Immunocompromised Patients with HBsAg α Determinant Mutants: Comparison of HBsAg Diagnostic Assays

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Key Words
Hepatitis B virus α determinant mutant · Hepatitis B surface antigen assay · Immunocompromised host · G145R · P120L · T123N

Abstract
Hepatitis B surface antigen (HBsAg) is considered the best marker for the diagnosis of hepatitis B virus (HBV) infection. Mutations of the s gene involving amino acid substitutions within the α determinant could affect the sensitivity of diagnostic tests. In the present study, HBsAg mutants were detected in 3 immunocompromised patients, previously found to be HBsAg negative and anti-HBs positive. All patients had high levels of HBV-DNA, whereas HBsAg tests gave discordant results. Immunosuppression can cause viral reactivation of occult HBV infection in these patients and favour the selection of HBsAg α determinant mutants.

The hepatitis B virus (HBV) affects more than 350 million people worldwide and is a leading risk factor for hepatocellular carcinoma. Although a hepatitis B vaccine has significantly reduced the overall annual incidence of HBV infection, the disease remains one of the major infectious problems affecting mankind [1]. HBV reverse transcriptase is an error-prone enzyme lacking 3′–5′ exonuclease proof-reading capacity [2], resulting in a large number of nucleotide substitutions during replication, with an estimated misincorporation rate of about $10^{10}$ incorrect nucleotide incorporations per day. HBV, like other viruses with error-prone polymerases (i.e. HIV and HCV), has a quasispecies distribution in infected individuals, and circulates as a mixture of genetically distinct but closely related variants. The quasispecies distribution of HBV implies that any newly generated mutation conferring a selective advantage will allow the corresponding viral population to overtake the other variants [2]. HBV mutants can emerge following the se-
lective pressure of antiviral therapy, immune response or vaccination [3, 4]. In fact, relevant mutations of HBV have been identified in HBV-DNA polymerase after prolonged treatment with nucleos( ti)de analogues, such as lamivudine or adefovir [3].

The emergence of HBV mutants capable of escaping the neutralization by anti-HBs antibodies (HBV escape mutants) has been mainly observed in two clinical settings: vaccinated newborns of HBV-infected mothers and transplant recipients receiving human monoclonal anti-HBs antibodies or hyperimmune hepatitis B immunoglobulins [5–7].

A Gly-145-Arg replacement due to a point mutation (G to A) at nucleotide 587 was the first mutation reported in the S gene of HBV; this mutant retains the ability to replicate at a high titer for years [8]. There have been other reports of hepatitis B surface antigen (HBsAg) gene mutants affecting amino acids from positions 120–144 of the α determinant and the pre-S region [8]. The amino acid substitutions lead to conformational changes affecting the binding of neutralizing antibody with two major consequences: mutant clones acquire a selective advantage in carriers treated with active or passive immunization and mutant viruses can lead to diagnostic failure [9, 10]. It is important to note that viral variants displaying changes in HBsAg seem to be very common among chronic HBV carriers [11].

We report 3 cases of patients with impaired immune status, 2 of them with oncohematological disease and 1 who had undergone kidney transplantation, showing re-activation of occult HBV infection with the emergence of HBV surface gene mutants. We also compare the capability of 5 different commercial HBsAg assays to detect these s gene mutants.

Patient 1 is a 67-year-old female diagnosed with lymphatic chronic leukemia in 1998. At that time the patient was HBsAg negative, anti-HBs positive (45 IU/ml) and anti-HBc (IgG) positive. The patient had normal amino-transferase values up to 2004, and had never received chemotherapy. In September 2004, the ALT level increased to 4 times the upper normal limit (UNL). At that time the patient was HBsAg positive, anti-HBs positive (titer not available), and HBeAg positive. In October 2004, ALT levels were still increased above 4 times UNL, and the patient was HBsAg positive, anti-HBs negative, and HBeAg positive. In November 2004, ALT levels were still increased above 4 times UNL, and the patient was HBsAg positive, anti-HBs negative, and HBeAg negative. In November 2004, she first came to our observation: ALT levels were 2.5 times UNL and serum bilirubin levels were normal; the results of HBV markers are reported in Table 1. A few days after hospitalization, the ALT level decreased to the normal range, and the patient asked to be discharged. At the 6-month follow-up (May 2005), the patient showed ALT levels of 2.5 times UNL; she was HBsAg negative, anti-HBs positive (50 mIU/ml), HBeAg positive, and HBV-DNA positive (Roche Cobas Monitor system).

