RATIONAL TESTING

Ordering and interpreting hepatitis B serology

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Cite this as: *BMJ* 2014;348:g2522
doi: 10.1136/bmj.g2522

This series of occasional articles provides an update on the best use of key diagnostic tests in the initial investigation of common or important clinical presentations. The series advisers are Steve Atkin, professor, head of department of academic endocrinology, diabetes, and metabolism, Hull York Medical School; and Eric Kilpatrick, honorary professor, department of clinical biochemistry, Hull Royal Infirmary, Hull York Medical School. To suggest a topic for this series, please email us at practice@bmj.com.

The authors explore how doctors in primary care can identify, investigate, and refer patients with hepatitis B infection

Tam is a 41 year old accountant who left Vietnam as a refugee with his mother in 1980. Normally fit and well, he presents to his general practitioner for assessment of heartburn. During the consultation he mentions that his 64 year old mother recently died of liver cancer. He thinks he was told many years earlier that he was a "healthy carrier" of the hepatitis B virus but has never had a follow-up consultation.

Who should be tested for hepatitis B virus?

Hepatitis B infection is a major public health problem around the world. Most cases of chronic infection are acquired through mother to child transmission at birth or through exposure to the virus in early childhood.[1] The major complications are hepatocellular carcinoma and decompensated cirrhosis, occurring in 15-25% of people with chronic hepatitis B infection.[2] The burden of disease occurs in countries of Asia, Africa, Central America, and eastern Europe. Testing for hepatitis B virus is recommended for all those born in countries with a high and intermediate prevalence (fig 1).[3-5] The box lists other priority populations for testing.

What are the next investigations?

The aim of testing is to establish whether the hepatitis B virus is present, whether there is immunity to the virus from previous infection or vaccination, or whether the patient is susceptible to the virus (has never been exposed or vaccinated) and therefore should be offered vaccination. The tests requested may vary according to the indication. For those at high risk of chronic infection, as in this scenario, a general practitioner should request tests for: hepatitis B surface antigen (HBsAg)—a marker of current infection; hepatitis B surface antibody (anti-HBs)—a marker of immunity through vaccination or previous exposure; and hepatitis B core antibody (anti-HBc)—a marker of previous exposure.

To ascertain if hepatitis B infection is the cause of abnormal liver function test results, testing for HBsAg is all that is required. However, if acute infection is suspected (for example, with recent possible parenteral or sexual exposure and a noticeable increase in serum alanine aminotransferase and aspartate aminotransferase, with or without jaundice), tests should be requested for HBsAg and anti-HBc IgM (a marker of recent exposure). For antenatal screening in the first trimester of pregnancy, guidelines recommend testing only for HBsAg.[6]

Counselling on the outcome of serological testing for viral hepatitis is crucial whenever tests are requested. Where appropriate, interpreters should be used to clarify the history and communicate about the disease. In some settings, such as in sexual health clinics, it may be appropriate to offer a first dose of hepatitis B vaccine at this consultation.

Interpretation of initial test results

The table shows the interpretation of initial tests for hepatitis B virus. The three recommended serological tests also enable determination of whether the patient has been previously exposed to hepatitis B virus, has mounted an immune response before vaccination, or is susceptible to hepatitis B infection.

HBsAg positive

If the result for HBsAg is positive, then the patient has current infection, which may be either acute or chronic.

The additional finding of a positive result for anti-HBc IgM antibody confirms the presence of acute hepatitis B infection, especially if supported by clinical suspicion and increased aminotransaminase levels. Most adults will clear an acute infection spontaneously and less than 5% will proceed to chronic infection. Less than 1% will

