection (HBsAg−/anti-HBc−/± anti-HBs+ following vaccination; HBV controls). Furthermore, HBsAg+ recipients were treated for their chronic hepatitis B (CHB patients), as clinically required and in accordance with current guidelines [22,27]. The study protocol was approved by our Institutional Review Board and informed consent was obtained from patients accordingly.

3.1. Clinical and laboratory procedures

Pre-transplant screening included the qualitative determination of serum HBsAg and anti-HBc and the quantitation of anti-HBs (CMIA, Abbott Diagnostics). Patients showing markers of previous or current HBV infection were also tested for serum HBeAg, anti-HBe and anti-HDV antibodies, quantitative HBVDNA, and liver function tests; they were studied with liver ultrasound (US) and, where indicated, esophago-gastroduodenoscopy and liver biopsy. The Ishak scoring system was used to quantify grade of hepatic necroinflammation (Histology Activity Index, HAI) and fibrosis [28]. After transplant, cases and controls were followed up every three months with clinical and laboratory examinations and, where indicated, serum HBVDNA and liver US every six months. HBsAg was tested once a year. In order to assess liver disease progression, we performed noninvasive assessment of liver fibrosis through transient elastography (TE) (Fibro Scan®, EchoSens, Paris, France) at the latest outpatient visit. Serum samples were drawn at the time of wait listing and stored at −80 °C. Further prospective serum samples were obtained from surviving patients at post-transplant weeks 8, 12, 24, 48 and yearly thereafter, and stored at −80 °C for subsequent use.

Transplanted patients received an immunosuppressive regimen with different combinations of a calcineurin inhibitor (cyclosporine A or tacrolimus), an antiproliferative agent (everolimus or mycophenolate), and oral prednisone. Serum through levels of immune suppressors were maintained accordingly to the International Society of Heart and Lung Transplantation guidelines [29]. Target cyclosporine A through levels were 150–250 ng/dl when used in conjunction with mycophenolic acid, and 100–200 ng/ml when associated to everolimus. Tacrolimus through levels were maintained within the range 10–15 ng/ml. Everolimus was kept at 3–8 ng/ml.

Symptomatic acute cellular rejection (grade > 2R) was treated with metil-prednisolone, 1 g/day for 3 days, followed by oral prednisone tapering. Antibody-mediated rejection was treated with immunoadsorption or plasma-exchange (once daily for 5 days, then on alternate days for maintenance) until removal of circulating anti-HLA antibodies, usually followed by rituximab at the dose of 500 mg once a week for 1–4 weeks (according to CD19/CD20 lymphocyte count). High dose immunoglobulins were also administered as needed.

3.2. Virological studies

Serum quantitative HBVDNA was measured once in every patient with evidence of prior contact or current infection with HBV by commercial real-time (RT) PCR (Cobas TaqMan HBVDNA, Roche Diagnostics). HBVDNA was also retrospectively tested on 100 µl of serum samples by a home-made, semi-quantitative, highly-sensitive nested PCR, targeting two viral regions, namely HBV surface (S) and HBV core (C), as previously described [30]. These assays were meant at detecting potential transient episodes of viremia appearance. With this test, HBVDNA semi-quantification was performed using a scoring scale ranging from 1 to 3 based on the intensity of the amplification band displayed on agarose gel electrophoresis. Home-made PCR sensitivity was about 19.2 IU/ml [31]. For cases and CHB patients, one pre-transplant and all available post-transplant serum samples were tested. In contrast, only one pre-transplant and a single, most recent, post-transplant serum sample were tested for controls. All serum samples were examined in 2014.

3.3. Statistical analysis

Due to the study design, statistical analysis of data was mainly descriptive. Numerical variables are shown as mean and standard deviation or median and range. Categorical variables are instead shown as number and percentage. Differences between groups were assessed by Mann-Whitney *U* test. Overall survival was estimated using the Kaplan-Meier method.