Patient 2 is a 61-year-old female diagnosed with non-Hodgkin lymphoma (low grade) in December 1999. In February 2000, she underwent splenectomy, at that time HBV markers were HBsAg negative, anti-HBs positive, anti-HBc positive, anti-HBe positive, and HBeAg negative. In September 2000, she was diagnosed with hypogammaglobulinemia and started replacement treatment with human immunoglobulin intravenously (400 mg/kg body weight/month). In March 2001, 4 fludarabine cycles

<table>
<thead>
<tr>
<th>Markers</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
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<tbody>
<tr>
<td>HBsAg</td>
<td>–</td>
<td>–/+</td>
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<tr>
<td>HBsAb</td>
<td>++</td>
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<tr>
<td>HBCAb IgG</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>HBeAg</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>HBeAb</td>
<td>–</td>
<td>–</td>
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<tr>
<td>HBV-DNA, copies/ml</td>
<td>$1 \times 10^7$</td>
<td>$4.7 \times 10^6$</td>
<td>$1 \times 10^7$</td>
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<tr>
<td>AST (0–40 IU/l), IU/l</td>
<td>52</td>
<td>47</td>
<td>50</td>
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<tr>
<td>ALT (0–40 IU/l), IU/l</td>
<td>69</td>
<td>73</td>
<td>61</td>
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Tests for hepatitis A virus, hepatitis C virus, hepatitis D virus, cytomegalovirus, HSV-1 and HSV-2, and Epstein-Barr virus IgM were negative. No serological markers of autoimmune (ANA, AMA, anti-LKM1) were found. The serological profile of the patients was evaluated with a Roche Elecsys Modular E170 analyzer. HBV-DNA was evaluated with a Roche Cobas Monitor system.
were administered followed in August 2002 by CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) therapy. After the first CHOP cycle, an increase in alkaline phosphatase and γ-glutamyltransferase was observed; serological HBV markers showed that the patient was HBsAg positive, anti-HBs positive (39 mIU/ml), anti-HBc (IgM and IgG) negative, HBeAg positive, and anti-HBe negative. In February 2003, a quantitative serum HBV-DNA test showed a viral load of >2.0 × 10^5 copies/ml, and despite positive HBV markers, chemotherapy was continued.

In March 2003, the patient was hospitalized for thrombocytopenia after the last course of chemotherapy. In May 2003, treatment with humanized anti-CD20 (rituximab) and lamivudine (100 mg/day p.o.) was started; in November 2003, INF-α (3 MUI/TIW) was added to the lamivudine treatment. In March 2004, serum HBV-DNA tested negative, and lamivudine and IFN were stopped; 2 months later (May 2004) rituximab was also stopped. The patient continued to receive intravenous immunoglobulin replacement because of hypogammaglobulinemia.

In June 2005, the patient was admitted to the Immunology Unit of the University of Naples Federico II. At that time the ALT level was above UNL and serological HBV markers were: HBsAg weakly positive, anti-HBs, anti-HBc (IgM and IgG) negative, HBeAg positive, anti-HBe negative, and HBV-DNA positive (4.7 × 10^5 copies/ml).

Patient 3, a 48-year-old male, who had received a kidney transplant in another hospital, was admitted to the Hematology Unit of the Second University of Naples in May 2005. A few hours later the patient was transferred to another hospital; thus, no further information or clinical data are available.

All patients when first observed at our hospital had an unusual HBV marker profile (table 1) that clearly required further investigations. The abnormal serological pattern with a high viral load was suggestive of mutation of the S antigen; therefore, viral DNA was obtained from the patients and sequenced. The TRUGENE HBV system (Bayer Diagnostics, Marburg, Germany) was used to sequence the S gene region and to genotype the HBV. A G145R (GGA → AGA) mutation in the a determinant of HBsAg was identified in patients 1 and 2. Two mutations, i.e. P120L (CCA → CTA) and T123N (ACC → AAC), were identified in patient 3. Genotype D was identified in the 3 patients; no mutation was found in the precore and core promoter regions.

We also evaluated the patients’ sera with 5 different diagnostic assays and obtained discordant results (table 2). The HBsAg mutant was detected in patients 1 and 3 with the HBsAg II EIA Cobas Core Roche and Ortho (ECi) Vitros HBsAg assays, but not with the Elecsys HBsAg assay (Roche; first generation), HBsAg V2 AxSYM (Abbott) or Bayer ADVIA Centaur assays. HBsAg was positive in patient 2 with the HBsAg II EIA Cobas Core Roche and Ortho (ECi) Vitros assays, whereas a gray zone result was obtained with the Elecsys assay (Roche) and a negative result with the HBsAg V2 AxSYM (Abbott) and Bayer ADVIA Centaur assays. The 5 diagnostic assays gave the same results for the other HBV serological markers (HBeAg, anti-HBs, anti-HBc, anti-HBe).

The sera of patients 1 and 2 were available at 18 months of follow-up; neither had received antiviral treatment during follow-up, and only patient 2 was still on immunoglobulin replacement. At this time, patient 1 was still HBsAg negative whereas patient 2 was unequivocally HBsAg positive; both patients still had a high viral replication (1.5 and 2.9 × 10^7 copies/ml, respectively). AST and ALT values were 55 and 64 IU/l, respectively, in patient 1, and 50 and 68 IU/l in patient 2. The G145R mutation was still present in both patients; however, another mutation (T126I) was identified in patient 2.

