Occult hepatitis B virus infection: a case of reactivation in a patient receiving immunosuppressive treatment for allogeneic bone marrow transplantation

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Introduction

The presence of hepatitis B virus (HBV) genome in HBsAg-negative subjects is known as occult HBV infection[1].

This particular form of hepatitis, already recognised in the 1980s, has been confirmed and studied using molecular biology techniques. In fact, occult infection is usually associated with the presence of anti-HBc and anti-HBs, but given the relatively high percentage (approximately 20%) of subjects who are negative for all markers[1], the introduction of a test to detect HBV DNA was fundamental.

Occult infection is related in some cases to mutant viruses that are not detectable by the commonly used tests; it has also been observed that the reactivation of HBV related to variants of the viral genome often has an unfavourable clinical prognosis[2,3].

Much more frequently, however, an occult infection is associated with strong suppression of viral replication, which is responsible both for the negativity for HBsAg and the undetectable or very low levels of HBV DNA in the serum, although this latter can be found in liver tissue[4,5].

An occult infection can have a important impact in various different clinical settings, including transmission through blood transfusion or organ transplants[6,7] and reactivation following immunosuppressive therapy.

Indeed, it has been shown that the host's immune response, co-infections (e.g. with hepatitis C virus) and epigenetic factors all play significant roles in occult infection[8].

We present the case of a Georgian child negative for HbsAg who, after receiving an allogeneic bone marrow transplant and immunosuppressive therapy, was found to be positive for HBV.

We then describe the investigations conducted in order to determine whether this was due to a new infection or reactivation of an occult infection.

Case report

A 13-year old patient, born in Georgia (Russia), was diagnosed with acute myeloid leukaemia (M5a) in April 2002. Induction therapy according to the BFM 95 protocol was administered in Georgia and the patient achieved a remission in June 2002; he continued with consolidation therapy (BFM 87 protocol) until August 2002.

During the cycles of chemotherapy the patient required constant transfusion support with both red cell concentrates and units of apheretic platelet.

In July 2003, the patient had a bone marrow relapse, which was treated with mitoxanthrone and cytarabine. In October 2003, the patient was referred to the Oncohaematology Department of the *G. Gaslini* Institute (Genoa, Italy) where he received chemotherapy consisting of fludarabine, daunoxome, cytarabine, and granulocyte colony-stimulating factor followed by fludarabine-cytarabine-glucocorticoids and maintenance therapy with thioguanine, until January 2004.

During this period the patient's tests for HbsAg were negative, confirming the negativity already found in Georgia. The patient underwent asportation of a lobe of a lung, because of documented pulmonary aspergillosis, during the period of administration of the chemotherapy.

In March 2004, the patient achieved a second complete and consolidated remission. Given the lack of a compatible family donor, the patient received a bone marrow transplant from an unrelated donor.

The conditioning regime included total body irradiation (TBI) (1200 cGy in 6 fractions), cyclophosphamide (120 mg/Kg in 2 days), melphalan (140 mg/m^2), while the prophylaxis against graft-versus-host disease (GvHD) consisted of antilymphocyte serum (Thymoglobulin, ATG, 3.75 mg/Kg on days -4, -3, and -2), cyclosporine (3 mg/Kg continuous infusion followed by oral administration) and methotrexate (10mg/m^2 on day +1 and 8 mg/m^2 on days +3, +6, and +11).

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The patient was infused a total of 3.79 x 10^8 /Kg bone marrow mononuclear cells from an HLA-compatible donor (matched for the A, B, C, and DRb1 loci). Engraftment occurred on day +19.

From day +24 complete donor chimerism (100%) was demonstrated (by evaluating DNA polymorphisms in peripheral blood and bone marrow); this finding was confirmed several times in association with a morphological complete remission of the acute leukaemia.

On day +68 the patient developed acute GvHD, predominantly involving the upper gastrointestinal tract (overall grade 1), which responded to low doses of steroids (0.5 mg/Kg/die), withdrawn about 1 year after the transplant.

In September 2004 (about 6 months after the transplant), a first positive test for HBsAg occurred; the viral infection was confirmed by molecular biology testing for HBV DNA (viral load 7.5x 10^8 copies/mL).

Following the first demonstration of positivity for HBsAg, the patient was monitored for markers of hepatitis B (HBsAg, anti-HBs, anti-HBc, anti-HBc IgM, HBeAg, anti-HBe Axsym System; Abbott Laboratories, Abbott Park, IL, USA) and underwent quantitative assay of HBV DNA (Cobas Amplicor HBV Monitor Assay, Roche Molecular System, Branchburg, NJ, USA).