4. Results

4.1. Baseline clinical features

Among 202 consecutive patients evaluated for possible heart transplant during the study period, 49 (24.2%) showed markers of previous or active HBV infection. Sixty-seven of them underwent heart transplant. They were mostly males (70.1%), with a median age at transplant of 52 years (range 29–68). They included 15 patients (22.4%) showing markers of either current (4 patients) or past (11 patients) HBV infection (Fig. 1). Among the 4 HBsAg positive patients, 1 had HDV coinfection with negative HBVDNA, and the other 3 had detectable serum HBVDNA; liver biopsy was performed at baseline showed mild liver fibrosis (F1-F2) in three patients, and moderate liver fibrosis (F4) in one, whereas median HAI was 6 (range 5–11). These CHB patients were started on antiviral treatment before or shortly after transplant, as shown in Table 3 shows the treatment regimens used and the timing thereof. The remaining 11 recipients were HBV cases (11/67

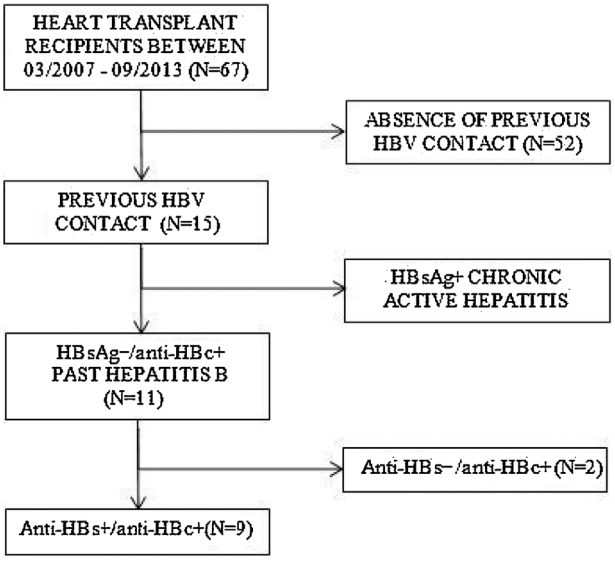

Fig. 1. Consort diagram of the studied patients.

recipients, 16.4%) and were all HBVDNA negative by RT-PCR. They were followed up from the day of transplant till December 2014 or death, if occurred before, with a median follow up of 30 months (range 1–69). The 11 HBV cases were mostly males (N = 7/11; 63.6%), with a median age at transplant of 55 years (range 30–60). Baseline ALT levels were slightly abnormal (1.5 UNL) in one of these cases, whilst GGT levels were above normal range in 9 (81.8%). None of these patients showed hypoalbuminemia or other signs of hepatic failure. Median level of total bilirubin was 1.1 mg/dl (range 0.7–2.0), with 54% of patients showing mild hyperbilirubinemia. No US signs of advanced liver disease were observed in any of them at the time of transplant.

The virological characteristics of the 11 HBV cases, 4 CHB patients and their donors are detailed in Table 1. The immune suppressive strategy adopted in these 15 patients is shown in Table 2; at one year post-transplant, all patients were still on low dose prednisone (2.5–5 mg/day).

4.2. Post-transplant follow-up

During the follow-up, 5 of the 11 HBV cases died for reasons unrelated to liver disease. The remaining 6 subjects were alive as of December 2014 (median follow-up after transplant: 35.5 months, range 15–69). At their last clinical evaluation, all 6 patients showed normal ALT, AST and albumin serum levels, 2 (33%) had slightly elevated GGT levels and 1 (16%) mild hyperbilirubinemia; only two patients had anti-HBs titers > 100 IU/L (Table 4). None of them experienced ALT flares or appearance of HBsAg during the follow-up. However, patient #8 was found to have a positive HBVDNA at month 22 of follow up, with a viremia equal to 209 IU/mL. Antiviral treatment with entecavir, 0.5 mg once daily, was started and is currently ongoing. One patient (#2) had a liver US coarse pattern with irregular margins compatible with the presence of advanced liver disease. He also had visceral obesity,

Table 2
Immunosuppressive strategy used after transplant in the 15 patients with past/current HBV infection.

ID	Cyclosporin	Tacrolimus	Mycophenolate	Everolimus	Prednisone
1	+		+		+
2	+		+		+
3		+	+		+
4	+		+		+
5	+		+		+
6	+			+	+
7	+			+	+
8	+			+	+
9	+		+		+
10	+		+		+
11		+	+		+
12	+		+		+
13	+		+		+
14	+		+		+
15	+		+		+

Table 3
Antiviral treatment and timing of administration in the 4 HBV-DNA positive recipients.

