De Novo Hepatitis B Surface Antigen (HBsAg) Positive, Core Antibody (Anti-HBc) Negative, Hepatitis B Virus Infection post-Liver Transplant from an Anti-HBc, HBsAg Negative Donor

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Abstract:

Liver transplant donors and recipients are routinely screened for Hepatitis B virus (HBV) infection with Hepatitis B surface antigen (HBsAg) and Hepatitis B core antibody (anti-HBc). Organs are accepted from donors who are HBV negative, with the caveat of increased monitoring when accepting organs from 'increased risk donors'. Transplant recipients are vaccinated if there is no sign of previous infection or immunity and monitored for reactivation in case of previous HBV infection. In cases where both the donor and recipient are HBV negative, no antiviral prophylaxis is used post-transplant. We present a case of a HBV immunized, anti-HBc negative patient who underwent orthotopic liver transplant from an anti-HBc negative donor. The patient did not receive post-transplant antiviral prophylaxis because of mutual anti-HBc-seronegative status. However, she developed HBV infection with isolated HBsAg, and persistently negative anti-HBc. Mutations in the core/pre-core regions of HBV gene were not implicated for the unique serology in this case. Immunosuppression post-liver transplant is the

likely etiology for isolated HBsAg seroconversion despite significantly elevated HBV DNA. Our experience suggests that HBV DNA screening of liver transplant donors and recipients, in addition to HBV DNA monitoring in recipients, may reduce transplant-associated HBV risk.

Key Words: Hepatitis, Hepatitis B, seroconversion, Hepatitis B surface antigen reactivity, liver transplant

Background:

Hepatitis B virus (HBV) infection is one of the most common infection in the world, with an estimated 257 million carriers worldwide (1). HBV is a public health-notifiable disease in Canada, under provincial and territorial surveillance. Chronic hepatitis B (CHB) infection in Canada is mostly attributed to migration from an endemic country or susceptible, unvaccinated adults with exposure risk factors. For those born in Canada, risk factors for HBV infection include having family members who are chronic HBV carriers, injection drug use, high-risk sexual activity, body piercing and tattoos, and a history of blood transfusions, particularly before 1971-72, when routine blood donor HBsAg screening was implemented in Canada (2,3). In addition, patients who are immunocompromised, due to HIV, chemotherapy, immunosuppressive post-transplant medications, or those with genetic abnormalities such as common variable immunodeficiency, remain at higher risk of HBV reactivation (4).

Hepatitis B surface antigen (HBsAg) is one of the most important laboratory screening measures for HBV infection. It is one of the first markers of an acute infection. However, HBsAg can also seroconvert following HBV vaccination. For example, Ly et al. and Rysgaard et al. demonstrated

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transient HBsAg seroconversion in 9 dialysis patients after HBV immunization (5,6). Vaccine-related HBsAg seroconversion typically reverts within a few weeks, without other seromarkers associated with acute HBV infection, such as Hepatitis B core antibody (anti-HBc), specifically IgM antibodies. Quantifiable HBV deoxyribonucleic acid (DNA) level is mandatory to guide treatment, and may indicate phase of HBV infection based on other serological markers (1,7).

The above serological profile is typically present in acute HBV infection. We present a case of HBV infection with positive HBsAg with detectable HBV DNA, but persistently negative anti-HBc in a previously HBsAg and anti-HBc-negative, HBV-immunized, post-liver transplant patient, whose source liver was from a HBsAg and anti-HBc-negative donor.

Case Report:

Our patient is a 27 year old female who underwent her first liver transplant for end-stage liver disease secondary to Wilson's disease. She suffered from graft loss due to ischemic cholangiopathy and underwent a second orthotopic liver transplant six years later. She was immunized against Hepatitis B virus (HBV) two years after her original transplant (twice) and then a year later, with persistently negative Hepatitis B surface antigen (HBsAg) and Hepatitis B core antibody (anti-HBc). Hepatitis B surface antibody (HBsAb) measured after the third vaccine was 46 [IU]/mL (Cobas® e 602, Roche Diagnostics). After her second liver transplant, she was discharged on the standard early immunosuppressive regimen including tacrolimus, mycophenolate mofetil, and tapering prednisone. Post-liver transplant, she continued to remain well with negative serologies for HBV infection, including negative HBsAg, negative anti-Hepatitis B core (anti-HBc) IgM and IgG antibodies, and HBsAb levels >10 [IU]/mL. However,

