Case Report

Reactivation of a hepatitis B virus surface-antigen mutant following an autologous stem-cell transplant for multiple myeloma in an anti-HBc-negative patient

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Introduction: Hepatitis B virus (HBV) surface-antigen mutants can present diagnostic difficulties when they are not recognized by commercial assays; HBV infection with undetectable core antibody is also described.

Case presentation: We report the case of a multiple myeloma patient who had no detectable serum HBV core antibody, HBV surface antigen (HBsAg) or HBV DNA prior to immunosuppressive treatment, who, after autologous stem-cell transplant, developed a reactivation of occult HBV with a HBsAg mutant, undetectable with a commercial HBsAg assay.

Conclusion: This case reminds clinicians to be mindful of the potential diagnostic difficulties of HBV serology in the immunosuppressed, and to remain vigilant for the possibility of the reactivation of HBV and the existence of HBsAg mutants, which can also reactivate, in the context of profound immunosuppression.

Keywords: Hepatitis B; diagnosis; serology; reactivation; escape mutant; tenofovir.

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Introduction

Chronic hepatitis B virus (HBV) infection is a major public health problem worldwide; over 350 million people are chronically infected, with an estimated one-third of the global population showing serological evidence of past infection (Liaw & Chu, 2009). The natural history of HBV infection depends on the host immune response and can result in either resolution or chronic infection. Chronic infection is usually defined as persistence of HBV surface antigen (HBsAg) in serum for greater than 6 months. HBV core antibody (anti-HBc) is usually detectable in both chronic and resolved (serum HbsAg-negative) infections.

However, occult HBV infection after resolution, defined as detectable HBV DNA in the liver with absence of detectable HBsAg in the serum, is a well-recognized entity (Raimondo et al., 2008). Reactivation of occult infection after immunosuppression is also well recognized, and can be associated with fulminant hepatitis and death (Sera et al., 2006). Current guidelines therefore recommend screening for both HBsAg and anti-HBc prior to chemotherapy or immunosuppressive therapy (European Association for the Study of the Liver, 2012).

However, diagnostic difficulty can arise in the presence of HBsAg mutants, which produce abnormal surface proteins that are not recognized by commercial HBsAg assays (Weber, 2005). Chronic HBV infection without detectable anti-HBc has also been described (Avettand-Fenoel et al., 2006). Here, we present a report of a multiple myeloma patient who had no detectable serum anti-HBc, HBsAg or HBV DNA prior to immunosuppressive treatment, who, after an autologous stem-cell transplant, appeared to reactivate an occult HBV infection with an HBsAg mutant that was undetectable with a commercial HBsAg assay.

Case report

In 2010, a 55-year-old man was diagnosed with light-chain multiple myeloma after developing lower back pain and was referred to our centre for management. He was born in the Philippines but had been resident in the UK since 2005. He had no past medical history, and as far as he knew had never been exposed to HBV or been vaccinated, and had not had HBV immunoglobulin (HBIG). He...
worked as a plant operator and was married with six children. He had never received a blood transfusion or blood products. Test results were negative for HBsAg (Siemens Centaur XP) anti-HBc (Siemens Centaur XP), as well as for hepatitis C virus antibodies and for human immunodeficiency virus p24 antigen and antibodies (Siemens Centaur XP).

He underwent chemotherapy with eight cycles of cyclophosphamide, thalidomide and dexamethasone from July 2010 to January 2011, before undergoing a high-dose melphalan autologous stem-cell transplant in April 2011, with a significant improvement in his serum light-chain levels. Around this time, his liver function tests, in particular his alanine transaminase (ALT) level, were noted to have become abnormal (Fig. 1), although no cause was identified. Around December 2011, his general practitioner sent his blood for repeat HBV serology to another laboratory, and it was found to be HBsAg positive using a different assay (Abbot Architect).

He was recalled and his serum HBV DNA was found to be positive (850 000 IU ml$^{-1}$). Although his HBsAg was still negative with the original assay (Siemens Centaur XP), it was HBsAg positive using three other commercial assays (Bio-Rad Ultra, Roche Elecsys and Abbot Architect). His anti-HBc was now positive (Siemens Centaur XP). He was positive for HBV e-antigen and negative for HBV e-antibody. Retrospective testing of a pre-transplant sample (from March 2011) was negative for both HBV DNA and HBsAg using three of the four commercial assays (Siemens Centaur XP, Bio-Rad Ultra and Roche Elecsys); unfortunately, there was not enough remaining serum to test for HBsAg using the Abbot Architect assay. Despite the prior anti-HBc negativity (Siemens Centaur XP), the sample was weakly positive for anti-HBc using a different assay (Bioelisa Biokit). A diagnosis of reactivation of occult HBV with an HBsAg mutant was made and he was started on tenofovir therapy.

The DNA sequence of the HBsAg gene was determined, which revealed HBV genotype C, consistent with postulated infection in the Philippines (Weber, 2005). It also identified typical mutations associated with HBsAg variants that cannot be detected by all HBsAg assays (L110I, I126T, F134S and G145R; Weber, 2005); no mutations associated with reverse transcriptase inhibitor resistance were identified in the reverse transcriptase gene.