ID	Time Of Antiviral Therapy Start (nr° of days before/ after tx)	Lamivudine	Adefovir	Entecavir	Tenofovir
3	−160	–	–	0.5 mg/day	–
4	−60	–	10 mg/day	–	–
12[a]	+5	100 mg/day	–	–	–
14	+3	100 mg/day	–		245 mg/day[b]

[a] Had HBV-HDV coinfection.
[b] Added to Lamivudine 14 months after heart transplant.

Table 1
Pre-transplant data of the 15 recipients with past or active HBV infection and their respective organ donors.

Recipients											Donors		
ID	Age	Sex	HBsAg	Anti-HBs (IU/L)	Anti-HBc	HBeAg	Anti-HBe	anti-HDV IgG	HBV-DNA	Year of HTx	Anti-HBs	HBcAb	HBV-DNA
1	55	M	–	1000	+	–	+	–	N.D.	2013	–	+	N.D.
2	60	M	–	15	+	–	–	–	N.D.	2009	–	–	N.D.
3	45	F	+	0	+	–	+	–	1.2×10^5 U/mL	2011	–	–	N.D.
4	55	M	+	0	+	–	+	–	101 U/mL	2009	–	–	N.D.
5	52	M	–	0	+	–	–	–	N.D.	2011	–	–	N.D.
6	56	F	–	85	+	–	–	–	N.D.	2012	–	+	N.D.
7	60	M	–	139	+	–	–	–	N.D.	2012	+	–	N.D.
8	59	M	–	2	+	–	–	–	N.D.	2011	+ (> 1000)	+	N.D.
9	58	M	–	0	+	–	–	–	N.D.	2011	–	+	N.D.
10	32	F	–	828	+	–	+	–	N.D.	2011	–	–	N.D.
11	48	F	–	260	+	–	–	–	N.D.	2010	–	+	N.D.
12	50	M	+	0	+	–	+	+	N.D.	2010	–	–	N.D.
13	30	F	–	+[a]	+	–	+	–	N.D.	2011	–	–	N.D.
14	39	M	+	0	+	–	+	–	2×10^3 U/mL	2008	–	–	N.D.
15	53	M	–	108	+	–	–	–	N.D.	2010	–	–	N.D.

N.D., target not detected; HTx, heart transplantation.
[a] Titer not available.

Table 4
Liver function tests, virological markers and ultrasound data at the last follow-up after transplant in the 6 HBV cases.

ID	ALT (IU/L)	γ-GT (IU/L)	Albumin (g/dl)	Tot Bil (mg/dl)	HBsAg	Anti-HBs (IU/L)	HBV-DNA (IU/mL)	Hepatomegaly	Liver Margins	US Pattern	Portal Vein Dilatation	Splenomegaly	Ascites
1[a]	14	14	4.1	1.5	–	> 1000	N.D.	No	Regular	Homogeneous	No	No	No
2	11	13	4.1	0.9	–	36.1	N.D.	Yes	Irregular	Coarse	Yes	No	No
6[a]	16	16	3.5	0.7	–	61.4	N.D.	No	Regular	Homogeneous	No	No	No
7	26	35	3.9	0.7	–	105.6	N.D.	No	Regular	Homogeneous	Mild (12 mm)	No	No
8[a]	12	17	4.4	0.8	–	10.8	209	No	Regular	Homogeneous	No	No	No
15	20	32	4.3	1	–	213.8	N.D.	No	Regular	Inhomogeneous	No	No	No

N.A. = not available; N.D. = not detected.
[a] Recipient of graft from an anti-HBc positive donor.

suggesting fatty liver infiltration as a possible cause as a possible cause of this US pattern.

During follow-up, we observed 3 episodes of rejection among cases (27%) and 3 episodes (27%) among controls.

4.3. Virological studies

By using a semi-quantitative PCR with high sensitivity, we subsequently tested for HBVDNA available sera from CHB patients, HBV cases and controls. The results of these virological analyses are shown in Fig. 2. Only 1 of the 11 HBV cases showed pre-transplant low levels of serum HBVDNA (HBV S region). HBV C region PCR turned positive twice after 4 and 5 years post-transplant in this patient, although HBV S PCR remained constantly negative. Of the remaining patients with negative pre-transplant results, four showed positive HBVDNA in post-transplant samples, targeting either C or S regions (Fig. 2). Interestingly, patient #8, who showed a positive HBVDNA with the commercial RT assay, had persistently positive HBVDNA by highly sensitive PCR, on both C and S regions, throughout follow up.