We evaluated the sera of patients 1 and 2 (collected at the first observation and at the 18-month follow-up) using a second generation of HBsAg assay developed by Elecsys HBsAg II assay (Roche). Both patients tested positive on the two occasions.

In this report we describe 3 cases of HBV a determinant mutants in patients with an impaired immune system (2 oncohemological patients and 1 kidney-transplanted patient) who tested HBsAg negative (with the first generation Elecsys HBsAg assay), anti-HBs positive, but have high viral replication (HBeAg and HBV-DNA positive). Two of the 3 patients had a history of HBV infection and were HBsAg negative and anti-HBs positive (titer above 10 mIU/ml). Data on viral replication of the 3 patients before reaching our hospital were not available. Therefore, we can speculate that the patients had an occult infection and that immunosuppression may have re-

<table>
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<tr>
<th>Patient</th>
<th>Elecsys</th>
<th>Cobas</th>
<th>AxSYM</th>
<th>Vitros</th>
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<td>1</td>
<td>-</td>
<td>+</td>
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<td>2</td>
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activated the HBV infection and selected a diagnostic escape mutant, as recently reported in a lymphoma patient [12]. It is noteworthy that patient 2 was treated with a humanized monoclonal antibody against CD20 (rituximab), which is known to induce reactivation of the occult HBV infection [13]. The same patient had also received periodic immunoglobulin replacement that could have contributed to the selection of a mutant viral quasispecies, which was responsible for the diagnostic test failure.

In February 2000, this patient showed anti-HBc-positive antibodies (IgG) whereas in August 2002, the patient was anti-HBc (IgM and IgG) negative. Anti-HBc clearance is very unusual. However, the patient was diagnosed with hypogammaglobulinemia (September 2000) and started replacement treatment with human immunoglobulin intravenously. Therefore, the reduced production of endogenous immunoglobulins associated with replacement therapy may explain this uncommon result.

It is important to note that the HBV surface mutations observed did not impair the HBV fitness since high viremia HBV (DNA >10^6 copies/ml) was observed in all patients.

Today, oncohematological patients routinely undergo testing for HBV infection and to monitor the risk of occult HBV reactivation; therefore, reliable diagnostic tests for screening and monitoring patients with impaired immune system are needed. However, in our study some HBsAg assays failed to detect S gene mutants of HBV. The first-generation HBsAg Elecsys test gave an indeterminate result in patient 2 and a negative result in patient 1. It is possible that in this patient wild-type HBV coexists with the mutant viral quasispecies in a proportion not detectable by direct sequencing. In fact, sequencing detects a viral population that represents more than 20% of the sample [2]. Therefore, we cannot exclude that the indeterminate result was due to a minor HBV wild-type population. The same assay gave an unequivocal positive result in the patient at 18 months of follow-up. This result could indicate an increase in the wild-type viral quasispecies.

The G145R mutants were missed by the Bayer ADVIA Centaur and HBsAg V2 Axsym (Abbott) assays. It has been reported that the Centaur assay does not detect this mutation [14], and so far the test has not been modified. The HBsAg V2 Axsym assay has been reported to detect the G145R mutant [14]. Therefore, it is possible that the HBsAg concentrations in our patients were below the threshold for assay detection. The results are in agreement with a recent report that Axsym HBsAg was negative in a case of occult HBV infection with the escape mutations G145R and C137W [12]. It is important to note that due to the limited availability of natural mutant samples most comparative studies of diagnostic assays have been performed using recombinant antigen mutants. Probably diagnostic assays that detect recombinant mutants could fail to detect natural mutant samples [15], which would explain the discrepancy between the studies. In contrast, the Roche Cobas and Ortho (ECi) Vitros assays gave positive results in all 3 patients. The results obtained with the Ortho Vitros assay are in agreement with previous findings [16]. The new Ortho Vitros HBsAg assay also gave positive results in patients 1 and 2 at 18 months of follow-up.

It is noteworthy that patient 3 carried two mutations in the HBsAg gene region, namely P120L and T123N, which have previously not been reported together in a human host. Both mutations lead to conformational changes and to diagnostic test failure [14]. In a previous study, the T123N mutation alone or in association with other mutations was missed by the Axsym and Centaur assays [14]. Interestingly, the P120L mutation caused the diagnostic failure of the Biomerieux Vidas HBsAg assay, whereas the Axsym assay detected HBsAg [17], which suggests that the combination of mutations can also contribute to diagnostic test failure.

In conclusion, patients with oncohematological disease can present an unusual serological profile probably due to immunosuppression that can cause reactivation of occult HBV infection. Because some diagnostic assays could fail to detect HBV surface mutants, as confirmed by the present report, the screening and monitoring of HBV infection in immunosuppressed patients should include an HBsAg assay that has a high sensitivity and specificity capable of detecting immune and diagnostic surface escape mutants.

Acknowledgment

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References


HBsAg Diagnostic Failure in Immunocompromised Patients

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