His HBV DNA viral load fell (Fig. 1) and was undetectable by December 2013; his ALT level also normalized. Unfortunately, his myeloma relapsed in January 2013, as evidenced by increasing serum light chains, and he was started on velcade and dexamethasone chemotherapy.

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**Fig. 1.** Schematic time line of the case showing ALT (IU ml$^{-1}$), HBV DNA PCR (IU ml$^{-1}$) and HBsAg and anti-HBc results over time.
He continues on this and tenofovir, which given his ongoing immunosuppression, is planned to continue lifelong.

He received two pools of platelets around the time of his stem-cell transplant and no other blood products. The donors were tested for serum HBsAg, anti-HBc and HBV DNA by the Blood Transfusion Service and were negative. His wife and children were tested for HBsAg (Abbott Architect) and were also found to be negative. He had no other risk factors for HBV exposure at the time of the transplant when HBsAg was detected.

### Discussion

This case demonstrates a reactivation of occult HBV infection with an HBsAg mutant. The diagnosis was delayed first because of a lack of serological evidence of HBV infection prior to immunosuppression (negative anti-HBc), and secondly because of difficulty in detecting circulating HBsAg with a commercial assay due to the presence of a variant surface antigen.

Given the country of origin of this patient and the genotype of the isolated virus, it seems most likely that the patient was exposed to HBV before his presentation with multiple myeloma – probably shortly after birth or during early childhood. Lack of anti-HBc in chronic HBV infection has been described but is uncommon, and is largely associated with immunosuppression; in 2169 HBsAg-positive chronic HBV patients in a French cohort, only 39 were negative for anti-HBc. Of the 19 patients for whom clinical data were gathered, almost all of them were immunosuppressed in some way (Avettand-Fenoel et al., 2006).

Lack of anti-HBc has been described in patients with haematological malignancy who reactivated occult HBV infections both prior to (Feeney et al., 2013) and after (Awerkiew et al., 2007) immunosuppressive therapy; in both of these cases, the core gene was wild type (WT), suggesting a failure of the immune response rather than core protein abnormality as the cause of lack of detectable anti-HBc. However, core gene heterogenicity as a cause of negative anti-HBc has been described (Zoulim et al., 1996). Lack of sensitivity of the anti-HBc assay has also been suggested as the cause of apparent lack of anti-HBc in some patients (Kantelhardt et al., 2009). It therefore seems likely in this patient that immunosuppression caused by the multiple myeloma resulted in the loss of detectable anti-HBc over time. This hypothesis is supported by the fact that a different assay gave a very weakly detectable anti-HBc using the patient’s pre-transplant sample; this weak anti-HBc positivity also makes it unlikely that the patient was newly infected with HBV after July 2010, an event that would otherwise explain the serological and molecular findings. The presence of an HBV genotype that is common in the Philippines and rare in the UK (Weber, 2005) also supports this hypothesis.

Surface-antigen mutant (or escape) viruses have amino acid substitutions within the ‘a’ determinant of the surface antigen, which restricts the binding of neutralizing antibodies. This has several implications; mutant viruses may be difficult to detect with some commercial HBsAg assays, as in our case. The mutations may also provide a selection advantage in patients vaccinated against HBV or treated with HBIG, and allow the development of chronic HBV infection despite these therapies, as seen in babies born to HBV-infected mothers undergoing HBV immunization and HBIG treatment (Carman et al., 1990) or liver transplant recipients given HBIG treatment to prevent reinfection of the transplanted liver (Ghany et al., 1998).

More relevant to this case, reactivation of occult HBV infection with an HBsAg mutant is also described in the context of immunosuppression with cytotoxic and mAb therapy, particularly rituximab (Awerkiew et al., 2007; Feeney et al., 2013; Sera et al., 2006; Westhoff et al., 2003) and usually in the presence of anti-HBs. In these situations, aggressive immunosuppression allowing virus replication in the presence of the patient’s anti-HBs can select for reactivation of HBsAg ‘escape’ mutants. Clinicians caring for such patients need to be alert to the possibility of HBsAg mutants arising.

It appears that no vaccine or HBIG had been administered to this patient and so the virus may be a naturally occurring strain (Alavian et al., 2013) and such HBsAg variants have been identified recently in the Philippines (Baclig et al., 2014). Such HBsAg variants are not only important in terms of detection by diagnostic assays, but may also escape vaccine-induced anti-HBs and may not be neutralized by HBIG preparations.

In conclusion, this case highlights the effectiveness of antiviral treatment for reactivation of HBV in an immunosuppressed patient. However, it also reminds clinicians to be mindful of the potential diagnostic difficulties of HBV serology in the immunosuppressed, and to remain vigilant for the possibility of the reactivation of HBV and the existence of HBsAg mutants, which can also reactivate, in the context of profound immunosuppression.

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### References