Based on these data, the cumulative incidence of HBVr during the study period was 12.5% (N = 1/8), with an incidence rate of 4.3 new cases/100 transplant*year (N = 1/22.8 recipients*year). During a mean follow up of 35.5 months, one viral reactivation and 4 virological events of uncertain significance were observed. From the clinical standpoint, no event of biochemical reactivation was observed.

All HBsAg+ patients pre-transplant (#3,4,12,14) had detectable HBVDNA in either quantitative or semi-quantitative assays. Only one of these 4 patients had still a detectable serum HBVDNA at week 48 post-transplant with the semi-quantitative assay, but not with RT-PCR. In contrast, all 11 recipients of HBV control group had negative samples before and at 48 weeks after transplant, using both C and S region primers (data not shown).

4.4. Clinical impact

At the last outpatient visit, only 1 of HBV cases showed signs of progression of liver fibrosis at US examination (patient #2), but he also had liver steatosis associated with visceral obesity. In this patient, virological events of uncertain significance were detected since the fourth year post transplant (Fig. 2), but without any reduction in the anti-HBs titer and without appearance of HBVDNA by RT-PCR. Accordingly, no antiviral treatment was started in this patient. No US signs of fibrosis/cirrhosis, as well as ascites or hepatocellular carcinoma were observed in the remaining 5 HBV cases. In order to obtain a non-invasive fibrosis assessment, TE was performed in 6 heart transplant recipients of each case and control group. As shown in Fig. 3, no difference in liver elastography between these two groups was observed. As of December 2016, 5/11 (33.3%) HBV cases and 15/52 (28%) heart transplanted

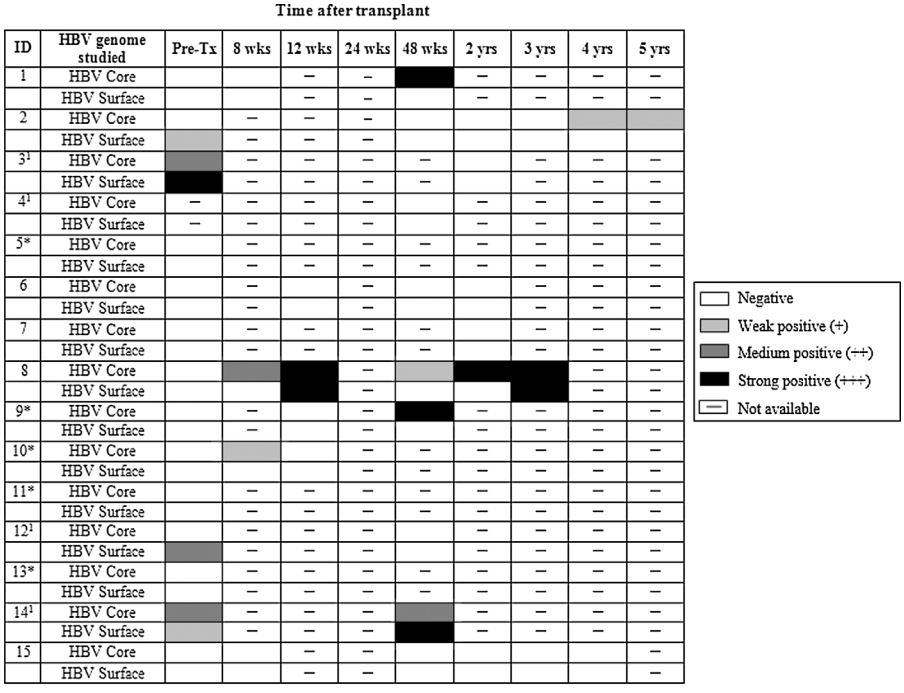

Fig. 2. Prospective detection of HBV-DNA before and after transplant in HBsAg−/anti-HBc+ and HBsAg+ patients.