A retrospective analysis of the patient's serological profile was conducted on stored serum samples. Quantification of HBV DNA and screening for HBsAg were carried out on all the stored samples. However, there was sufficient serum in only some of the samples to carry out further tests for markers.

Serum tests and molecular biology investigations were also carried out in samples from donors who had provided the products used for the patient's transfusion support.

Table I shows the results of the investigations of hepatitis B markers and HBV DNA carried out on the patient's samples.

The first positive test for HBsAg occurred in September 2004 and was confirmed by positivity for HBeAg and a high viral load (HBV DNA).

Subsequent controls of the patient also showed positivity for anti-HBc IgG and IgM, suggesting that this was the initial, acute phase of the disease. However, a retrospective study carried out on stored serum showed that in October 2003, at the time of the patient's admission to our Department, he was positive for anti-HBs and anti-HBc, markers that indicate a previous infection.

The first sample positive for HBV DNA was from February 2004, that is, 1 week prior to the infusion of the allogeneic bone marrow cells. The viral load, although being below the level of the linearity of the kit used, was detected by automatic extrapolation by the instrument and quantified as 39.9 copies/mL. The load detected in the subsequent samples increased progressively; the first finding of HBsAg positivity was in a sample collected in May 2004.

Despite this serological picture of acute hepatitis B, the patient showed no clinical or ultrasound signs of compromised liver function, presenting only a slight increase in serum transaminase levels: the maximum concentration of alanine aminotransferase reached 157 U/mL at 9 months after the transplant.

In an attempt to identify the cause of the positivity for hepatitis B markers, the donors who had supplied the transfusion products given to the child were analysed; it was not, however, possible to have any information on screening tests for infections concerning the donors used in Georgia.

Between the time the patient was admitted to our hospital and the positive test for HbsAg, he had received 116 units of red cell concentrates and platelets collected from 108 donors. In order to optimise the investigations, we decided to recall only the 60 donors whose blood components has been used for transfusion support prior to the date of the first positive test for HBV DNA. Full investigations of all hepatitis B markers and HBV DNA were carried out on these donors, who, with the exception of three, were all regular donors. No markers of hepatitis B were found except in three cases who were positive for anti-HBs, anti-HBc, anti-HBe and five who were positive only for anti-HBs due to vaccination. HBV DNA was negative in all cases.

As far as regards antiviral therapy, the patient was not prescribed lamivudine, despite the already published reports [9-11] on the utility of this drug for the prophylaxis and treatment of hepatitis B infection in immunocompromised patients, confirmed by the indications issued in May 2005 by the Italian Association for the Study of the Liver (AISF)[12]. It was not considered appropriate to start therapy with lamivudine in this case, in the knowledge that the patient would have to return to Georgia where it is difficult to obtain the drug and monitor HBV DNA.

In fact, since February 2005, the patient has been living in Georgia again; we know that his clinical condition is good and that his blood-chemistry tests are within the norm. We were not, however, able to have further information on the HBV serological profile. Nevertheless, during the child's return to our hospital in 2005 because of pulmonary problems, we were able to confirm the persistence of the HBV, in the absence of clinical signs of compromised liver function.

Table I - Hepatitis B markers and HBV DNA levels in the patient

Date	HBsAg	anti-HBs	HBeAg	anti-HBe	anti-HBc	Anti-HBcAb IgM	HBV DNA
23/10/2003	Negative						
01/11/2003 Stored serum sample	Negative				Positive		Not detected
09/12/2003 Stored serum sample	Negative	Positive 83 mUI/mL					Not detected
02/01/2004 Stored serum sample	Negative						Not detected
05/02/2004 Stored serum sample							Not detected
17/02/2004	Negative	Positive 10 mUI/mL					
24/02/2004 Stored serum sample							39.9 copies/mL
TRANSPLANT							
01/03/2004 Stored serum sample	Negative						71.7 copies/mL
02/04/2004 Stored serum sample	Negative						107 copies/mL
10/05/2004 Stored serum sample	Positive 4,93 S/N						3.2×10^3 copies/mL
31/05/2004 Stored serum sample	Positive 129,84 S/N						8.9×10^4 copies/mL
06/09/2004 308 S/N	Positive	Negative	Positive	Negative	Negative	Negative	7.5×10^8 copies/mL
23/09/2004	Positive	Negative	Positive	Negative	Negative	Negative	5.7×10^8 copies/mL
21/10/2004	Positive	Negative	Positive	Negative	Positive	Positive	6.5×10^8 copies/mL
27/05/2005	Positive	Negative	Positive	Negative	Positive	Positive	5.2×10^7 copies/mL
14/12/2005	Positive	Negative	Positive	Negative	Positive	Positive	5.3×10^7 copies/mL

