Virology Question and Answer Scheme (VIROQAS)

The importance of being earnest: Following up a low level hepatitis B surface antigen (HBsAg) result

Cillian F. De Gascun*, Marianne Fraher, Margaret Crean, Jeff Connell, William W. Hall

National Virus Reference Laboratory, University College Dublin, Belfield, Dublin 4, Ireland

A R T I C L E   I N F O

Article history:
Received 16 October 2009
Received in revised form 6 May 2010
Accepted 8 May 2010

Case description

A 24 yr old Lithuanian woman (LA) resident in Ireland presented to the antenatal service of her local (regional) hospital for the first time at 36 weeks gestation in July 2008. Blood was drawn (sample date 08/07) and sent to the National Virus Reference Laboratory (NVRL) for antenatal screening for evidence of infection with human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV). The sample was weakly positive in the HBsAg Abbott Architect (reading/cut-off: 0.29/0.05) and was confirmed by neutralization in the Abbott Murex HBsAg assay. No other marker of HBV infection (including HBV e antigen (HBeAg), e antibody (anti-HBe), HBcIgM, and anti-HBc) was detected. HIV and HCV serological results were negative.

What is your interpretation of these results?
What further investigations are indicated?
How would you treat this lady’s newborn baby?

See evidence-based opinion overleaf

* Corresponding author. Tel.: +353 1 716 1349.
E-mail address: cfbdeg@hotmail.com (C.F. De Gascun).

1386-6532/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jcv.2010.05.001
Evidence-based opinion

What is your interpretation of these results?

The serological profile would be consistent with three possible scenarios: (i) early HBV infection; (ii) cross-reactivity as the result of recent vaccination with recombinant HBsAg; or (iii) non-specific cross-reactivity within the assay, perhaps due to the physiological state of pregnancy, as has been reported with enzyme-linked immunosorbent assays (ELISA) for other viral infections. Reactivity in the HBsAg assay was neutralized with specific anti-sera and this would suggest the presence of HBsAg, due either to early infection or recent vaccination.

What further investigations are indicated?

Health-care providers should test all pregnant women for HBsAg during an early prenatal visit (e.g., first trimester) in each pregnancy, even if they have been previously vaccinated or tested.4–6 In addition, those women who engage in behaviours that put them at high risk for infection (e.g., injection-drug use, multiple sexual partners in previous 6 months, evaluation or treatment for a sexually transmitted infection [STI]), and those with clinical hepatitis symptoms should be re-tested at the time of admission to the hospital for delivery. At present, there are no recommendations for the use of hepatitis B DNA testing in pregnancy. However, maternal HBV viraemia has been implicated as the most important factor associated with failure of neonatal vaccination to prevent transmission of infection.7,8

In the case presented here, no further investigations were performed on the initial sample. However, the low level HBsAg result was phoned to the referring hospital and a follow-up sample – intended primarily to exclude early HBV infection – requested. A small gradual increase in reactivity in the Abbott Architect HBsAg assay was observed in three follow-up samples received on 14/07 (sample 2), 22/07 (sample 3), and 10/08 (sample 4), with OD/CO ratios of 0.40/0.05, 0.65/0.05, and 2.16/0.05, respectively (Table 1). However, all other markers of HBV infection remained undetected. LA presented to her primary care physician for the infant’s 6-week check-up on 01/10/08 (sample 5). As requested in the hospital discharge letter, blood was drawn for HBV serology. HBsAg was not detected. In addition HBeAg was not detected; however HBcIgM (>200 mIU/ml), anti-HBc, anti-HBe, and anti-HBs were detected. Based on these results, HBV DNA investigations using the Roche COBAS Amplicor HBV Monitor were performed retrospectively on previous samples received in which sufficient volume remained. HBV DNA was detected in the first (July 8) and second (July 14) samples with viral loads of 1530 IU/ml and 2200 IU/ml, respectively. There was insufficient sample volume remaining to test samples 3 and 4; HBV DNA was not detected in sample 5. Furthermore, a final sample collected 14/10 (sample 6), confirmed a serological profile consistent with recent but resolving HBV infection: again, HBsAg DNA was not detected.

