Journal of Clinical Virology 96 (2017) 54–59

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Full Length Article

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

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ARTICLE INFO

Keywords:
Occult hepatitis B infection (OBI)
Heart transplant
Hepatitis B reactivation
Virological event

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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http://dx.doi.org/10.1016/j.jcv.2017.09.011
Received 22 July 2017; Received in revised form 21 September 2017; Accepted 22 September 2017

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