Lessons from practice

Unusual hepatitis B virus findings in blood donors

Clinical records

Patient 1: Occult hepatitis B virus infection with hepatitis B core antibodies

A 66-year-old, hepatitis B virus (HBV) unvaccinated male blood donor exhibited repeatedly reactive multiplex nucleic acid test (NAT) results for human immunodeficiency virus type 1, hepatitis C virus and HBV, and non-reactive discriminatory HBV NAT and hepatitis B surface antigen (HBsAg) results. Twenty-eight previous donations since 2010 were HBV NAT non-reactive. Total hepatitis B core antibody (anti-HBc) results were repeatedly reactive and HBsAg antibody (anti-HBs) results were also reactive, while e-antigen (HBeAg), HBeAg antibodies (anti-HBe) and anti-HBc IgM were non-reactive. National Reference Laboratory (NRL) HBV NAT results were reactive. These results were consistent with occult HBV infection (OBI). The donor, his parents, and his partner were born in Taiwan.

Patient 2: Occult hepatitis B infection without hepatitis B core antibodies

A 50-year-old, HBV unvaccinated female blood donor exhibited reactive multiplex NAT, reactive discriminatory HBV NAT and non-reactive HBsAg results. All five previous blood donations made 3–7 months earlier were NAT non-reactive. Total anti-HBc was non-reactive while anti-HBs was reactive. There was insufficient specimen for further testing so additional specimens were collected 5 days later. These specimens exhibited the same results again, plus total anti-HBc, anti-HBc IgM, anti-HBe and HBeAg were non-reactive, and NRL HBV NAT was reactive. These results were consistent with OBI. The donor, her parents, and her partner were born in Vietnam.

Patient 3: Acute hepatitis B virus infection

A 46-year-old, HBV unvaccinated male blood donor exhibited reactive multiplex NAT, reactive discriminatory HBV NAT and non-reactive HBsAg results. Thirteen previous donations since 2010 were HBV NAT non-reactive. There was insufficient specimen remaining for NRL HBV NAT so additional specimens were collected 4 days later. These specimens showed reactive discriminatory and NRL HBV NAT results, but the multiplex NAT result was non-reactive. Anti-HBs, total anti-HBc, anti-HBc IgM, HBeAg and anti-HBe were non-reactive on the initial and repeat specimens. On referral, an infectious diseases department at a tertiary referral hospital initially interpreted the results as falsely reactive. However, repeat testing 2 months later at another laboratory found an HBV viral load of 72 IU/mL, while HBsAg, anti-HBc and anti-HBs remained non-reactive. Anti-HBs became reactive 5 months later, while HBsAg, anti-HBc and HBV NAT were non-reactive. These results were consistent with window period acute HBV infection and subsequent clearance. The donor and his parents were born in Australia, but he reported unprotected sexual intercourse with women and having a barber shave his face with a cut-throat razor while travelling in China and the Philippines earlier that year.

Discussion

Australian Red Cross Lifeblood currently screens approximately 1.6 million donations annually for blood-borne infections.[1] It therefore encounters rare sets of testing results such as those illustrated by these three cases of HBV infection. Such testing results are not commonly seen by clinicians, even those dealing directly with HBV-infected people. In summary, these patients had non-reactive HBsAg, but HBV DNA was repeatedly detected, and they possessed risk factors for HBV infection and sometimes other markers such as anti-HBc (Box). Another scenario in which this may occur is HBsAg mutant HBV infection, whereby HBsAg is not detected by the assay.

Testing algorithms for blood-borne viruses in blood donation laboratories differ substantially from those of diagnostic laboratories because they are specifically designed to screen blood donors, who are asymptomatic, rather than diagnose infection in patients with clinical or laboratory markers of disease. The HBV testing algorithm at Lifeblood consists of donor testing for HBsAg and HBV DNA, with donor deferral and supplementary testing if either or both tests are reactive (Supporting Information).[2] This highly sensitive testing algorithm sometimes yields results which do not conform to the usual serological patterns of HBV infection.

One such atypical HBV result pattern is OBI, defined by low levels of replication-competent HBV DNA in the liver and/or blood without detectable HBsAg. HBV NAT results are reactive, with or without anti-HBc and/or anti-HBs reactivity. The prevalence of OBI is unknown because it is easily mistaken for cleared/resolved HBV infection in clinical settings where HBV NAT is rarely performed if HBsAg is non-reactive. Further, HBV DNA may be undetectable or only intermittently detectable via NAT in OBI if the viral load is below the lower limit of detection, typically around 10 IU/mL.[3] Serological patterns observed in OBI include: anti-HBc reactive and anti-HBs non-reactive (50.1%); anti-HBc reactive and anti-HBs reactive (40.1%); anti-HBc non-reactive and anti-HBs reactive (9.5%); and anti-HBc non-reactive and anti-HBs non-reactive (0.3%).[4] Atypical anti-HBc non-reactive OBI is associated with younger donor age, higher HBV vaccination rates, higher anti-HBs levels, higher HBV viral loads, and

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Summary of patient demographics, results, risk factors and diagnoses