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on routine screening in May 2020, she was found to have a reactive HBsAg with negative anti-HBc. Repeat testing was confirmatory, including concordant serology results on a different platform (Abbott ARCHITECT), and demonstrated a HBV DNA level of 11,600 [IU]/mL (Cobas® HBV; Roche Diagnostics) with negative anti-HBc. There was no elevation of liver enzymes, including alanine aminotransferase (ALT) and she remained asymptomatic post-transplant. She was started on nucleoside antiviral treatment with Entecavir, with follow-up demonstrating a persistently negative anti-HBc.

As the liver donor and recipients were both HBsAg and anti-HBc negative prior to transplantation, antiviral prophylaxis was not administered immediately post-transplant. In addition, the donor was also immunized prior to transplant. In terms of risk factors for Hepatitis B infection, our patient did not have any invasive dental procedures or blood transfusions since re-transplantation, and did not engage in any high risk activities. Contact tracing of family members and partner demonstrated non-reactive HBsAg and anti-HBc in all.

To investigate the potential for a mutation in the pre-core/core region, amplicon-based next-generation sequencing targeting the core/pre-core region (nucleotides 1672 – 1946; coreF:TAAGAGGACTCTTGGAC; coreR:AAGAAGTCAGAAGGCAA) was performed with the MiSeq Reagent Kit v2 Nano (Illumina). No mutations in the pre-core/core region were identified.

Discussion:

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Acute HBV infection usually presents with positive HBsAg and positive anti-HBc IgM antibodies (7). As HBV infection resolves, HBsAg becomes undetectable, but anti-HBc IgG persists. However, if HBsAg persists for longer than 6 months, spontaneous clearance is less likely. Chronic HBV infection is characterized by persistence of HBsAg and anti-HBc IgG antibodies (7). We have presented an interesting case of HBV infection 10 months after liver transplantation with unusual serology of positive HBsAg but persistently negative anti-HBc IgM and IgG antibodies. Although isolated HBsAg positivity can be found in the initial incubation period after infection, this is the first reported presentation of persistently isolated HBsAg positivity despite a significantly detectable HBV viral level of over 11,000 [IU]/mL in an immunized patient who received a liver transplant from an anti-HBc negative patient. This could be due to agent or host factors, including infection with a HBV mutant, immune tolerance or lack of response by the host immune system.

The HBV C-gene (C-ORF) contains two main regions – the core region (183 amino acids) which encodes the viral nucleocapsid, and the pre-core gene (29 amino acids) which encodes the HBe-antigen in combination with the core gene (8). Fiordalisi et al. sequenced the HBV pre-C/C regions and found familial heterogeneity in the samples, with single base deletions causing generation of stop codons, thereby leading to synthesis of truncated proteins (9). These truncated proteins are not recognized by the immune system, therefore, lack of antibody formation. Kreutz et al. found that core-deletion mutants were often observed in those with chronic HBV infection (related to low level of viremia) (10). Another related mutation is in the X-ORF (X-region) of HBx (protein X), which is known to transactivate transcriptional regulatory elements, which include enhancer and core protein elements (11). Patients with X-ORF mutation may suppress

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the replication and expression of HBV DNA, therefore leading to suppression of anti-HBc synthesis (11). They may also demonstrate low or negative HBsAg, and negative anti-HBc despite low HBV DNA replication. Despite this established phenomenon, sequencing of our patient's samples did not demonstrate a mutation in the core or pre-core region of the HBV genome.

