



Case report

HBV transmission from an occult carrier with five mutations in the major hydrophilic region of HBsAg to an immunosuppressed plasma recipient



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ARTICLE INFO

Article history:

Received 20 April 2013  
 Received in revised form 11 June 2013  
 Accepted 12 June 2013

Keywords:

HBV infection  
 Occult HBV infection  
 HBsAg mutants  
 Transfusion

ABSTRACT

We describe the case of transmission of an HBsAg-negative hepatitis B infection to an immunosuppressed patient by plasma donation from an HBsAg-negative subject, but with very low serum HBV DNA (about 50 IU/ml) and five mutations in the major hydrophilic region of HBsAg.

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1. Why this case is important?

Over the last four decades, the risk of transfusion-transmitted hepatitis B virus (HBV) has steadily decreased. The residual risk of transfusion-transmitted HBV is mainly related to blood donations negative for the hepatitis B surface antigen (HBsAg) and nucleic acid amplification technique (NAT) collected during the pre-seroconversion 'window period' or due to occult HBV infection (OBI) or to viral mutants in the antigenic "a" determinant of HBsAg.

Occult HBV infection is characterized by undetectable HBsAg in serum and HBV DNA detectable in the liver tissue and at low levels or undetectable in serum, with or without anti-HBc positivity [1,2]. Its reactivation is a well-known clinical event in onco-hematological patients receiving chemotherapy [3]. The HBsAg is an envelope glycoprotein that is currently the primary element

for diagnosis and target of immunoprophylaxis of HBV infection. The dominant epitopes of HBsAg, which are the targets of neutralizing B-cell responses, reside in the "a" determinant [amino acids (aa) 124–147] within the major hydrophilic region (MHR). Amino acid substitutions in the MHR can cause reduced binding of anti-HBs antibodies, resulting in immune escape. The most common MHR mutation, G145R, was initially described in 1990 [4]. The emergence of single or multiple aa substitutions at this and other positions within the MHR has been observed in infants born to HBsAg-positive mothers who received the HBV vaccine, in liver transplant recipients who received hepatitis B immunoglobulin (HBIG) and in patients who experienced HBsAg loss after anti-HBV therapy [5].

We describe here the case of an HBsAg-negative acute hepatitis B infection in an immunosuppressed patient receiving plasma from an HBsAg-/NAT-negative donor.

2. Case description

The blood donor was a 56-year-old Italian male who had donated since 2008 at the Transfusion Service of the Istituto Nazionale Tumori, Pascale, Naples, Italy. He had not been vaccinated against HBV and had no history of known risk factors for HBV infection. At the time of all blood donations, he was negative for HBsAg and NAT determination and with normal serum

Abbreviations: HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; NAT, nucleic acid amplification technique; OBI, occult HBV infection; MHR, major hydrophilic region; HBIG, hepatitis B immunoglobulin; ALT, alanine aminotransferase; PC, polymerase chain reaction.

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**Table 1**  
Serological, virological and biochemical data of the donor before and after the blood donation (June 25, 2010) and of the plasma recipient (Patient no 1) at the time and after the plasma transfusion (August 16, 2010).

Data of the donor										
	25/5/2008	18/12/2008	24/4/2009	18/12/2009	25/6/2010	28/01/2011	14/2/2011	7/4/2011	30/6/2011	7/11/2011
Time from index donation (months)	-25	-18	-14	-6	0	+6	+6.5	+8	+12	+16
HBsAg	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
HBsAb (IU)	/	/	/	/	/	55	49	50	54	46
NAT	Negative	Negative	Negative	Negative	Negative	Positive	/	/	/	/
HBV DNA (IU/ml)	/	/	/	/	/	57	46	Negative	Negative	Negative
ALT, x n.v.	0.6	0.75	0.65	0.7	0.8	0.96	0.9	1.1	0.9	0.85
Data of the plasma recipient (Patient no 1)										
Time from index donation (months)	/	/	/	04/08/2010	26/10/2010	02/03/2011	22/03/2011	09/9/2011	30/12/2011	
HBsAg	/	/	/	Negative	Negative	Negative	Negative	Negative	Negative	Negative
HBsAb (IU)	/	/	/	2	110	9	7	2	3	
Total anti-HBc	/	/	/	Negative	Negative	Negative	Negative	Positive	Positive	Positive
Anti-HBe	/	/	/	Negative	Negative	Negative	Negative	Positive	Positive	Positive
HBV DNA (IU/ml)	/	/	/	/	/	3380	19,200	Negative	Negative	Negative
ALT, x n.v.	/	/	/	1.1	1	2.8	2.7	1.1	1	

NAT, nucleic acid amplification technique; ALT, alanine aminotransferase; x n.v., times normal value.

alanine-aminotransferase (ALT) values. All the recipients of each blood donation were checked for HBsAg- and HBV-DNA-positivity, but none resulted positive.

On June 25, 2010 the donor again attended the transfusion service for a blood donation (Table 1). Since at this time he was HBsAg-, anti-HCV- and anti-HIV-negative, and negative for HBV DNA, HCV RNA and HIV RNA by NAT and had normal serum ALT, the blood was transfused to three hospitalized patients. Precisely, the plasma unit was transfused to Patient n° 1 from the hematology unit in August 2010, the platelets unit to Patient n° 2 from the gynecology unit in June 2010, and the red cells unit to Patient n° 3 from the hematology unit in July 2010. All three recipients were HBsAg-negative at the time of the transfusion and had not been vaccinated against HBV. Patients 1 and 3 had severe immunosuppression due to chemotherapy for a non-Hodgkin lymphoma.

