Occult hepatitis B infection by a recombinant D/C virus in an immunosuppressed patient

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\textbf{ABSTRACT}  
Approximately 300 million people worldwide were living with chronic hepatitis B virus infection as of 2016, however, this number does not account for those who might be living with occult hepatitis B virus infection due to difficulty diagnosing this condition. The multiple genotypes and the ability of the hepatitis B virus to acquire mutations that down-regulate its expression make occult hepatitis B virus infection a very elusive diagnosis. This is especially worrisome when there is a need to start immunosuppressive therapies, since there is a risk of reactivation in undiagnosed patients. We present a case of female patient who was referred to the consultation because she was about to start chemotherapy with an anti-CD20 agent and had a positive anti-HBc and anti-HBs. During routine workup an occult hepatitis B virus infection was diagnosed. Upon further study mutations in the PreCore and Basal Core Promoter regions were identified, as well as, a double genotype D/C. Therapy with tenofovir was initiated before the patient was started on chemotherapy. This case highlights the importance of comprehensive studying of patients who present with apparently resolved chronic hepatitis B virus infection, especially when they are about to start immunosuppressive therapies.

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\textbf{Introduction}  
As of 2016, it was estimated that approximately 300 million people were living with chronic hepatitis B virus (HBV) infection worldwide (defined as hepatitis B surface antigen [HBsAg] positivity), and of these, only about 10% (29 million) were diagnosed, and only 5% of the eligible ones received antiviral treatment [1]. However, this estimation has shortcomings, as it does not include patients with occult hepatitis B infection (OBI), that are defined as having negative HBsAg, negative or positive hepatitis B core antibody [anti-HBc] with negative or positive hepatitis B surface antigen [anti-HBs] along with detection of HBV DNA in the liver or serum. The down-regulation of the expression of HBsAg can be triggered by various viral (i.e. mutations, co-infections [hepatitis C virus, human immunodeficiency virus]), epigenetic (histone deacetylation and chromatin organization) and host factors (i.e. immune response, HLA polymorphisms) [2,3]. In the last few years, there have been several reports concerning the importance of mutations in the HBV genome and their role in the molecular mechanisms of OBI, the most important ones being mutations in the “Sa” determinant of HBsAg, mutations in the pre S1, pre S2 and C regions and mutations that affect RNA splicing [4,5].

OBI poses a diagnostic challenge, especially among patients who are about to start immunosuppressive therapies such as chemotherapy (i.e. rituximab – anti-CD20), high dose corticosteroids and anti TNF-alfa antibodies (i.e. adalimumab and infliximab), that can alter the competence of the immune system and lose control over viral replication in the liver, which most of the times can lead to a reactivation of the infection and subsequent overt hepatitis (often fulminant) after immune reconstitution [2–6]. Recent guidelines recommend the screening of HBV infection in all patients that are undergoing immunosuppressive therapies, in order to start treatment or prophylaxis if necessary [6].

\textbf{Case report}  
A 74-year old female patient was referred to the infectious diseases screening and prevention consultation of immunosuppressed individuals because she was going to start chemotherapy
with obinutuzumab for a small-cell lymphocytic lymphoma and had a positive anti-HBc in the initial screening. The patient presented with no symptoms aside from tiredness, and had a personal history of vertigo, chronic constipation, anxiety and deafness. Her chronic medication included trazodone, beta-histine, zolpidem and mexazolam. She did not report any allergies, did not smoke or took intravenous drugs and reported never having received a blood transfusion and never living outside of Portugal. She only had unprotected sexual intercourse with her deceased husband. Physical and neurological exams were unremarkable, aside from bilateral axillary lymphadenopathy.

In the initial screening she had anemia (Hgb 121 g/dL), normal renal and hepatic functions and a C-reactive protein of 0.07 mg/dL. She was immune to toxoplasmosis and hepatitis A and had previous infections by cytomegalovirus (CMV) and Epstein-Barr virus (EBV). Hepatitis C virus (HCV), human immunodeficiency virus (HIV) and syphilis screening were all negative. HBsAg, quantitative HBsAg (0.00) and HBeAg were all negative. She had positive anti-HBc, anti-HBe and anti-HBs (50.19). Her HBV DNA was 642 IU/mL. The CT scan of the abdomen was unremarkable. After these results, HBV DNA was repeated, and similar results were obtained. It was then opted to perform additional testing and surveillance until the patient was started on chemotherapy.