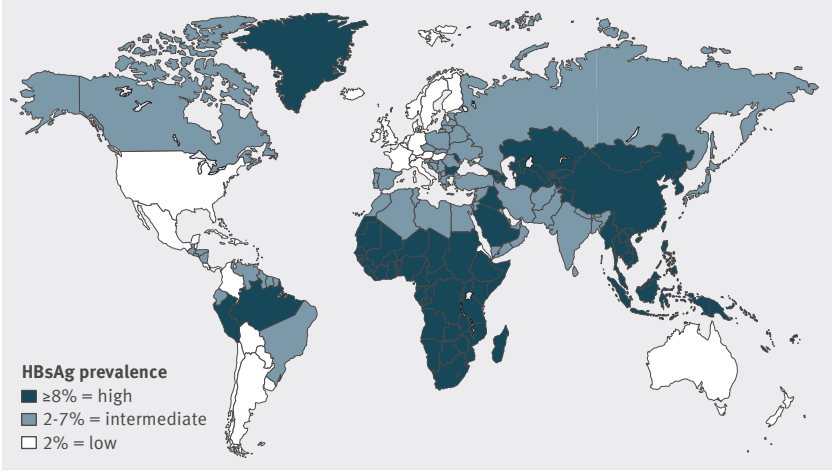

Fig 1 | Geographical distribution of chronic hepatitis B infection. HBsAg=hepatitis B surface antigen

LEARNING POINTS

- Opportunistic testing for hepatitis B virus (with HBsAg, anti-HBc, and anti-HBs) in people at high risk for hepatitis B virus is essential for diagnosis and appropriate management
- Most patients found to be positive for HBsAg will have chronic hepatitis B; test for HBeAg, anti-HBe, hepatitis B virus DNA, and ALT to determine the phase
- Patients with chronic hepatitis B and hepatitis B virus DNA levels >2000 IU/mL, increased ALT level, or any signs of advanced fibrosis or cirrhosis, or pregnant women found to be seropositive for HBsAg require specialist review
- Patients with inactive hepatitis B may not require specialist referral, but should be monitored at least once a year for change of disease state

Interpretation of hepatitis B virus serology performed at initial testing

Test	Result	Interpretation
HBsAg	Positive	Acute hepatitis B infection: usually noticeable increases in serum alanine aminotransferase and aspartate aminotransferase levels. Infection likely to resolve spontaneously in >95% of adults. Occasionally, patients with a severe flare-up of chronic hepatitis B infection may develop low titre anti-HBc IgM
Anti-HBs	Negative	
Anti-HBc	Positive	
Anti-HBc IgM	Positive	
HBsAg	Positive	Chronic hepatitis B infection: patient has had infection longer than 6 months, often since birth. At risk of complications, including cirrhosis and hepatocellular carcinoma. Further assessment is indicated
Anti-HBs	Negative	
Anti-HBc	Positive	
Anti-HBc IgM	Negative	
HBsAg	Negative	Resolved hepatitis B infection: immune to reinfection. May represent occult hepatitis B virus. May be at risk of reactivation of the virus with immunosuppression; for example, treatment containing rituximab
Anti-HBs	Negative or positive	
Anti-HBc	Positive	
HBsAg	Negative	Successful vaccination: immunity considered if antibody titre >10 mIU/mL
Anti-HBs	Positive	
Anti-HBc	Negative	
HBsAg	Negative	Susceptible: vaccination recommended if at risk of exposure to hepatitis B virus
Anti-HBs	Negative	
Anti-HBc	Negative	

HBsAg=hepatitis B surface antigen; anti-HBs=hepatitis B surface antibody; anti-HBc=hepatitis B core antibody.

POPULATIONS REQUIRING TESTING FOR HEPATITIS B VIRUS

- Patients at high risk of previous exposure to hepatitis B virus
- People born in a country with an intermediate or high prevalence of chronic hepatitis B infection (fig 1)
- Infants born to women with chronic hepatitis B infection
- Family, sexual, or household contact of someone with hepatitis B infection
- Adults at high risk of infection (for example, past injecting drug use, multiple sexual partners, men who have sex with men, sex workers)
- Diagnosis of another infection with shared mode of acquisition (for example, hepatitis C virus or HIV)
- Patients presenting with relevant medical conditions
- People with abnormal liver function test results or evidence of acute or chronic liver disease
- People with hepatocellular carcinoma
- Pregnant women (routine antenatal screening test)
- People undergoing chemotherapy or immunosuppressive therapy (risk of reactivation of hepatitis B virus)

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Previous articles in this series

- Using haemoglobin A1c to diagnose type 2 diabetes (*BMJ* 2014;348:g2867)
- Investigating an incidental finding of lymphopenia (*BMJ* 2014;348:g1721)
- Estimated glomerular filtration rate (*BMJ* 2014;348:g264)
- Investigating polyuria (*BMJ* 2013;347:f6772)
- Investigating low thyroid stimulating hormone (TSH) level (*BMJ* 2013;347:f6842)

develop acute liver failure (indicated by coagulopathy and encephalopathy).