	Patient 1	Patient 2	Patient 3
Age	66 years	50 years	46 years
Sex	Male	Female	Male
First time or repeat donor	Repeat	Repeat	Repeat
Most recent negative donor HBV screening	2 years	3 months	1 year
Initial results	• HBV NAT: R (S/Co: 15.5, 9.9); NR; R* • HBsAg: NR • Anti-HBs: R (16.1 IU/L) • Anti-HBc: R (S/Co: 2.1, 3.3, 3.3) • Anti-HBc IgM: NR • HBeAg: NR • Anti-HBe: NR	• HBV NAT: R (S/Co: 15.4); R (S/Co: 25.4); NT* • HBsAg: NR • Anti-HBs: R (184.4 IU/L) • Anti-HBc: NR • Anti-HBc IgM: NT • HBeAg: NT • Anti-HBe: NT	• HBV NAT: R (S/Co: 15.2); R (S/Co: 25.1); NT* • HBsAg: NR • Anti-HBs: NR • Anti-HBc: NR • Anti-HBc IgM: NR • HBeAg: NR • Anti-HBe: NR
Subsequent results	na	• HBV NAT: R (S/Co: 15.5); R (S/Co: 27.3); R* • HBsAg: NR • Anti-HBs: R (218 IU/L) • Anti-HBc: NR • Anti-HBc IgM: NR • HBeAg: NR • Anti-HBe: NR	• HBV NAT: NR; R (S/Co: 24.4); R* • HBsAg: NR • Anti-HBs: NR • Anti-HBc: NR • Anti-HBc IgM: NR • HBeAg: NR • Anti-HBe: NR • HBV NAT: R (72 IU/mL) • HBsAg: NR • Anti-HBs: NR • Anti-HBc: NR • HBV NAT: NR • HBsAg: NR • Anti-HBs: R (27 IU/L) • Anti-HBc: NR
Risk factors	Born in Taiwan; partner born in Taiwan	Born in Vietnam; parents and partner born in Vietnam	Cut-throat razor shave at barber and unprotected sexual intercourse with women in China and the Philippines
Diagnosis	Occult HBV infection	Occult HBV infection	Window period acute HBV infection and clearance

Anti-HBc = hepatitis B core antibody; Anti-HBe = hepatitis B e-antigen antibodies; Anti-HBs = hepatitis B surface antigen antibodies; HBeAg = hepatitis B e-antigen; HBsAg = hepatitis B surface antigen; HBV = hepatitis B virus; na = not applicable; NAT = nucleic acid test; NR = non-reactive; NT = not tested (insufficient specimen); R = reactive; S/Co = signal-to-cutoff ratio. * HBV NAT results: non-discriminatory human immunodeficiency virus type 1, hepatitis C virus and HBV multiplex NAT; discriminatory HBV NAT; National Reference Laboratory HBV NAT. ◆

fewer HBV core and S protein mutations.[4] Although the risk of cirrhosis, hepatocellular carcinoma and transmission in OBI is unknown but likely to be low, patients with OBI should probably undergo long term monitoring and appropriate specialist consultation. Transfusion-transmitted infection (TTI) has been described and risks have been estimated; it is therefore important that blood operators detect and recognise it.[5,6]

HBV NAT may also detect acute HBV infection during the window period, when HBsAg and anti-HBs are present in approximate equilibrium such that both are rendered non-reactive on serological assays, as seen with patient 3. Modelling indicates a negligible HBV TTI residual risk (less than 1 in 1 million per unit transfused), predominantly due to undetected OBI, although window period acute HBV infection also contributes.[5,7] Despite the use of DNA tests with lower limits of detection of 2–4 IU/mL, transfusion transmission from an acutely infected donor remains possible.[8] Lookback of blood recipients was not performed for patients 1 and 2 because the risk of HBV TTI was estimated to be very low given all previous HBV NAT assayed donations were non-reactive and both patients were anti-HBs reactive. Lookback was performed for patient 3, establishing that the recipient of the previous blood donation was not HBV infected.

General practitioners and other specialists will occasionally be referred blood donors with unusual results such as those described in this article. It is therefore important that clinicians consider such results significant, rather than falsely positive.

Lessons from practice

- Blood donor screening laboratories use different testing algorithms to diagnostic laboratories and may yield results which do not conform to the usual patterns with which clinicians are familiar.
- Hepatitis B virus (HBV) infection without detectable surface antigen may occur in window period acute HBV infection or occult HBV infection. This can also occur with infection with a hepatitis B surface antigen (HBsAg) mutant strain.
- Occult HBV infection is characterised by HBV nucleic acid test (NAT) reactivity and HBsAg non-reactivity, with or without hepatitis B core antibody and/or HBsAg antibody reactivity. It is easily mistaken for cleared/resolved HBV infection on serological testing and may be missed by HBV NAT if the viral load is below the limit of detection.
- If HBV NAT and/or serological testing yields atypical or unexpected results, consultation with medical specialists familiar with unusual forms of HBV infection and continued follow-up of these patients is recommended.

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Supporting Information

Additional Supporting Information is included with the online version of this article.