Genotype and pre-core mutation testing were performed. To carry out these tests we used a Laminar flow chamber for handling samples, Nuclisens B specific protocol for DNA extraction performed on Easy Mag-BioMerieux-France and PCR operated on Biometra Termocycler. To identify nucleotide polymorphisms in the Basal Core Promoter (BCP) and PreCore region of HBV we use Inno-Lipa HBV PreCore assay – Innogenetics/Fujirebio-Belgium. It was identified a PreCore codon 28 mutation (G1896A), as well as, a BCP mutation (T1762A/T1764). Using the Inno-Lipa HBV Genotyping assay a genotype D was clearly identified, but concomitantly a line of specific oligonucleotide probe of genotype C was also visible. In order to clarify this result, genotype testing was performed again, and the same results were obtained. The blood was then sent to and external laboratory in Ghent, Belgium for the sequencing of the HBV. The sequencing revealed a polymorphism, being the highest match with HBV genotype D (93 %), when compared with sequences in available internet databases.

Since the patient was about to start chemotherapy, she was prescribed tenofovir 245 mg once daily. The medication was well tolerated, and HBV viremia became undetectable. Screening was offered to all of her four children. The oldest daughter (55yo) presented with chronic hepatitis B infection (HBsAg +, anti-HBc +, anti-HBs –) and is being accompanied in the infectious diseases consultation, the second daughter (53yo) and the third son (50yo) presented with a resolved infection (HBsAg +, anti-HBc +, anti-HBs +) and the younger daughter (48yo) was immune through vaccination (HBsAg +, anti-HBc +, anti-HBs +). We were unable to screen her husband since he had passed away a few years prior.

Discussion

Occult hepatitis B virus infection (OBI) prevalence varies, from 1 % to 30 % around the world, having in western Europe a prevalence below 1:5000 inhabitants, and is defined as the existence of HBV DNA in the liver or serum with either presence (seropositive) or absence (seronegative) of one or both anti-HBc and anti-HBs [4,7]. Normally, the absence of HBsAg and presence of anti-HBc and anti-HBs is characterized as immunity due to natural infection. However, this serologic result can also be found in patients carrying OBI when a structural arrangement or down-regulation of the expression of HBsAg is present [4,5]. In our patient, the obtained results were congruent with natural immunity to HBV, yet after HBV DNA was performed it revealed an occult hepatitis B infection.

The mechanisms that potentially inhibit HBV and induce OBI are various. Some depend on the host and his immunological ability to control and contain the infection within the liver through efficient CD4+ and CD8+ response, the production of interferon type 1 (IFN) and TNF-a that help suppress viral replication, as well as, an epigenetic factor that regulates the acetylation and deacetylation of H3/H4 histones that bound the covalently closed circular DNA (cccDNA) [3]. Others depend on viral factors, in which mutations play a significant role. The most common mutations affect the pre-S/S regions and can affect HBsAg by two different ways: the first one affects the “Sa” determinant of HBsAg and may lead to a failure of detection of this protein by commercially available tests; the second one affects the pre-S1/S2 regions and can significantly decrease or abolish the replication of the virus [3-5]. There can also exist mutations of the C, X and P genes of the virus, but they are less frequently observed. In OBI, the most frequent mutations of the C gene, affect the basal core promoter (BCP) region and the Pre-core (Pre-C) region. The ones that are usually observed are the double mutation A1762T/G1764A (or T1762A/T1764) of the BCP, that contributes to the suppression of precore mRNA and G1896A of the Pre-C that stops the production of hepatitis B e-antigen (HBeAg) and down-regulates HBV replication [5,8]. Our patient presented with both mutations, which can explain the absence of HBeAg and HBsAg and quantitative HBsAg in the multiple tests that were performed. The presence of G1896A (pre-core codon 28) and the BCP A1762T/G1764A mutations could have been the probable mechanism that led to an occult hepatitis B infection in this patient.

