

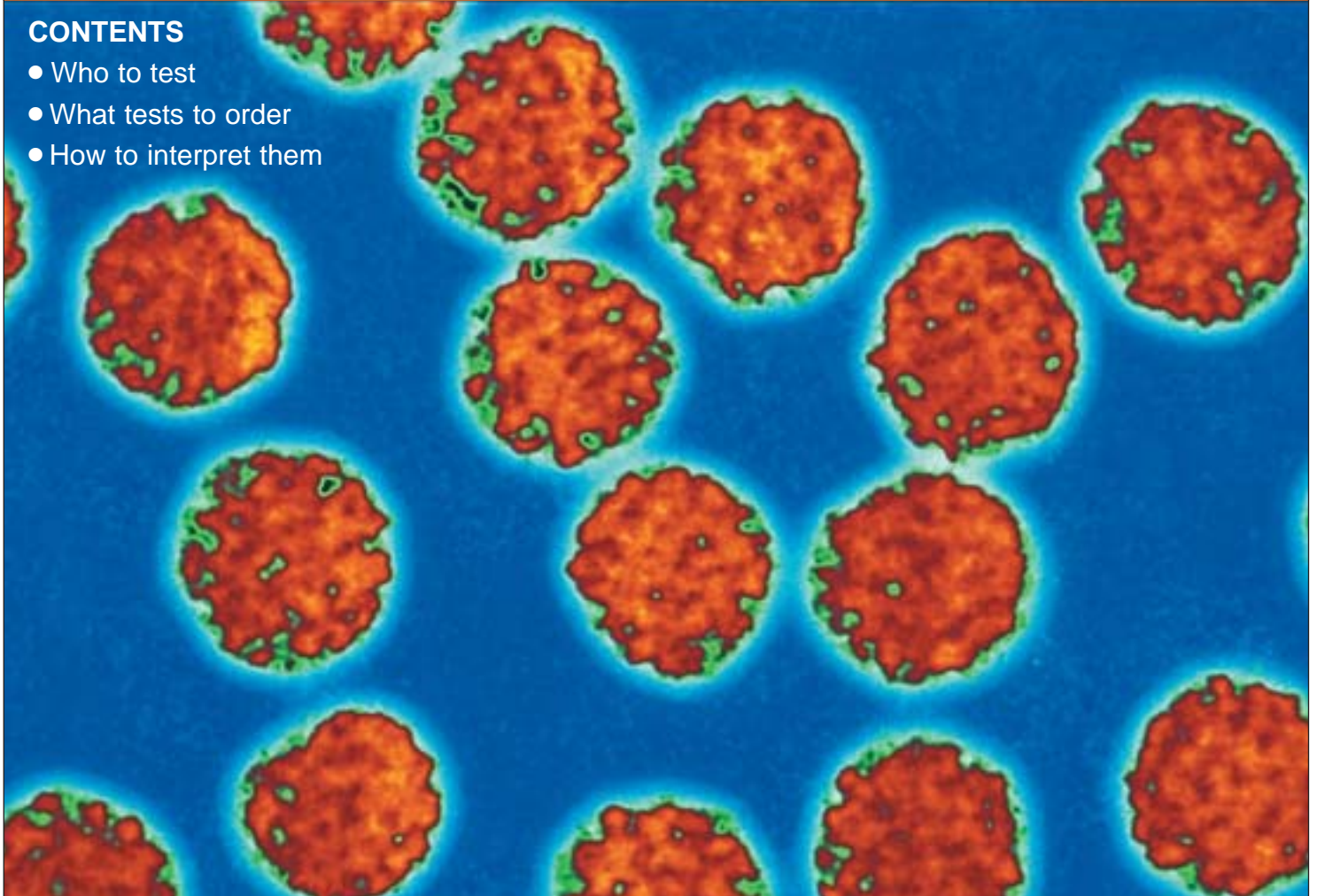
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Common Sense Pathology

A REGULAR CASE-BASED SERIES ON PRACTICAL PATHOLOGY FOR GPs

CONTENTS

- Who to test
- What tests to order
- How to interpret them



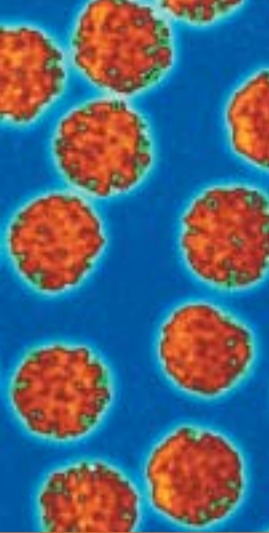
Recent advances in diagnosis and management of
VIRAL HEPATITIS

A JOINT INITIATIVE OF



Australian
Doctor.





