Pathology (January 2014) **46(1)**, pp. 69–92

CORRESPONDENCE

Detection of hepatitis B virus DNA in serum is rare in the absence of hepatitis B surface antigen: impacts for detection and monitoring of chronic hepatitis B

Sir,

Hepatitis B (HBV) is one of the most common infectious diseases worldwide, with 2 billion people having evidence of past or present infection worldwide and over 200,000 chronically infected in Australia alone.[1,2] It has been estimated that in 2010 there were 312,400 deaths due to HBV-associated cirrhosis worldwide.[3]

Serological testing is the gold standard for diagnosis, with detection of hepatitis B surface (HBsAg) and envelope antigen (HBeAg) consistent with active replicating virus, and anti-hepatitis B surface antibody (anti-HBs Ab) found following successful immunisation or control of natural infection. Over time it has become clear that loss of HBsAg with or without the development of anti-HBs Ab does not represent 'clearance' of HBV as previously thought. In some patients, low level viral replication within the liver may persist associated with, or in the absence of, low level detectable HBV DNA in the serum.[4] The prevalence of so called 'occult HBV' varies geographically but was found in 5–10% of European blood donors with isolated anti-hepatitis B core Ab.[5] The long-term clinical impacts have not yet been determined but these patients are at risk of HBV reactivation in the setting of immunosuppression.[6]

Quantification of HBV DNA is used to confirm the presence of circulating HBV and to monitor and guide treatment of chronic HBV, but its ability to predict HBsAg re-emergence and active hepatitis in the setting of immunosuppression remains undefined.[7] Due to a perceived increase in requests for HBV DNA testing in patients traditionally thought at low risk for active HBV (HBsAg negative, anti-HBs Ab positive) a retrospective review of HBV DNA testing was performed to provide local data as to the reliability of serological and biochemical markers as predictors of HBV DNA positivity, and thus guide its ongoing use.

All patients who had HBV DNA quantification at the Alfred Hospital from September 2008 to September 2010 were identified from the laboratory records and included.

The Alfred Hospital is an adult tertiary referral centre with large hepatology, haematology and infectious diseases units. It is the state referral centre for the management of HIV and performs a number of solid organ and haematological transplants each year.

Study information was collected from the electronic pathology system and included age, gender, serological testing and the result of HBV DNA including viral load when positive. Only serological testing performed at the Alfred Hospital and ordered on the same day or prior to the HBV DNA was recorded (i.e., the results that would be available to the laboratory or physician when ordering the test). The most recent liver function test, specifically albumin, gamma-glutamyl transferase (GGT) and alanine aminotransferase (ALT), were recorded, as was the patient's hepatitis C (HCV) and HIV sero-status and the medical specialty of the requesting physician. In patients with multiple HBV DNA requests over the 2 year study period each was considered a new event and recorded separately.

HBV DNA requests were processed at the state reference laboratory using the Abbott RealTime HBV assay (Abbott Molecular, USA), detection limit 15 IU/mL.[8] All serological testing is performed on site using the Abbott ARCHITECT i2000SR system. Anti-HBs Ab was considered positive when the titre was 10 IU/mL or greater.

Results were summarised using chi-squared tests to evaluate differences in proportions and the Mann–Whitney U test for continuous data. Statistical significance was regarded as $p < 0.05$, two tailed and all statistical analyses were performed using Stata 11.0/IC (Stata, USA). The project received approval from the Alfred Hospital Ethics committee.

From 443 patients, 1101 samples were identified; seven were subsequently excluded because the sample had not been processed, hence 1094 samples (436 patients) were included. Patient characteristics are shown in Table 1. Ninety-nine (22.7%) were HIV positive; 65 (14.9%) HCV positive and 27 (6.2%) were co-infected with HIV and HCV. Twenty patients were concurrently HIV, HCV and anti-HBc Ab positive; 227 (52.1%) and 132 (30.3%) had not been screened for HIV or HCV, respectively.

In total, 473 (43.2%) samples had detectable HBV DNA; 192 (44.0%) of all patients at some time during the study period. Samples from HIV positive patients had lower HBV viral loads and were less likely to have detectable HBV DNA (see Table 1). A HBsAg result was not available in 43 (10.3%) patients. Anti-HBs Ab and anti-HBc Ab data were unavailable in 111 (25.4%) and 113 (25.9%), respectively.

All patients with detectable HBV DNA were HBsAg positive. That is, there were no cases of occult hepatitis B detected by HBV DNA testing over the study period. Of the samples that were HBsAg positive [865 (87%)], 426 (49.2%) had detectable HBV DNA.

Of the anti-HBs Ab positive samples [103 (13.0%)], 25 had detectable HBV DNA (all 25 concurrently HBsAg positive). The mean anti-HBs Ab titre in samples with detectable HBV DNA and anti-HBs Ab was 18.1 mIU/mL (range 10–74), compared with 285 mIU/mL (range 11–1000) in samples with detectable anti-HBs Ab but negative HBV DNA ($p < 0.001$). There were 37 samples from 26 patients with isolated anti-HBc Ab positivity (HBsAg and anti-HBs Ab negative); all were HBV DNA negative, 10 of these patients were HIV positive and the remainder were under the care of a haematologist.

The relationship between HBV DNA detectability and liver function tests are shown in Table 2. Overall samples with detectable HBV DNA had higher ALT and lower GGT levels. This result was not true for the subset of patients who were HIV positive in whom a detectable HBV DNA was associated with lower albumin levels but no differences in ALT or GGT.

Hepatitis B is a major cause of morbidity and mortality worldwide. This study confirms the importance of serology in diagnosing and monitoring HBV and, strikingly, over a 2 year period found no instances of detectable HBV DNA in serum in the absence of concurrent HBsAg.