In the last few years, several reports identified the possibility of recombinant hepatitis B viruses, since it has one of the most diverse and heterogeneous genomes. In 2012, in Eastern India, a novel D/C recombinant was discovered and classified as subgenotype D9. Subgenotype D9 was the first to have a genotype D backbone with genotype C mosaicism in the pre-C/C region, as well as, a higher prevalence of BCP double mutation A1762T/G1764A [9]. After that, in 2013, two larger studies of blood donors and hospital patients identified a recombinant genotype A3/G and a genotype D2/D3 in Burkina Faso and Sao Paulo, respectively [10,11]. At the same time, in Japan a co-infected HIV/HBV patient was diagnosed with a recombinant G/A2 hepatitis B strain that had very few similarities to the previous ones reported from that country [12]. In Africa, also in 2013, an analysis was made to compare the recombinant D/E genotypes present in the continent [13].

The sequencing performed to our patient’s virus by an external laboratory provided us with the highest match with HBV genotype which was genotype D (93 %), however the INNO-LIPA HBV genotyping performed in our own hospital identified both D and C genotypes. Allying this information with the identification of both BCP double mutation A1762T/G1764A and Pre-C mutation G1896A (both common to genotype D and C), we assumed that our patient was probably infected by subgenotype D9. Nevertheless, extensive genotyping was not performed, and we cannot identify with absolute certainty the genotype of the HBV. Even more uncommon is that most of these recombinant genotypes were observed mainly in developing countries, and our patient has no record of ever having been outside of Portugal. In fact, the only risk factor that we could identify was unprotected sex with her husband. One explanation might be that her husband, could have had contact with the virus through sexual intercourse in Mozambique (Africa) during the Portuguese colonial war and posteriorly could have transmitted it.

There have also been reports that the presence of these mutations (BCP double mutation A1762T/G1764A and Pre-C mutation G1896A), in particular the double mutation A1762T/G1764A, was associated with a significant increase in the risk of hepatocellular carcinoma (HCC). Still, most studies found out that the stronger correlation is between the double mutation and HBV
genotype B and C [8,14]. Our patient presented with an HBV genotype D with genotype C mosaicsisms, having similarities to the D9 subgenotype identified in India some years ago. In this case the patient had an unremarkable CT scan, so the risk for HCC is difficult to ascertain but it can be believed that the recombination process could have more influence over the clinical outcome than the appearance of several separate mutations [9].

Occult hepatitis B virus infection, like in our patient’s case, poses a diagnostic challenge since we have to be more alert when it comes to choosing what test should be performed to identify the infection. According to the European Association for the Study of Liver (EASL) clinical practice guidelines on the management of hepatitis B virus infection, all candidates for immunosuppressive therapies (in particular rituximab) should be tested for HBV markers before they are started on immunosuppression, but they only include the possibility of testing for HBV DNA in patients with negative HBsAg and positive anti-HBc [15]. We argue that in all patients who are about to undergo chemotherapy, high dose corticosteroids or immunosuppressive therapy and have negative HBsAg, positive anti-HBc and positive Anti-HBs should be offered HBV DNA testing in order to rule out a possible OBI. In our case, if HBV DNA had not been performed prior to initiation of chemotherapy with obinutuzumab (anti-CD20), the result could have been catastrophic.

Lastly, it is crucial to mention the importance of screening all of her offspring in order to prevent further transmission of the virus to younger generations. Having done so, in our patient’s case, we were able to identify at least two daughters that were at risk. This allowed us to start monitoring both of them, due to the risk of having chronic hepatitis B and subsequently hepatocellular carcinoma (HCC).

Author statement

We present a case of an occult hepatitis B virus infection in a patient who was about to start chemotherapy with an anti-CD20 agent. The recombinant genotype D/C of the HBV and the acquired mutations in the PreCore and Basal core Promoter regions made it difficult to diagnose this infection. We emphasise the importance of diagnosing occult hepatitis B virus infections in patients who are about to start immunosuppression in order to start immediate treatment and avoid complications.

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Consent

Written informed consent was obtained from the patient for publication of this case report. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

Declaration of Competing Interest

None.

CRediT authorship contribution statement

Gonçalo Pereira Cruz: Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing. Celene Sargent: Data curation, Writing - review & editing. Maria Conceição Ventura: Writing - review & editing. Joaquim Oliveira: Supervision, Writing - review & editing. José Saraiva da Cunha: Writing - review & editing.

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