A positive HBsAg test result more commonly reflects the presence of chronic infection, particularly when screening someone at high risk for the virus, or when assessing someone with features of chronic liver disease or before chemotherapy. Patients with chronic infection will also be positive for anti-HBc (total or IgG) but negative for anti-HBc IgM. The results for anti-HBs will usually be negative unless the patient is in the process of seroconversion from HBsAg positive to negative, where both HBsAg and anti-HBs may be present for a time. All patients who are positive for HBsAg for longer than six months are considered to have chronic hepatitis B infection. The concept of a "healthy carrier" of the hepatitis B virus is no longer thought to be valid as clinical status can change over time and patients remain at risk for complications of liver disease. Long term monitoring is recommended in all those who are positive for HBsAg.

HBsAg negative

Previous exposure with resolution of infection is characterised by being negative for HBsAg and positive for anti-HBc and anti-HBs. This serological pattern means that patients have been previously exposed to hepatitis B virus but undergone HBsAg seroconversion to anti-HBs, which is associated with disease resolution, improved clinical outcomes, and immunity to reinfection. Some patients may already have developed cirrhosis before HBsAg seroconversion, however, and remain at increased risk for hepatocellular carcinoma. Furthermore, patients who are negative for HBsAg and positive for anti-HBc may have occult infection and be at risk of reactivation of the virus and a flare-up of hepatitis in the setting of intense immunosuppression, particularly in association with treatment regimens that contain rituximab.

Response to previous vaccination is characterised by a positive anti-HBs antibody result alone. Anti-HBs titres greater than 10 mIU/mL after vaccination are considered to be protective.

If all three serological markers are negative, patients are susceptible to hepatitis B virus because they have been neither exposed nor vaccinated. Vaccination should be offered where there are risks for infection through sexual transmission, percutaneous exposure, occupation, or household or close contact.

Tam is from a priority population. When tested he is found to be positive for both HBsAg and anti-HBc and negative for anti-HBs. It is most likely that he has chronic hepatitis B infection rather than showing recent exposure to the virus, in view of his Vietnamese heritage and because he recalls having been told that he was a carrier of the virus.

Further evaluation of patients with chronic hepatitis B infection

Some clinical guidelines recommend referral of all patients who are seropositive for HBsAg[3]; however, many patients with chronic hepatitis B infection have inactive disease and may not require referral to a specialist, particularly when access to specialist services are limited. It is therefore essential that general practitioners understand the clinical course of chronic infection, which may change over time as a result of the relative balance between viral replication and the host's immune response. Liver inflammation and fibrosis in chronic infection is due to the immune response, with an increase in alanine aminotransferase level being a marker of necroinflammation. Antiviral treatment may be indicated in patients with increased alanine aminotransferase levels and significant viraemia, particularly when hepatic fibrosis is evident.

The clinical course of chronic hepatitis B infection has been recognised to occur in four phases (immune tolerance, immune clearance, immune control, and immune escape, fig 2).[7] Loss of HBsAg represents a fifth phase of chronic infection.[8] It is important that at each assessment of patients with chronic infection (which should be conducted at least annually in untreated patients) an attempt is made to determine the phase of infection so that specialist referral can be made if appropriate. To determine the phase of disease, once it is known that a patient is HBsAg