While the role of HBV DNA measurement in treatment initiation and monitoring in HBsAg positive infections has been well established,[9] it may be a poor option in patients

Print ISSN 0031-3025/Online ISSN 1465-3931

Table 1 Patient characteristics

	Total	HIV status		*p* value
		Negative*	Positive	
n (%)	436 (100)	337 (77.3)	99 (22.7)	–
Male	302 (69.3)	207 (61.4)	95 (95.9)	–
Age, mean years (range)	49.1 (19 – 82)	49.9 (19 – 82)	46.3 (26 – 70)	–
Speciality of requesting physician				
Gastroenterology	269 (61.7)	261 (77.4)	8 (8.0)	–
Infectious diseases	117 (26.8)	30 (8.9)	87 (87.8)	–
Haematology	24 (5.5)	20 (5.9)	4 (4.0)	–
Transplant	8 (1.8)	8 (2.3)	0 (0.0)	–
Oncology	4 (0.9)	4 (1.1)	0 (0.0)	–
Miscellaneous	14 (3.2)	14 (4.1)	0 (0.0)	–
HCV Ab positive	65 (21.3)	38 (18.1)	27 (28.7)	<0.001
HBV DNA detectable	473 (43.2)	398 (47.6)	75 (29.1)	0.001
HBV DNA titre (IU/mL),† median (range)	2482 (17–5.32 $\times 10^9$)	3860 (19–5.32 $\times 10^9$)	594 (17–3.51 $\times 10^9$)	0.008
HBsAg detectable	865 (79.0)	650 (77.7)	215 (83.3)	0.27
anti-HBs Ab detectable	104 (9.5)	78 (9.3)	26 (10.1)	0.21
anti-HBsAb titre‡ (mIU/mL), median (range)	40 (10–1000)	17 (10–1000)	244 (12–1000)	<0.001
anti-HBcAb detectable	742 (67.8)	546 (96.9)	196 (85.9)	<0.001
HBeAg detectable	146 (13.3)	101 (12.1)	45 (17.4)	0.01
anti-HBe Ab detectable	595 (54.4)	531 (63.5)	64 (24.8)	<0.001

n = 436 patients; 1094 samples.
* Patients without HIV testing performed presumed to be negative.
† In samples with detectable HBV DNA.
‡ In samples with detectable anti-HBs Ab.
HBsAg, hepatitis B surface antigen (available in 391/436 patients); anti-HBs Ab, anti-hepatitis B surface antibody (available in 325/436 patients); anti-HBc Ab, anti-hepatitis B core antibody (available in 323/436 patients); HBeAg, hepatitis B envelope antigen (available in 348/436 patients); anti-HBe Ab, anti-hepatitis B envelope antibody (available in 348/436 patients); HIV, human immunodeficiency virus; HCV Ab, hepatitis C virus antibody; HBV DNA, hepatitis B virus DNA.

with HBsAg negativity with, to date, a lack of correlation with clinical outcomes or progressive disease.[7]

It is interesting that there was a small number of patients with concurrent anti-HBs Ab, HBsAg and HBV DNA detectable; a pattern that in some cases remained true with repeat testing 12 months apart (data not shown). In all cases the titre was <100 mIU/mL with an average titre of 18.1 mIU/mL, suggesting that the currently recommended cut-off of 10 mIU/mL may not be the level at which protection against native infection is conferred.[10] Conversely, no patient with an anti-HBs Ab titre of ≥100 mIU/mL had detectable HBV DNA, thus this may be a useful cut-off above which HBV DNA monitoring is not required; further research is necessary to confirm this association.

There were a number of differences between the uninfected and the HIV positive patients. It is likely that the decreased rates of detectable HBV DNA and lower viral loads are a factor of the use of tenofovir as part of the preferred first line antiretroviral regimen. It was more surprising that the rates of detection of anti-HBs Ab were similar between groups, with the HIV positive patients having higher average surface antibody titres. This may represent a selection bias in that HIV positive patients with detectable anti-HBc Ab may be more likely to have HBV DNA testing performed regardless of anti-HBs Ab result.

In this study elevated ALT was associated with detectable HBV DNA, however this pattern was not true in HIV positive patients and prospective studies in patients undergoing chemotherapy have demonstrated that HBV viraemia precedes the development of hepatitis by approximately one week (range 0–11). Thus a normal ALT lacks sensitivity to rule out HBV reactivation in the early stages of disease.[11]

It was disappointing to note that only half of the patients had been screened for HIV. Given the well described potential for

Table 2 Relationship between liver function tests and detection of HBV DNA

	Total			HIV negative*			HIV positive		
	Detectable HBV DNA	Undetectable HBV DNA	*p* value	Detectable HBV DNA	Undetectable HBV DNA	*p* value	Detectable HBV DNA	Undetectable HBV DNA	*p* value
n (%)	473 (43.2)	621 (56.7)	–	398 (47.6)	438 (52.4)	–	75 (29.1)	183 (70.9)	–
ALT (U/L), median (range)	31 (5–7762)	27 (7–734)	<0.001	32 (5–7762)	26 (7–734)	<0.001	29 (11–236)	30 (9–573)	0.99
GGT (U/L), median (range)	31 (8–1076)	40 (9–3255)	<0.001	26 (8–1076)	34.5 (9–3255)	<0.001	82 (17–1010)	67 (15–2383)	0.66
Albumin (g/L), median (range)	40 (18–48)	40 (10–50)	0.20	40 (8–48)	39 (10–50)	0.39	38 (19–46)	40.5 (15–49)	<0.001

* Patients who had not been screened for HIV were presumed to be negative.
ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; HBV DNA, hepatitis B virus DNA.