Seven months later, in January 2011, the donor came back to the transfusion service for a new blood donation. He denied any risk factor for parenteral infection, but at this time the NAT determination was positive for HBV, although he was still HBsAg-negative and had normal serum ALT. The serological study of HBV infection showed that the donor was anti-HBs-positive (55 IU/ml), total anti-HBc- and anti-HBe-positive, and negative for anti-HBc IgM and HBeAg. The serum HBV load was very low (57 IU/ml) (Table 1). Therefore, the blood unit was eliminated and the donor was indefinitely suspended from blood donation. The sero-virological pattern of the donor was confirmed in the subsequent evaluations from February 2011 to June 2011, with a very low HBV viral load (46 IU/ml) in February and negative in April and June (Table 1).

The serological and virological methods applied are reported in the Supplementary Data. Samples positive for HBV by NAT were evaluated also by real-time Polymerase Chain Reaction (PCR) with a detection limit estimated at around 40 copies/mL [6].

In order to assess the sero-virological status of the three recipients of the blood components from the donor, Patients n° 1, 2 and 3 were recalled in March 2011 and tested for HBV markers. The plasma recipient (Patient n° 1) was HBsAg-negative but was HBV-DNA-positive (3380 IU/ml) with abnormal serum ALT levels, confirmed in two determinations (Table 1). However, from September 2011 the patient again became HBV-DNA-negative with normal serum ALT value (Table 1).

The platelets and red cells recipients (Patients no 2 and 3, respectively) remained HBsAg- and HBV-DNA-negative (see Supplementary Tables 1 and 2), but four months after the platelets

transfusion in June 2010 Patient n° 2 showed a marked increase in the anti-HBs titers (from 10 to 1000 IU/ml) (see Supplementary Table 1).

The sera of the donor and of Patient no 1 were analyzed for HBV genotype and HBV pre-S/S coding region (see Supplementary Data). Both the donor and patient no 1 had genotype D and the HBV pre-S/S coding region sequences were identical. Five amino acid substitutions were found in the MHR: T116N, T123I, D144V, G145K, I150T.

### 3. Other similar and contrasting cases in the literature

Published data reporting the infectivity of OBIs by transfusion are rare [7]; at our knowledge this is the first case of transmission of OBI to an immunosuppressed patient by plasma donation from an HBsAg-negative subject, but with very low serum HBV DNA (about 50 IU/ml) and five mutations in the major hydrophilic region of HBsAg.

### 4. Discussion

The risk of transfusion-transmitted HBV is currently related mainly to blood donations collected during the pre-seroconversion 'window period' or to an OBI or to viral mutants in the antigenic "a" determinant of HBsAg. In the case presented here, the sero-virological status of the donor is suggestive of an OBI. In fact, according to the Taormina criteria [1], OBI is associated with undetectable or low level HBV DNA in the serum of HBsAg-negative individuals, as in our subject who was HBsAg-negative with a negative or very low serum HBV load (two determinations <60 IU/ml).

The plasma recipient, who had severe immunosuppression and was being treated with chemotherapy because of non-Hodgkin lymphoma, became HBV-DNA-positive with abnormal serum ALT values 5 months after the transfusion but remained HBsAg-negative. He had the same HBV genotype (D) and the same pre-S/S coding region sequence as the donor. His severe immunosuppression status was probably why he showed higher titers of serum HBV DNA (3380–19,200 IU/ml) than the donor. The platelets and red cells recipients remained HBsAg- and HBV-DNA-negative, but the platelets recipient showed a marked increase in the anti-HBs titers (from 10 to 1000 IU/ml) 4 months after the donation, a demonstration of contact with HBV.

Interestingly, the pre-S/S coding region sequence of the donor and the plasma recipient showed five amino acid substitutions in the MHR: T116N, T123I, D144V, G145K and I150T. Previous studies have shown that naturally occurring MHR variants are common in different geographical areas [5], and that many of the mutations in the MHR reduce the sensitivity of HBV detection assays and result in false negative results, thus increasing the risk of an erroneous diagnosis of occult HBV infection [1,8] or of infection being transmitted via transfusion [9]. At least to our knowledge, 3 of the 5 mutations found in the donor and the plasma recipient have not been previously described. A mutation at position 123, T123N, has been found to be responsible for both diagnostic and HBIG therapy failure [10], but no data are available on the T123I mutation. The D144A and G145R mutations have been reported to impair the antibody recognition by several kits for HBsAg [11]. We found different aa substitutions in the same positions (D144V, G145K), but since the position is the same as the above-mentioned mutations, they may also have been responsible for the impaired recognition by the antibodies. Verheyen et al. recently compared two quantitative HBsAg assays for the detection of HBsAg mutants expressed *in vitro* (Architect, Abbott and Elecsys, Roche,) and showed that the HBsAg mutation G145K was under quantified by the Elecsys system [12], the one used at our institution. Svicher et al. evaluated the HBsAg mutations associated with OBI in 24 Italian blood donors with genotype D and showed that the T116N mutant closely correlated with OBI and strongly affected HBsAg detection [13]. These authors suggest that, by altering HBV antigenicity and/or viral-particle maturation, this mutation together with others (S143L, R122P, Q101R, S167L) may affect the reliability of the universal diagnostic assays for HBsAg detection.

This is a case of transmission of occult HBV infection through plasma transfusion. The immunosuppressive status of the recipient probably favored the transmission, and the accumulation of the five mutations in the MHR affected the reactivity of the diagnostic assay used for HBsAg. Thus, to avoid the risk of transmitting occult HBV infection and the HBV mutants, NAT and the detection of HBsAg by two tests or the detection of the anti-hepatitis B core antigen may be a wise strategy.

## Funding

None.

## Conflict of interest

All the authors of the manuscript declare that they have no conflict of interest in connection with this paper.

## Ethical approval

Not required.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jcv.2013.06.020>.

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