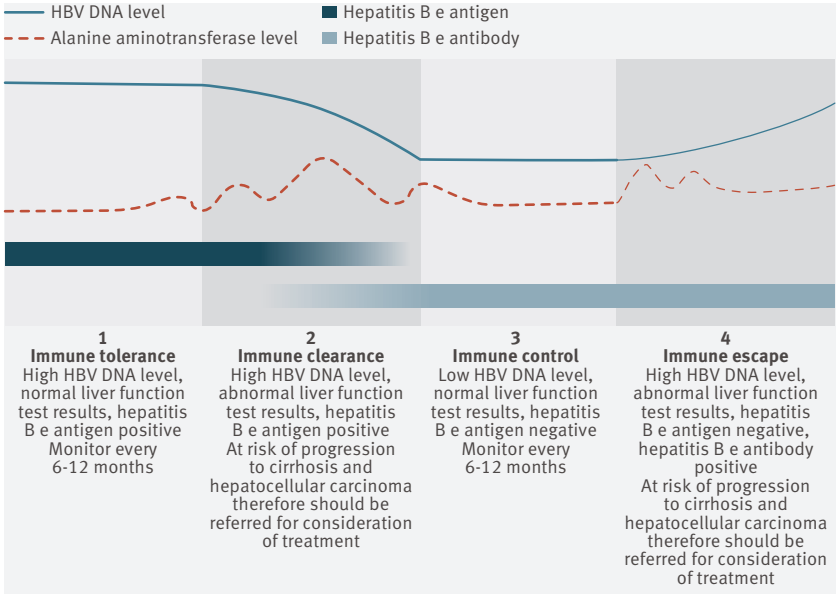

Fig 2| Clinical course of chronic hepatitis B infection. Reproduced with permission of the Australasian Society of HIV Medicine

positive, requires testing for hepatitis B e antigen (HBeAg, a marker of wild type infection usually associated with high circulating viral levels), hepatitis B e antibody (anti-HBe, a marker of clearance of HBeAg), hepatitis B virus DNA level (a marker of the level of circulating virus), and serum levels of alanine aminotransferase and aspartate aminotransferases, in particular the former.

Immune tolerance phase

The immune tolerance phase usually occurs during the initial 15-30 years of infection and is shown by positivity to hepatitis B e antigen (HBeAg), high hepatitis B virus DNA levels, but normal alanine aminotransferase levels. Treatment is not usually indicated, and children, adolescents, and young adults in particular can be monitored in general practice. Monitoring of alanine aminotransferase levels, HBeAg, and anti-HBe at least every six months has been recommended, with specialist referral if fluctuations in alanine aminotransferase levels are observed. Around 1.7-4.5% of children and adolescents infected at birth have cirrhosis at liver biopsy and hepatocellular carcinoma can occur.[9]

Immune clearance phase

The immune clearance phase is associated with positivity for HBeAg, high levels of hepatitis B virus DNA (usually >20 000 IU/mL, and increased alanine aminotransferase levels. During this phase, patients may spontaneously seroconvert from being positive to negative for HBeAg with development of antibodies to HBeAg (anti-HBe). A prolonged immune clearance phase may be associated with progressive fibrosis and development of cirrhosis. These patients require specialist review and consideration of antiviral treatment with the aim of reducing progression to cirrhosis, liver failure, and hepatocellular carcinoma.

Immune control phase

The immune control phase is characterised by being negative for HBeAg, positive for anti-HBe, low or undetectable levels of hepatitis B virus DNA (usually <2000 IU/mL) and alanine aminotransferase levels within normal range. These patients usually do not require specialist review unless cirrhosis is a concern. Surveillance for liver cancer by ultrasonography every six months may be indicated. Patients in this phase are sometimes referred to as inactive carriers.

Immune escape phase

The immune escape phase represents reactivation of the hepatitis B virus and is characterised by chronic infection with negativity for HBeAg, increased levels of hepatitis B virus DNA (usually >2000 IU/mL), and fluctuating alanine aminotransferase levels. Such patients are at high risk of progression to cirrhosis, liver failure, and hepatocellular carcinoma, and require specialist review.

Further assessment also includes:

- Physical examination for abdominal and peripheral signs of chronic liver disease and portal hypertension.
- Other blood tests to establish disease severity, including full liver function tests, full blood count, and coagulation studies. The finding of an aspartate aminotransferase level higher than that of alanine aminotransferase or a low platelet count may indicate the presence of cirrhosis. A raised bilirubin level, low albumin level, or increased prothrombin time or international normalised ratio may indicate the presence of cirrhosis with development of liver failure.
- Exclusion of additional viral infections that may impact the course of hepatitis B infection, including testing for hepatitis C (anti-HCV), hepatitis D (anti-HDV) (which only occurs in people with hepatitis B infection), and HIV.
- Determination of immunity to hepatitis A (anti-HAV), allowing for vaccination of susceptible people.
- Eliciting a family history to determine if other family members have a history of hepatitis B infection or hepatocellular carcinoma. The testing and vaccination status of family, household, and sexual partners should be ascertained and vaccination offered to those at risk.

When to refer to a specialist

Referral practice varies around the world, depending on the prevalence of hepatitis B infection, the design of health services, and the knowledge and engagement of primary care doctors. Not all patients with chronic infection require referral to a specialist, as long as the general practitioner is comfortable about identifying inactive disease (that is, the immune control phase) and commits to undertaking regular assessment and monitoring of these patients. In some areas, including in developed countries with large migrant populations, shared care programmes have been introduced to ensure the appropriate triage and management of patients.