* died during follow up
[1]HBsAg positive patients

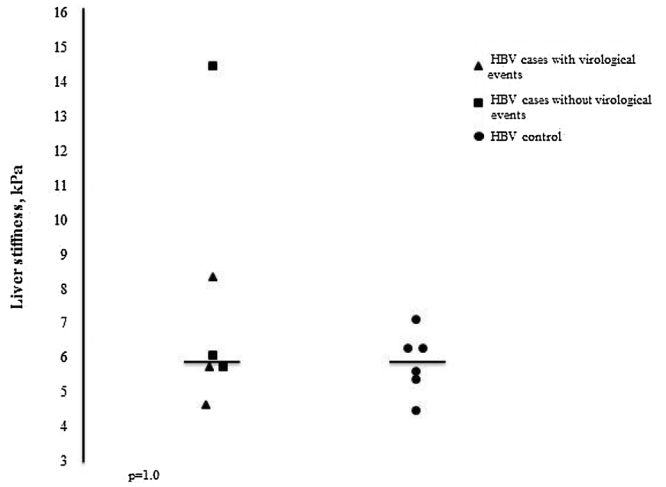

Fig. 3. Scatter-plot of fibroscan values (k Pa) in 6/11 HBV cases, with and without virological events, and controls.

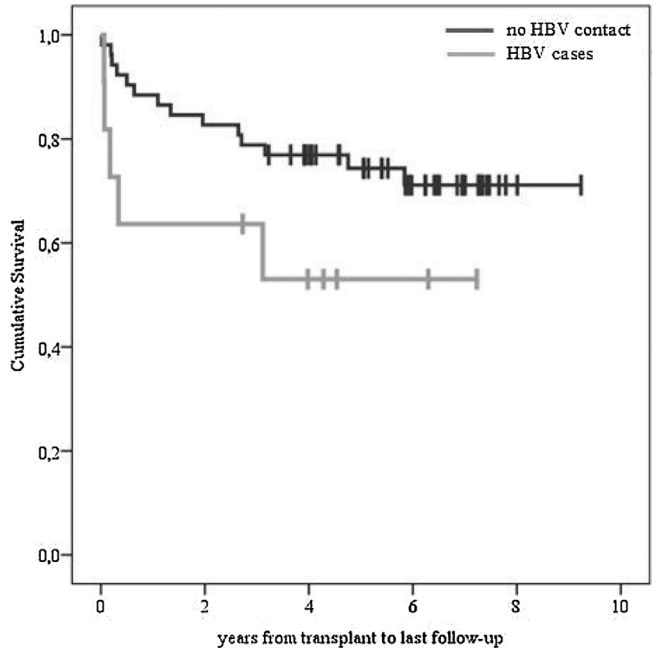

Fig. 4. Kaplan-Meier survival curve of the 11 HBV cases and 52 heart transplant recipients without prior HBV infection (no HBV contact).

patients who did not have any previous HBV infection died. A non-significant difference in survival between these two groups was observed by Kaplan Meier analysis (Mantel-Cox Log Rank Test p = 0.097) (Fig. 4).

5. Discussion

There are few published data on HBVr in solid organ transplant recipients. The available evidence comes from retrospective studies on heterogeneous patient populations with different types of grafts and variable definitions of HBVr; with these limitations, the rate of HBVr was generally low [30,32–35]. No specific indications are available about HBVr in heart recipients [22,23,27]. In this study, we evaluated the prevalence of markers of previous HBV infection in a cohort of heart transplant candidates, as well as the incidence and clinical significance of HBVr in a small group of heart transplant recipients. In our epidemiological setting, prevalence of past HBV infection was substantial, but not coupled with virological events. During follow-up, HBV viremia was frequently detected by a highly sensitive assay. However, this did not translate into neither seroreversion nor progression of liver disease. Although not applied on all prospective serum samples, HBV-specific RT-PCR was largely negative in HBsAg–/anti-HBc–. In contrast, positive results for HBVDNA were found overall in more than half of HBV cases (N = 5/8; 62.5%) using a more sensitive nested PCR assay. We defined these as 'virological events of uncertain significance', because they were fluctuating, not always detected by amplifying both HBV regions targeted (core and surface), and seemingly not associated with clinical events. In fact, the single patient with HBVDNA measurable by RT-PCR, without HBsAg reappearance, was put on antiviral therapy without delay to prevent evolution into clinical reactivation; in this case, it remains unknown whether virological progression would occur without treatment.