Immune tolerance is another mechanism attributed to lack of anti-HBc production. There are several proposed mechanisms by which the host immune response fails to respond to acute HBV infection with HBc antibodies. One proposed mechanism is that of T-cell anergy. T cell anergy refers to development of immune system tolerance after an initial exposure to the antigen. After the initial exposure, T-cells are functionally inactive but remain alive in a hypoactive state (12). T-cell anergy is essential in development of self-tolerance and regulation of the cellular immune response. The role of T-cell anergy has been implicated in the lack of anti-HBc development in infants born to HBsAg carrier mothers (13). Ni et al. studied 10 children with persistently seropositive HBsAg, HBe-antigen, and HBV DNA (13). They were exposed to HBV perinatally, and therefore, developed immune tolerance to HBc antigen in early infancy and maintained isolated HBsAg seroconversion. As the immune tolerance waned, the children developed anti-HBc antibodies between ages of 2 and 8 years. Laperche et al. postulated a similar reasoning for the lack of HBc antibodies in two blood donors who had positive HBsAg, but lacked anti-HBc and had a normal ALT level (14). Both the donors were immunocompetent and did not demonstrate any mutations in their HBV core or pre-core genes, but were thought to have developed immune tolerance due to HBV exposure in utero.

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In addition to T-cell anergy, lack of anti-HBc response has also been attributed to immunosuppression. Melegari et al. studied 12 children undergoing chemotherapy, most of whom had hematological malignancies (15). All children were thought to have acquired nosocomial HBV infection from undetected infective blood (15), characterized by positive HBsAg, HBeAg, and HBV DNA but negative anti-HBc IgM and IgG throughout chemotherapy. Avettand-Fenoel et al. studied 39 patients with HBV infection, characterized by isolated HBsAg positivity, and negative anti-HBc (16). The patients in the study were immunosuppressed due to HIV infection, solid organ transplant (kidney, heart, or bone marrow), while 1 patient had HCV co-infection. More recently, Brousseu et al. reported a case of acute HBV infection in a patient receiving rituximab for low-grade non-Hodgkin lymphoma with isolated HBsAg positivity (17). That patient developed anti-HBc 6 months after first positive HBsAg, with the delay attributed to immunosuppression due to Rituximab. Both Avettand-Fenoel et al. and Brousseu et al. showed that patients developed HBc antibodies once immunosuppression was less pronounced or after chemotherapy completion (16,17), further establishing the role of immune suppression in lack of anti-HBc response in acute HBV infection.

Our patient was immunosuppressed with tacrolimus, mycophenolate mofetil, and prednisone post-liver transplant. The profound immunosuppression is the likely reason for lack of seroconversion of anti-HBc despite significant HBV DNA viral load and isolated HBsAg presentation. She remains on antiviral treatment with Entecavir and 10 months after initial HBsAg seroconversion, she remains anti-HBc negative.

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Our patient likely acquired her HBV infection via the donor source liver as she did not endorse any risk factors for acute HBV infection and, indeed, all of her familial and social contacts were tested and found to be negative for HBV. HBsAg and anti-HBc are used for screening donors and recipients prior to liver transplantation, to identify their HBV infection status and determine the role of antiviral prophylaxis after transplantation. Both the donor and recipient had negative serologies for HBsAg and anti-HBc, and the donor was immunized for HBV prior to transplant with detectable HBsAb >10 [IU]/mL, therefore, no antiviral prophylaxis was initiated. HBV DNA detection is the most accurate tool available for detection of HBV infection. It is usually obtained by quantitative molecular assays, which are limited due to resources, such as cost or availability. Our case raises concern that screening with HBsAg and anti-HBc alone may not be sufficient to rule out source donor liver HBV infection. Additionally, it highlights the need to consider post-transplant antiviral prophylaxis against HBV to prevent potential post-transplant viremia and/or consider post-transplant monitoring with nucleic acid amplification testing.

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References:

1. Coffin CS, Fung SK, Alvarez F, Cooper CL, Doucette KE, Fournier C, et al. Management of Hepatitis B Virus Infection: 2018 Guidelines from the Canadian Association for the Study of the Liver and Association of Medical Microbiology and Infectious Disease Canada. Vol. 1, Canadian Liver Journal. 2018. p. 156–217.
2. Rotermann M, Langlois K, Andonov A, Trubnikov M. Seroprevalence of hepatitis B and C virus infections: Results from the 2007 to 2009 and 2009 to 2011 Canadian Health Measures Survey. Vol. 24, Health reports. Ottawa: Statistics Canada; 2013. p. 3–13.
3. Zhang J, Zou S, Giulivi A. Epidemiology of Hepatitis B in Canada. Vol. 153, American journal of epidemiology. Johns Hopkins University Press; 2001. p. S213.
4. Moller B, Hopf U, Stemerowicz R, Henze G, Gelderblom H. HBcAG expressed on the surface of circulating Dane particles in patients with Hepatitis-B virus-infection without evidence of anti-HBC formation. Vol. 10, Hepatology (Baltimore, Md.). Philadelphia: W B Saunders Co; 1989. p. 179–85.
5. Rysgaard CD, Morris CS, Drees D, Bebber T, Davis SR, Kulhavy J, et al. Positive hepatitis B surface antigen tests due to recent vaccination: a persistent problem. Vol. 12,