Recent advances in diagnosis and management of **VIRAL HEPATITIS**



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Introduction

Recent advances in treatment options for chronic hepatitis and a better understanding of the pathogenesis of primary hepatocellular carcinoma (HCC) have had a significant effect on the objectives and methodology of diagnostic testing in patients with liver disease. Table 1 summarises the investigations needed to make a diagnosis of viral hepatitis.

This issue of *Common Sense Pathology* is a joint initiative of *Australian Doctor* and the Royal College of Pathologists of Australasia.

It is published by Reed Business Information
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Chatswood DC NSW 2067.
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E-mail: mail@australiandoctor.com.au
Web site: www.australiandoctor.com.au
(Inc. in NSW) ACN 000 146 921
ABN 47 000 146 921 ISSN 1039-7116

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www.rcpa.edu.au

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While the views expressed are those of the authors, modified by expert reviewers, they are not necessarily held by the college.

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Cover: Hepatitis B viruses. Alfred Pasiaka/Science Photo Library

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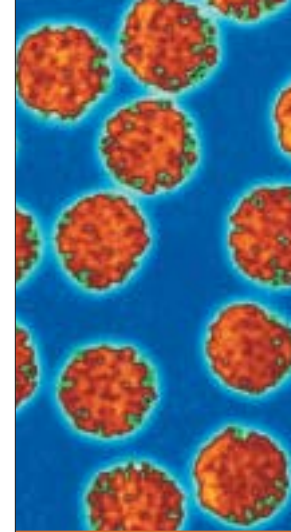


Table 1. Investigation of viral hepatitis

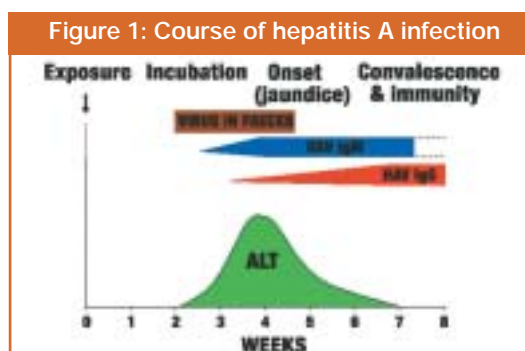
	ACUTE	CONVALESCENT	CARRIERS
HEPATITIS A	27 nm particles in faeces from two days before to one week after onset; IgM antibody detectable a few days after onset	IgG (total) antibody peaks at 2-3 weeks after onset and persists indefinitely	No carriers
HEPATITIS B	HBsAg, anti-HBc, HBeAg HBV DNA all in serum; HBsAg in hepatocyte cytoplasm; HBc in nucleus; episomal HBV DNA and replicative forms in liver	HBeAg and HBV DNA lost, anti-HBe present in serum; then HBsAg is lost; anti-HBs signifies recovery and immunity; anti-HBc and anti-HBs persist indefinitely (? for life); integrated HBV DNA may persist in liver	HBsAg in serum and liver; either HBe or anti-HBe in serum; always anti-HBc; sometimes anti-HBs; HBV DNA integrated in hepatocyte
HEPATITIS C	HCV RNA appears in serum before anti-HCV, then both co-exist; IgM may or may not be detectable	HCV RNA lost from serum; anti-HCV persists	HCV RNA and anti-HCV persist in serum; intermittent viraemia characteristic
HEPATITIS D	Delta antigen and HBsAg in serum and liver; anti-delta follows	Both HBsAg and delta antigen lost from serum and liver; anti-delta persists	Anti-delta and HBsAg present in serum; delta antigen may be detectable in liver
HEPATITIS E	Hepatitis E antigen in faeces; anti-HEV IgM in serum	Development of anti-HEV IgG	No carrier state
HEPATITIS F	As for E but tests not readily available		
HEPATITIS G	HGV RNA in serum	No antibody test yet available	No way of distinguishing acute cases from carriers

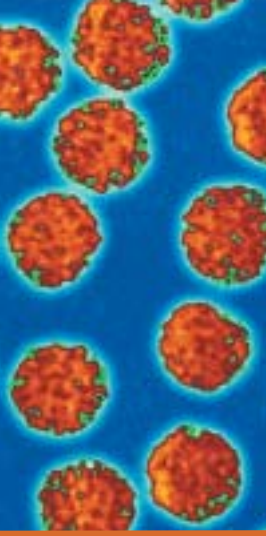
Hepatitis A

Hepatitis A is an enterovirus transmitted by the faecal oral route and it does not establish chronic infection.