In general, our limited data suggest that HBVr in heart transplant recipients with HBsAg − /anti-HBc is uncommon. Current guidelines do not address the issue as to whether transplant recipients with previous markers of HBV infection should undergo active HBVDNA screening after transplant [22]. Moreover, there is no recommendation on antiviral prophylaxis to prevent reactivation [22]. Indeed, detection of HBVDNA during follow-up in heart transplant recipients would have great importance if it led to acute clinical manifestations or favored progression of liver disease. In our group of patients, none of these outcomes were observed. The only patient (#2) showing US signs consistent with liver cirrhosis had other concomitant causes of liver disease, and it is impossible to ascribe his liver disease to HBsAg − /anti-HBc + . Furthermore, all recipients were on several medications that can contribute to liver damage. The absence of progression of liver disease would be best ascertained by liver histology [36]. This would require pre- and post-transplant liver biopsies, that were deemed not justified in patients with markers of previous HBV infection. Although less accurate, TE [37] may provide non-invasive assessment of liver disease stage. Unfortunately, we did not perform pre-transplant elastography. At the last follow-up, no significant differences in liver stiffness between heart graft recipients in presence or absence of markers of past HBV infection were found, and the Kaplan Meier curve showed past HBV infection did not impact patients' survival.

Risk factors for HBVr in heart transplant recipients remain unclear. In HBsAg– /anti-HBc + kidney recipients, an anti-HBs titer < 100 IU/mL was associated with a higher risk of HBVr [35]. In our HBV group, measurable levels of HBVDNA by RT-PCR were observed only in the single patient with the lowest anti-HBs titer (10.8 UI/ml). If reproduced elsewhere, this result would confirm a weak humoral immune response may play a role in the lack of HBV replication control in heart transplant recipients with markers of previous HBV infection. Three of the 5 HBV cases with virological events received the heart from an anti-HBc-positive donor, but only the one with lower anti-HBs titer presented with a measurable HBVDNA viremia. The risk of HBV transmission from an anti-HBc positive non-hepatic donor ranges from 0% to 5.2% in different studies; it is significantly lower than that of hepatic donors, especially if the recipient is immune, underlining the importance of pre-transplant vaccination [24]. Prophylaxis is not recommended for recipients of an anti-HBc positive non-hepatic organ who have natural or vaccine immunity. Prophylaxis with lamivudine is suggested for about 12 months for non-immune recipients of an anti-HBc + /HBVDNA + non-hepatic organ, instead [24,38].

In the absence of donor's samples, no comparative virological analysis was possible in our patients. Notwithstanding, it could be interesting to further characterize HBV sequences of HBV cases to search for specific mutations possibly present, and to analyze their role in the dynamics of HBV infection.

This study has several limitations: 1) invasive assessment of liver disease was not performed; 2) a low number of patients with prospective samples was available for analysis; 3) there were a few missing serum samples in individual cases; 4) an in-depth molecular typing of HBVDNA sequences obtained has not been completed. Other methods,

as detection of HBVDNA in Peripheral Blood Mononuclear Cells (PBMCs) and/or liver, could be more accurate to identify the persistence of HBV replication at very low levels. Unfortunately, we did not store prospective PBMCs and, being patients HBsAg-negative, we did not perform liver biopsy. Finally, the amount of stored serum available was limited, thus we could not perform DNA extraction from larger volumes.

Notwithstanding, in the absence of larger studies or even case reports, we believe our data could provide a starting point to implement analysis of an important but inadequately known topic, while studies on a larger number of patients are surely warranted. In conclusion, while HBVr is infrequent, 'virological events of uncertain significance' occur in a much higher number of heart transplant recipients with markers of previous HBV infection. The clinical impact of such events is uncertain, and a longer follow up on a larger cohort of patients is needed.

Competing interests

None declared.

Ethical approval

Not required.

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Author's contributions

Martina Vitrone and Emanuele Durante-Mangoni worked on concept/design of the study; Martina Vitrone, Domenico Iossa, Luca Rinaldi, Rosa Molaro, Antonio Parrella, Roberto Andini, Enrico Ragone, Ciro Maiello worked on data collection and data analysis/interpretation; Pia Clara Pafundi: worked on statistics; Rosa Zampino and Emanuele Durante-Mangoni: drafted and critically revised the manuscript.

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