Uncorrected Proof

BMC clinical pathology. England: BioMed Central Ltd; 2012. p. 15.

6. Ly D, Yee HF, Brezina M, Martin P, Gitnick G, Saab S. Hepatitis B surface antigenemia in chronic hemodialysis patients: effect of hepatitis B immunization. Vol. 97, The American journal of gastroenterology. Elsevier Inc; 2002. p. 138–41.

7. Pondé RAA, Pondé RAA. Atypical serological profiles in hepatitis B virus infection. Vol. 32, European journal of clinical microbiology & infectious diseases. Berlin/Heidelberg: Springer-Verlag; 2013. p. 461–76.

8. Bruss V. Hepatitis B virus morphogenesis. Vol. 13, World journal of gastroenterology : WJG. Pleasanton: Baishideng Publishing Group Inc; 2007. p. 65–73.

9. Fiordalisi G, Primi D, Tanzi E, Magni E, Incarbone C, Zanetti AR, et al. Hepatitis-B virus-C gene heterogeneity in a familial cluster of anti-HBC negative chronic carriers. Vol. 42, Journal of medical virology. New York: Wiley-Liss; 1994. p. 109–14.

10. Kreutz C. Molecular, immunological and clinical properties of mutated hepatitis B viruses. Vol. 6, Journal of cellular and molecular medicine. Oxford, UK: Blackwell Publishing Ltd; 2002. p. 113–43.

11. Kramvis A, Kew MC. The core promoter of hepatitis B virus. Vol. 6, Journal of viral hepatitis. Oxford, UK: Blackwell Science Ltd; 1999. p. 415–27.

12. Chen M, Sällberg M, Hughes J, Jones J, Guidotti LG, Chisari F V, et al. Immune Tolerance Split between Hepatitis B Virus Precore and Core Proteins. Vol. 79, Journal of Virology. Washington: American Society for Microbiology; 2005. p. 3016–27.

13. Ni Y-H, Hsu H-Y, Chang M-H, Chen D-S, Lee C-Y. Absence or delayed appearance of hepatitis B core antibody in chronic hepatitis B surface antigen carrier children. Vol. 17, Journal of hepatology. Copenhagen: Elsevier B.V; 1993. p. 150–4.

Uncorrected Proof

14. Laperche S, Guitton C, Smilovici W, Courouce AM. Blood donors infected with the hepatitis B virus but persistently lacking antibodies to the hepatitis B core antigen. Vol. 80, Vox sanguinis. Oxford, UK: Blackwell Science Ltd; 2001. p. 90–4.

15. Melegari M, Jung M-C, Schneider R, Santantonio T, Bagnulo S, Luchena N, et al. Conserved core protein sequences in hepatitis B virus infected patients without anti-HBc. Vol. 13, Journal of hepatology. Copenhagen: Elsevier B.V; 1991. p. 187–91.

16. Avettand-Fenoel V, Thabut D, Katlama C, Poynard T, Thibault V. Immune suppression as the etiology of failure to detect anti-HBc antibodies in patients with chronic hepatitis B virus infection. Vol. 44, Journal of Clinical Microbiology. Washington: American Society for Microbiology; 2006. p. 2250–3.

17. Brousseau N, Murphy DG, Gilca V, Larouche J, Mandal S, Tedder RS. Acute hepatitis B virus infection with delayed appearance of hepatitis B core antibody in an immunocompromised patient: a case report. Vol. 11, Journal of medical case reports. England: BioMed Central; 2017. p. 111.

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