In acute HAV Infection, anti-HAV IgM is present even before the onset of symptoms and persists for about 12 weeks (Figure 1). Vaccination seldom produces measurable HAV IgM.

Anti-HAV IgG is present by the time of symptom onset and persists for life. The threshold for the ELISA test is about 100 IU/L, which is well





above the protective level. Two doses of standard (or one dose of high dose) HAV vaccine are needed to reach and sustain this level in the long term.

The hepatitis A virus (HAV) is grown in cell cultures to produce the antigens used in diagnostic ELISA tests and in the inactivated vaccine.

Hepatitis B

Hepatitis B infection in immune competent adults frequently produces symptomatic hepatitis, the risk depending to a large extent on the viral dose. In contrast, infection in infants and immune-suppressed individuals is usually subclinical and persistent. Chronic infection is often associated with defective viral replication with less liver damage or infectivity than ongoing productive infection. Productive infection is also more likely to cause primary HCC.

Transmission occurs by parenteral inoculation of blood or body fluids, or by mucosal, especially sexual, contact. Maternal transmission occurs mainly during delivery.

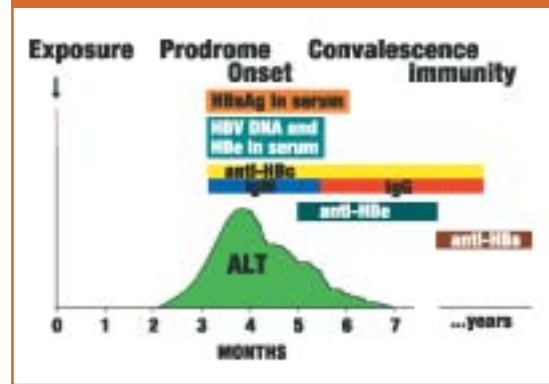
The hepatitis B virus (HBV) has a double-shelled structure that encloses the viral-encoded enzyme DNA polymerase as well as the small circular double-stranded DNA genome. Infectivity assays are restricted to research laboratories and diagnostic assays are based on detection of components of the virion in blood or liver and on measurement of antibodies. Antibody to the outer layer — hepatitis B surface antigen (HBsAg) — confers immunity and the vaccine is based on this antigen. Antibody to the inner hepatitis B core (HBcAg) appears early in infection and persists for life whether or not the virus is cleared from the liver. Anti-HBc IgM is routinely present in acute infection, but may reappear during episodes of activity in chronic carriers. The test reagents, like the vaccine, are produced by recombinant DNA technology.

Whenever the virus is growing in the liver, infectious particles can be found in the serum. At the same time, a soluble protein (HBeAg antigen) is also excreted into the serum. This protein is a product of the virus core gene and is often used as a marker of active virus replication. Loss of HBeAg and appearance of anti-HBe occur at the end of virus replication. However, measuring serum HBV DNA is the preferred method of detecting active virus because infectious mutant viruses, which lack the signal needed to drive the excretion of HBeAg antigen, are relatively common in Australia.

Acute hepatitis B

The time course of acute hepatitis B infection is

Figure 2: Course of hepatitis B infection



shown in Figure 2. Five to ten per cent of adults and 80-90% of neonates fail to eliminate HBV within 6-12 months but the degree of ongoing liver damage as judged by ALT and/or liver biopsy varies from trivial to life threatening.

Initial diagnosis: HBsAg is the primary test. If negative, it is most unlikely the patient has hepatitis B. In cases where there is overwhelming clinical suspicion but a negative HBsAg result a negative anti-HBc test will confirm the negative antigen result.

Monitoring progress: At onset both HBeAg and HBV DNA are present, and loss of these markers and the appearance of anti-HBe are good indications that the infection is resolving. Final recovery cannot be assumed until the patient develops anti-HBs weeks or even months later.

Distinguishing acute from chronic hepatitis B: The patient's progress is the key parameter, and chronic HBV infection is usually defined as persistence of HBsAg in serum for longer than six months. Anti-HBc IgM is regularly present in acute hepatitis but may recur in patients with chronic infection, particularly during episodes of reactivation.

Chronic hepatitis B

Initial diagnosis: HBsAg is again the primary test.