In patients who are seropositive for HBsAg the following features clearly, however, require specialist review:

- Chronic hepatitis B infection with increased or fluctuating alanine aminotransferase levels (more than upper limit of normal range), suggesting active disease and a risk for progression to cirrhosis, regardless of whether patient is positive or negative for HBeAg.
- Any features suggesting advanced fibrosis or cirrhosis, such as abnormal liver echotexture or splenomegaly on ultrasonography, a low platelet count, or more

overt signs of liver failure, including increased bilirubin levels, low albumin levels, or prolongation of prothrombin time.
- All women found to be seropositive for HBsAg during routine antenatal screening.

Specialist review will include an assessment for the presence of hepatic fibrosis, either by liver biopsy or increasingly through non-invasive assessment, such as transient elastography, a simple technique using a probe applied to the right chest wall to measure liver stiffness.[10] A decision will then be made as to whether antiviral treatment is indicated.

In pregnant women with high levels of hepatitis B virus DNA, antiviral treatment may be considered in the third trimester, to further reduce the risk of mother to child transmission. Infants born to women who are positive for HBsAg require vaccination and hepatitis B immunoglobulin within 12 hours of birth.

Because of the strong association between chronic hepatitis B infection and hepatocellular carcinoma, international guidelines recommend routine surveillance with ultrasonography every six months in some patients with chronic hepatitis B to identify small tumours that may be amenable to curative treatment.[8 11] When general practitioners decide that patients with inactive chronic infection do not require referral to a specialist, there should be an undertaking to ensure that such patients undergo surveillance for hepatocellular carcinoma, and that patients found to have a liver lesion are referred for further assessment and management.

Outcome

Tam is found to be positive for HBeAg and negative for anti-HBe, his hepatitis B virus DNA level is 2.5 million IU/mL and his alanine aminotransferase level is increased, at 105 U/L (normal range 0-40). These results indicate that Tam is in the immune clearance phase of infection, has active disease that might warrant antiviral treatment, and requires specialist review.

Tam is surprised to learn that he has active disease and is at risk for liver cancer. He is referred for specialist assessment, which reveals active disease with moderate hepatic fibrosis. He starts antiviral treatment and is enrolled in regular surveillance for hepatocellular carcinoma in view of his family history of this cancer and his age. His 36 year old wife, who was also born in Vietnam, is tested and found to be negative for HBsAg but positive for anti-HBs and anti-HBc, reflecting past exposure and development of immunity.

Contributors: Both authors contributed equally to the conception and design, or analysis and interpretation of data; drafting the article or revising it critically for important intellectual content; and final approval of the version to be published. They are the guarantors.
Competing interests: None declared.
Provenance and peer review: Commissioned; externally peer reviewed.
Patient consent not required (patient anonymised, dead, or hypothetical).

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ANSWERS TO ENDGAMES, p 36

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ANATOMY QUIZ Coronal T2 weighted magnetic resonance image of the brain

A: Superior sagittal sinus
B: Right lateral ventricle
C: Third ventricle
D: Right sylvian fissure
E: Left posterior cerebral artery
F: Basilar artery

STATISTICAL QUESTION

Ecological studies: advantages and disadvantages

Statement *a* is true, whereas *b* and *c* are false.

PICTURE QUIZ An adolescent athlete with groin pain

1 There is bony irregularity and displacement of the lesser trochanter of the left femur.
2 The most likely diagnosis is an avulsion fracture of the lesser trochanter.
3 Young athletes, aged 11-17 years are most at risk. The secondary ossification centres for the lesser trochanter of the femur appear at age 11 and fuse by age 17. During this period, any substantial traction force exerted by the inserting musculotendinous unit—the iliopsoas insertion to the lesser trochanter—may result in a bony avulsion injury.
4 Avulsion fractures of the lesser trochanter in adolescents are generally treated conservatively. Management aims to restrict the precipitating activity or event and offload the affected area, with partial weight bearing using crutches until symptoms resolve (usually one to two weeks).
5 Surgery may be needed when there is a substantial degree of displacement of the avulsed fragment from the femoral origin. Proximal displacement of more than 2 cm would warrant an operative opinion.