Discussion

The finding of a conversion from negativity to positivity for HBsAg in a multiply transfused subject must lead to in-depth investigations of both the patient and the donors used to provide the transfusion support in order to differentiate a new hepatitis B infection acquired through the blood derivatives administered from a reactivation of an occult infection.

Recent studies in areas in which HBV is endemic have shown that the presence of anti-HBc and anti-HBs does not correlate significantly with the presence of occult HBV infection[9] and that, vice versa, HBsAg negative, anti-HBs negative and anti-HBc negative donors may have an occult HBV infection that can only be detected by molecular biology tests, in the absence of which the infection could be transmitted to a recipient[3].

On this background, we decided to repeat the tests on the donors used for our patient's transfusion support: the investigations carried out enabled us to exclude an infection transmitted through the blood components administered in our hospital.

The retrospective analyses carried out on the patient's

stored serum samples also allowed us to exclude the bone marrow donor as the source of the infection, since the first evidence of viral DNA (albeit below the limit of sensitivity of the test) was found in a sample collected before the infusion of the bone marrow stem cells.

The results of all the investigations carried out suggest that our patient's infection could have been due to reactivation of hepatitis B, consequent to the strong immunosuppression caused by the high-dose chemotherapy, which led to the loss of immunological control of the HBV infection.

Numerous cases of reverse seroconversion in patients with resolved hepatitis B (HBsAg negative, anti-HBs positive, anti-HBc positive)[14-17] and in patients positive only for anti-HBs[8] have been described by many authors: this reactivation of HBV seems to the result of immunosuppression that causes a decrease in the production of anti HBsAg IgG immunoglobulins[18].

In particular, the marked immune deficit occurring in transplant recipients as a result of chemotherapy and GVHD treatment causes the reactivation of latent infections, normally controlled by immunological surveillance. There have, however, also been case reports of reverse seroconversion in HBsAg-negative and anti-HBs-positive patients undergoing autologous bone marrow transplantation[19] in which the immunosuppressive therapy is less aggressive.

In cases of resolved hepatitis B, the loss of HBsAg and the appearance of anti-HBs and anti-HBc indicate that the virus has disappeared from the circulation; however, definite cases of reactivation suggest that the virus, although no longer detectable, may remain latent in the liver[4,5].

This phenomenon may be related to both host and viral factors; in fact, in some cases[2,3] analysis of the viral genome after reactivation has shown mutations in the core region.

There are, however, certainly more cases in which the reverse seroconversion is related to the host's immune status; in other words, the viral reactivation usually occurs in heavily immunosuppressed patients, as in the case we present, or immediately after a decrease or withdrawal of immunosuppression[14].

It is also worth noting that there have been no reports of reactivation of hepatitis in patients who have received bone marrow stem cells from anti-HBs positive donors[16], suggesting that the passive transfer of specific antibodies or the passage of specific immune system cells can play an important role in controlling the HBV infection[13].

In the light of these considerations, it could be appropriate to administer hepatitis B vaccination to anti-HBs negative donors prior to their donation of haematopoietic stem cells[16,20].

The case we report also offers the occasion to emphasise the importance of medium-term and long-term haemovigilance: patients who have received transfusions and, even more so, multiply transfused patients must be monitored for late adverse events by controls of their serological profile.

Our patient, who had received numerous transfusions in another hospital, underwent screening for infections on admission to our hospital and again after 4 months; at the next screening control, about 7 months later, it was found that the patient's serological profile had changed. Only the possibility of being able to test stored samples of the patient's serum for hepatitis markers and HBV DNA enabled us to make the differential diagnosis between a new infection and reactivation of an occult infection.

Given the possibility of reactivation of an occult infection, we emphasise the importance of testing all patients for HBV DNA prior to haematopoietic stem cell transplantation in order to start prophylactic antiviral treatment should evidence of such an infection be found.