Monitoring progress: The outlook for patients is much more serious if viral replication is ongoing in the liver. Such patients have HBV DNA and usually also HBeAg in their serum, and should be flagged for regular follow up. They also need counselling about their infectivity. Sexual contacts are at greatest risk and should be vaccinated if non-immune. Within households, inapparent parenteral infection may follow sharing of razors, combs and even brushes, and vaccination of

immediate family members is advisable. There is no risk of infection from ordinary social contact or food handling in the workplace or at school. Infected health care workers, however, may not perform exposure prone procedures. Over time many patients, estimated at about 5% per year, progress from HBeAg positive to anti-HBe negative status. Both their infectivity and the risk of chronic liver damage recedes with such seroconversion and annual testing is useful in charting virological status.

Immunity: Anti-HBs confers immunity provided it is present in sufficient titre, over 10 IU/L. There has been much discussion about the persistence of immunity when anti-HBs falls below this level. Epidemiological evidence suggests that protection is maintained in individuals who responded adequately to vaccination, but there are few direct observations of the outcome of exposure to highly infective inocula.

Cirrhosis and hepatocellular carcinoma:

Virological tests do not assist screening for these conditions beyond flagging patients with ongoing viral replication. Serum markers such as α -feto-protein are of only modest sensitivity and specificity for early diagnosis of HCC. Biopsy remains the gold standard but modern imaging techniques are also contributing to diagnosis and management.

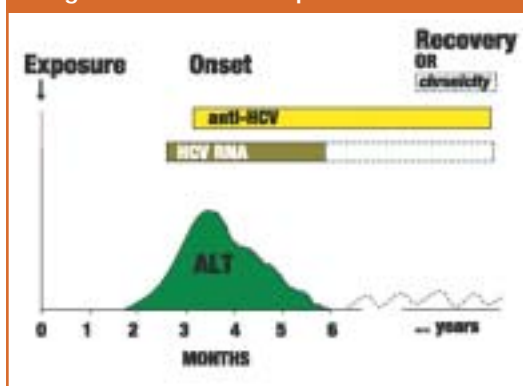
Sensitivity to antiviral drugs: The sequence motifs which define the targets of commonly used antiviral drugs such as lamivudine are detected by direct sequencing of PCR products amplified from serum samples. This service is available from specialist laboratories only.

Hepatitis C

Hepatitis C virus (HCV) is related to the pesti-viruses and flaviviruses which cause numerous arthropod-borne infections in humans and animals. Molecular techniques were used in its initial discovery and have yielded the current tests for both virus and antibody. After the early stages of infection, virus and antibody co-exist in the serum, though it is increasingly recognised that a proportion, perhaps half, of infected patients eventually clear virus but have persistent antibody. No marker of immunity is yet known. The time course of HCV infection is shown in Figure 3.

HCV antibody screening uses a mixture of recombinant HCV antigens in an ELISA test, and

Figure 3: Course of hepatitis C infection



for confirmation samples are re-tested using a kit from a different manufacturer based on the same principle but employing different reagents. An immunoblot analogous to the HIV Western Blot is also available for difficult samples.

Acute HCV infection

HCV RNA is present when the ALT becomes abnormal but anti-HCV rises more slowly and may not be detectable for several weeks. Some patients (about 40%) clear viraemia and become HCV RNA negative between six and 12 months after onset, but their anti-HCV persists in the long term.

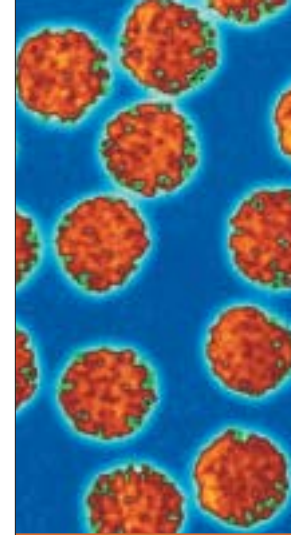
Chronic HCV infection

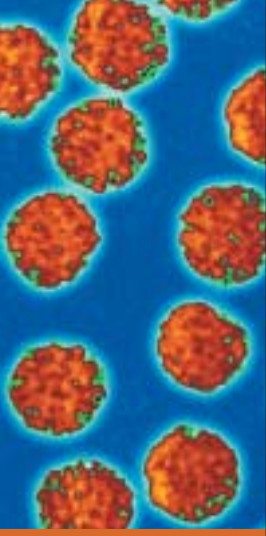
Anti-HCV testing will identify almost all chronic infection except in immunosuppressed patients who may fail to produce antibody. Genotype influences the response to therapy with interferon and antivirals, which is assessed by reduction in the level of viraemia. Both genotype and viral load are assessed as part of the workup of a patient being considered for treatment. Viral load determination is based on quantitative PCR technology while genotyping depends on analysis of the nucleotide sequence of a relatively well-conserved region of the viral genome – usually in the 5' non-coding region. As with HBV, virological testing has little to offer in assessment of the likely level of cirrhosis or the presence of HCC in the liver.

