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CASE REPORT

Pitfall of hepatitis B surface antigen testing in a kidney transplant recipient presenting hepatitis B reactivation

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Summary Diagnosis of hepatitis B virus (HBV) infection based on hepatitis B surface antigen (HBsAg) detection can be hampered in the setting of HBV reactivation in immunocompromized patients with prior serology indicating past cured infection, and can be associated with severe or fulminant and fatal hepatitis. We present a case of HBV reactivation in a renal transplant patient in whom HBsAg failed to be confirmed as a true positive result. One year after transplantation, systematic testing showed HBsAg positivity with a titer at 244 pg/mL, anti-hepatitis B core antibody and concurrent anti-hepatitis B surface antibody positivity. Confirmation of HBsAg detection by seroneutralization did not confirm HBsAg positivity, indicating that HBsAg detection was a false positive result. Notwithstanding, HBV DNA titer in serum was concurrently 8.6 Log IU/mL. HBV DNA sequencing showed a genotype D and several amino acid substitutions within HBsAg, including some previously involved in impaired diagnosis and altered immunogenicity. Although no perturbation of liver biochemical markers was observed, treatment with tenofovir was introduced. One month later, HBV DNA level had decreased by 2.6 Log IU/mL and no clinical and biochemical symptoms of hepatitis had occurred. The present case underlines that serologic diagnosis of HBV reactivation can be tricky in transplant recipients with a prior serology indicating past HBV infection. This prompts to perform HBV DNA testing in case of positive HBsAg testing, regardless of the result of neutralization by anti-HBs antibodies.

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Introduction

The diagnosis of hepatitis B virus (HBV) infection is based on hepatitis B surface antigen (HBsAg) detection, which can be hampered in case of infection with HBV HBsAg mutants [1—3]. Such diagnosis failure is of particular concern in the setting of HBV reactivation in immunocompromized patients with prior serology indicating past cured infection, i.e. with positive anti-hepatitis B surface (anti-HBs) and anti-hepatitis B core (HBc) antibody testing [4—7]. We present a case of HBV reactivation in a renal transplant patient in whom HBsAg failed to be confirmed as a true positive result.

Case report

A 65-year-old man received a kidney transplant in September 2009. He had been treated by hemodialysis for 6 years after a post-infectious glomerulonephritis. Systematic HBV serologic testing performed in September 2009 indicated past cured HBV infection. Thus, it showed HBsAg negativity and anti-hepatitis B core antibody positivity, while anti-HBs antibody titer was 27 mIU/mL (Architect Abbott assays, Abbott Diagnosis, Abbott Park, IL); anti-hepatitis e (HBe) antibody testing was negative. Because the anti-HBs antibody titer was low for a hemodialyzed patient, he received a booster vaccination 1 month before the graft. The kidney donor was negative for HBV (anti-HBc antibody, anti-HBs antibody, and HBsAg testing were negative). Post-graft immunosuppressive regimen associated an induction with rabbit anti-thymocyte globulins and a maintenance treatment with tacrolimus, mycophenolate mofetil, and prednisone. A 3-month prophylaxis with valganciclovir and a 6-month prophylaxis with sulfametoxazole-trimethoprim was prescribed. During the first year, the transplantation was complicated by a post-transplant diabetes, hypertension, and an acute pyelonephritis episode. One year after the graft, the patient was well and his creatininemia had stabilized around 160 μmol/L.

A systematic testing 1 year post-transplantation showed HBsAg positivity, HBsAg titer being 244 pg/mL (positivity threshold, 0.05), anti-HBc antibody positivity, with concurrent anti-HBs antibody positivity (titer, 21 mIU/mL). Liver function tests were normal. Indeed, alanine aminotransferase level (ALT) was 19 IU/L (usual values, 8—45), aspartate aminotransferase level was 22 IU/l (12—35), alkaline phosphatase level was 79 IU/L (53—128), gamma-glutamyl transferases level was 21 IU/L (9—55), total bilirubinemia was 15 μmol/L (5—34), and lactate dehydrogenase level was 225 IU/L (125—248). As recommended in case of first positivity of HBsAg testing [8], a confirmation assay was performed to assess the level of neutralization by anti-HBs antibodies, by pre-incubating the patient's serum with neutralizing anti-HBs antibodies before retesting. This procedure aims to assess the specificity of initial HBsAg detection. Indeed, in case of true positivity, pre-incubation with anti-HBs antibodies hampers further interaction of HBsAg with anti-HBs antibodies in the HBsAg detection assay, which leads to a decrease in the signal corresponding to HBsAg detection (threshold for interpretation of HBsAg true positivity, neutralization > 50%). In contrast, in case of false positivity, the signal corresponding to HBsAg detection does not decrease substantially (neutralization < 50%). In the present case, the confirmatory assay did not confirm HBsAg positivity (neutralization was 13%), in spite of serial dilution of the serum (at final 1:10 and 1:100 dilutions) that aimed at enabling neutralization by anti-HBs antibodies in case of a high HBsAg titer. Indeed, the initial signal of HBsAg detection fall of only 13% (positivity threshold for HBsAg neutralization, > 50%). Thus, according to the manufacturer's instructions, HBsAg positivity was interpreted as a false positive result. Notwithstanding, HBV DNA testing was concurrently performed because of the earlier HBV serologic profile indicating past HBV infection and the known risk of HBV reactivation in renal transplant recipients. HBV DNA could be detected and viremia was 8.6 Log IU/mL (Abbott Real-time PCR). HBsAg detection was controlled with two other assays: AxSYM Abbott assay (Abbott Diagnostics) showed HBsAg positivity but absence of confirmation by the neutralization assay, while VIDAS HBsAg Ultra assay (Biomérieux, Marcy l'Étoile, France) showed HBsAg positivity (Titer, 290.80 pg/mL; positivity threshold, 0.13) and confirmation by neutralization at a dilution of 1:1000 (100% of neutralization; positivity threshold, > 50%). HBV DNA sequencing performed with in-house assays, as described previously [9], showed a genotype D. Additionally, several amino acid substitutions were present within HBsAg in comparison with that of reference genotype D HBV (Fig. 1), including some previously involved in impaired diagnosis or concurrent HBsAg and anti-HBs antibody detection (P120PT, G130R, D144AE) [1,9—12]. Although no perturbation of liver biochemical markers was observed, treatment by tenofovir was introduced. One month later, HBV DNA level had decreased by 2.6 Log IU/mL, liver biochemical markers were still normal, and no clinical symptom of hepatitis had occurred.