Table 1
Hepatitis B serological progression from July to October 2008.

<table>
<thead>
<tr>
<th>Sample date</th>
<th>HBsAg (reading/cut-off)</th>
<th>Anti-HBs (mIU/ml)</th>
<th>HBeAg</th>
<th>Anti-HBe</th>
<th>Anti-HBc</th>
<th>HBcIgM</th>
<th>HBV DNA (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 8</td>
<td>0.29/0.05</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>July 14</td>
<td>0.40/0.05</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>1530</td>
</tr>
<tr>
<td>July 22</td>
<td>0.65/0.05</td>
<td>ins</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>2200</td>
</tr>
<tr>
<td>August 10</td>
<td>2.16/0.05</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>ins</td>
</tr>
<tr>
<td>October 1</td>
<td>Negative</td>
<td>128</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>nd</td>
</tr>
<tr>
<td>October 14</td>
<td>Negative</td>
<td>228</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>nd, not detected; ins, insufficient sample volume for test.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

This case highlights the importance of follow-up testing when a weak positive HBsAg result is generated: of particular importance is the requirement to perform anti-HBc investigation even if HBsAg is not detected on follow-up. If the first and final samples in this case were tested for HBsAg only, it is probable the patient would not have been identified as HBV infected. Whilst it is unusual HBeAg was not detected in this case, this may have been detectable for a short time between samples 4 and 5 (10/8 and 1/10). Of note, the patient was infected with wild type virus as anti-HBe was detected and also the probable source of infection – subsequently identified as the patient’s partner who was diagnosed with chronic HBV infection on a sample dated 01/10/08 – tested HBeAg positive.

The patient described was asymptomatic through the course of the acute HBV infection. Although anicteric infection in itself is not that unusual,10 this case does highlight the importance of antenatal screening programmes as a means of identifying individuals unknowingly infected with a vertically (and horizontally) transmissible infectious disease. That being said, it is also possible that the non-specific HBV-associated symptoms were in this case attributed by the patient to pregnancy.

With the benefit of hindsight, the diagnosis of HBV infection could have been confirmed earlier with DNA testing. However, it has been our policy not to utilise molecular assays for screening/diagnostic (as against confirmatory/monitoring) purposes, as low level DNA results may be generated that are the result of non-specificity within the assay rather than true infection.11
The HBV status of the infant in this case was assessed at 8 months of age, following completion of the course of vaccination against HBV. At that time, the infant tested negative for HBsAg, HBeAg, anti-HBe, anti-HBc, and HBV DNA: anti-HBs was detected at a level of 134 mIU/ml, indicating the child had not become infected with HBV (despite not having received HBlg at birth). While maternal acute hepatitis B infection does not necessarily pose a significant risk to the neonate if the mother has had time to clear her hepatitis before delivery, the fact that acute HBV in the third trimester of pregnancy – as reported here – did not result in neonatal infection is unusual. However, it may be explained in this case by the low level HBV viraemia observed in the mother, as transmission is more likely to occur when the mother’s viral load is high. Nevertheless, in keeping with the Green Book Recommendations, HBlg (in addition to HBV vaccination) should have been administered to the neonate at birth to reduce the risk of neonatal infection.

Conclusion

This case illustrates the importance of not dismissing weakly positive HBsAg results, and raises the issue of whether HBV DNA testing should be routinely performed in this patient cohort with limited serological evidence of HBV infection. The use of serological assays – even those that are highly sensitive and specific – is compromised by the generation of false-positive results especially when testing low-risk populations, e.g., in national screening programmes. In the case presented here, the potential outcome was highly significant.

Competing interest

None declared.

Ethical approval

Not required.

Funding

None.

References