References

1) Torbenson M, Thomas DL. Occult hepatitis B. Lancet Infect Diseases 2002; **2**: 479-86.
2) Gerolami R, Henry M, Borentain P, et al. Fulminant hepatitis B associated with a specific insertion in the basal core promoter region of hepatitis B virus DNA after immunosuppressive treatment. Clin Infect Dis 2005; **40**: e24-7.
3) Kitano K, Kobayashi H, Hanamura M, et al. Fulminant hepatitis after allogenic bone marrow transplantation caused by reactivation of hepatitis B virus with gene mutations in the core promotor region. Eur J Haematol 2006; **77**: 255-8.
4) Blum HE, Offensperger WB, Walter E, et al. Latent hepatitis B virus infection with full-length viral genome in a patient serologically immune to hepatitis B infection. Liver 1988; **8**: 307-16.
5) Mason AL, Xu L, Guo L, et al. Molecular basis for persistent hepatitis B infection in the liver after clearance of serum hepatitis B surface antigen. Hepatology 1988; **27**: 1736-42.
6) Blanpain C, Knoop C, Delforge ML, et al. Reactivation of hepatitis B after transplantation in patients with pre-existing anti-hepatitis B surface antigen antibodies: report on three cases and review of literature. Transplantation 1998; **66**: 883-6.
7) Endo T, Sawada K, Fujimoto K, et al. Reactivation of hepatitis B virus after autologous peripheral blood stem cell transplantation in patients with positive hepatitis B surface antibodies. Rinsho Ketsueki 2000; **41**: 322-8.
8) Raimondo G, Pollicino T, Cacciola I, et al. Occult hepatitis B virus infection. J Hepatol 2007; **46**: 160-70.
9) Di Marco V, Marzano A, Lampertico P, et al. Clinical outcome of HbeAg-negative chronic hepatitis B in relation to virological response to lamivudine. Hepatology 2004; **40**: 883-91.

10) Lau GK, Yiu HH, Fong DY, et al. Early is superior to deferred pre-emptive lamivudine therapy for hepatitis B patients undergoing chemotherapy. Gastroenterology 2003; **125**: 1742-9.
11) Fabrizi F, Dulai G, Dixit V, et al. Lamivudine for the treatment of hepatitis B virus-related liver disease after renal transplantation: meta-analysis of clinical trials. Transplantation 2004; **77**: 859-64.
12) Marzano A, Angelucci E, Andreone P, et al. for the Italian Association for the Study of the Liver (A.I.S.F.). Prophylaxis and treatment of hepatitis B in immunocompromised patients. Dig Liver Dis 2007; **39**: 397-408.
13) Hui CK, Sun J, Au WY, et al. Occult hepatitis B virus infection in hematopoietic stem cell donors in a hepatitis virus endemic area. J Hepatol 2005; **42**: 813-9.
14) Kempinska A, Kwak EJ, Angel JB. Reactivation of hepatitis B infection following allogeneic bone marrow transplantation in a hepatitis B-immune patient: case report and review of the literature. Clin Infect Dis 2005; **41**: 1277-82.
15) Seth P, Alrajhi AA, Kagevi I, et al. Hepatitis B virus reactivation with clinical flare in allogeneic stem cell transplants with chronic graft-versus-host-disease. Bone Marrow Transplant 2002; **30**: 189-94.
16) Dhedin N, Douvin C, Kuentz M, et al. Reverse seroconversion of hepatitis B after allogeneic bone marrow transplantation. A retrospective study of 37 patients with pretransplant anti-HBs and anti-HBc. Transplantation 1998; **66**: 616-9.
17) Onozawa M, Hashino S, Izumiyama K, et al. Progressive disappearance of anti-hepatitis B surface antigen antibody and reverse seroconversion after allogeneic hematopoietic stem cell transplantation in patients with previous hepatitis B virus infection. Transplantation 2005; **79**: 616-9.
18) Yeo W, Johnson PJ. Diagnosis, prevention and management of hepatitis B virus reactivation during anticancer therapy. Hepatology 2006; **43**: 209-220.
19) Endo T, Sawada K, Fujimoto K, et al. Reactivation of hepatitis B virus after autologous peripheral blood stem cell transplantation in patients with positive hepatitis B surface antibodies. Rinsho Ketsueki 2000; **41**: 322-8.
20) Brugger SA, Oesterreicher C, Hofmann H, et al. Hepatitis B virus clearance by transplantation of bone marrow from hepatitis B immunised donor. Lancet 1997; **349**: 996-7.

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