Discussion

Impaired HBsAg diagnosis can be a particular clinical concern in the context of HBV reactivation in renal transplant recipients with a serology at time of graft indicating past HBV infection. Indeed, such event can be associated with severe or fulminant and fatal hepatitis [13—15], and delayed diagnosis of HBV reactivation might delay the introduction of anti-HBV therapy.

A few studies have reported previously impaired confirmation of HBsAg positivity by means of seroneutralization. Le Pendeven et al. reported a case of hepatitis B reactivation in a transplant renal recipient with HBV serology indicating post-infection [4]. At the time of HBV reactivation, HBsAg testing was highly positive but HBsAg failed to be seroneutralized (Monolisa HBsAg confirmation, Biorad, Marnes, France), which delayed the viral diagnosis. Concurrently, HBV DNA level was 4.107 copies/mL. HBV serology and HBV DNA testing were performed retrospectively on available serum samples. HBsAg was detected and only neutralized by the VIDAS assay in two sera collected 2 and 6 months before hepatitis onset. Moreover, HBV DNA could be detected in the three serum samples that were tested, as far as 22 months before HBV reactivation. HBV DNA sequencing showed a genotype E with the presence of the G145R mutation within the ''a determinant'' of the HBV surface

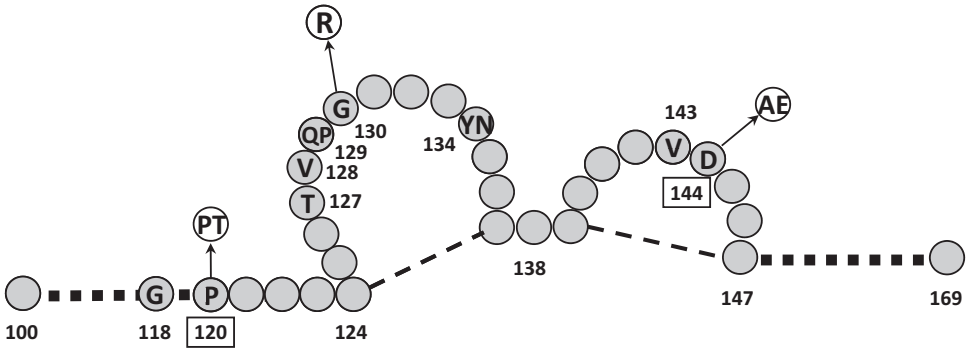

Figure 1 Amino acid changes in the major hydrophilic region of hepatitis B surface antigen from the patient. Substitutions from wild-type to mutated amino acid are indicated by an arrow. Dotted lines indicate more than one amino acid; punctured lines indicate disulphide bridges.

gene, which has been related to escape to immune response after vaccination and had been frequently associated with impaired HBsAg detection [1—4,9—11]. In the present case, amino acid substitutions did not include G145R but others reported previously in cases of diagnosis failure [1,3] and HBV reactivation in renal transplant recipient [10,12]. Noteworthy, in the case of Le Pendeven and in the present case, anti-HBs antibody and HBsAg testing were concurrently positive. This was previously observed in immunocompromized patients and was associated with increased amino acid variability within HBsAg and the presence of amino acid substitutions associated with altered HBsAg immunogenicity or impaired HBsAg diagnosis [1,9—13]. Such atypical serologic pattern may contribute to complicate the diagnosis of HBV infection. In another study conducted in the USA in 2003—2004, Chen and Kaplan reported absence of confirmation of HBsAg-positive results with the Immulite 2000 test (Diagnostic Product Corporation) by the neutralization assay in 433 (52%) of 826 HBsAg-positive serum samples [16]. Additionally, they found that HBsAg neutralization test was negative in 89 (70%) among 127 randomly selected weakly reactive samples, as defined by a test/cutoff optical density ratio comprised between 1.0 and 2.5 and, noteworthy, HBV DNA was detected in six (6.7%) of these 89 sera, as assessed with the Roche COBAS Amplicor assay v.2.0 (Roche Diagnostics). O'Brien reported in 2000 a far lower proportion, 1.1%, of 4,467 HBsAg-positive serum samples tested by an enzyme immunoassay that were not neutralized by anti-HBs antibodies [17]. It should be noted that in the present case, HBsAg level that was measured and failed to be confirmed by seroneutralization was high.

In summary, the present case underlines that serologic diagnosis of HBV reactivation can be tricky in transplant recipients with a prior serology indicating past HBV infection. This prompts to perform HBV DNA testing in case of HBsAg positivity, regardless of the result of neutralization by anti-HBs antibodies.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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None.

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