Hepatitis D

Hepatitis D virus (HDV) infection may occur simultaneously with acquisition of hepatitis B or by superinfection in an established carrier. Delta infection usually results in severe liver damage and fortunately it can only grow in cells already infected with HBV.

Hepatitis D infection is detected by the presence





of anti-HDV in a HBsAg positive carrier. A test for delta antigen, found in serum during acute delta infection, has been developed but is not widely available.

Hepatitis E

Hepatitis E virus (HEV) is an enterovirus-like agent responsible for much of the severe hepatitis in Africa, the Middle East and the Indian sub-continent. It spreads by the faecal oral route, has a high mortality in pregnant women and has caused outbreaks involving thousands of cases when urban water supplies have been contaminated. In Australia, it is occasionally encountered in travellers. A closely related virus infects pigs in many countries including Australia. Tests for antibody in serum using tissue culture-derived antigen are available. Anti-HEV IgM is present at the onset of illness.

Case study 1

PJ, a 38-year-old school teacher, returned home a week ago after a year in Indonesia. She now feels nauseous and generally unwell and is slightly jaundiced. Her two children, aged seven and 10, travelled home with her and are well, as is her husband who came home six weeks ago.

Hepatitis A is the obvious clinical diagnosis for PJ.

What tests are needed and which other family members should be tested?

HAV IgM tests on PJ and the children will confirm her diagnosis and show whether the children were infected at the same time. If the children are negative and PJ has been unwell for less than a week it may be worth offering them passive prophylaxis. Immunoglobulin is only effective if given within six days of exposure.

How can you determine whether her husband is susceptible?

In this case, HAV IgG (or total anti-HAV) provides the answer. If he is negative, passive prophylaxis would be useful.

What if PJ's HAV IgM had been negative?

Further HAV testing would not be warranted (see Figure 1) — hepatitis B or E would now be possibilities.

Practice point

This is clearly a situation which could have been prevented by hepatitis A vaccination before travelling. The recent increase in prevalence of hepatitis A in Australia raises the issue of vaccination for health care workers, especially as combined HAV/HBV vaccines are available.

Case study 2

RY brings her new baby to the surgery with a letter from the hospital saying she is HBsAg positive. The baby has received hepatitis B specific immunoglobulin and his first dose of hepatitis B vaccine. The family emigrated from China six years ago and all were vaccinated on arrival in Australia. The letter specifies arrangements for continuation of the vaccination schedule, but RY asks about the implications of her hepatitis B status for her own future and that of the rest of the family.

How would you start, assuming that physical examination of mother and baby is normal?

Repeat the HBsAg test to show whether she is indeed chronically infected, and ALT to seek liver damage.

If RY is still HBsAg positive six months after her first test but her ALT is within normal limits is further testing needed?

HBeAg and/or HBV DNA testing on the same sample is needed to show if HBV is actively replicating in her liver.

Why is this necessary?

Because if she is HBV DNA negative you can reassure her that she is unlikely to develop serious liver disease or to infect those around her, but if positive it is worth follow up by repeat testing at yearly intervals. If serum HBV DNA persists and especially if the ALT becomes elevated, RY should be assessed by liver histology and consideration given to antiviral treatment.

Although HCC is much more common in men, the risk is still high in middle-aged female carriers who are actively replicating hepatitis B with cirrhosis. Programs of intensive monitoring, for example with liver ultrasound are being established in a few centres. If the HBeAg and DNA are negative, yearly re-testing would be a suitable option.

What tests, if any, would be needed for the baby?

None until the antibody level from the immunoglobulin administered at birth has declined. However, between 5-10% of infants born to HBV DNA positive mothers become infected despite vaccination. This is best assessed at 12 months of age with an HBsAg test and if negative, an anti-HBs test on the same specimen will demonstrate an adequate response to vaccination.

What follow up is needed for vaccinated babies born to HBsAg negative or even HBsAg positive/HBV DNA negative mothers?

Testing is unnecessary because the infection rate in these infants is so low.

What about her husband and two other children, now aged eight and 10?

Despite vaccination on arrival in Australia all members of the household are at some risk because it is quite possible they were already infected before arriving in Australia. Over 80% of adults and approximately half the children from countries where hepatitis B is endemic will have some markers of infection. HBsAg (and flow on HBe and HBV DNA) are the most useful starting point for all three because the critical distinction is between active and past infection.

How would you act on a positive result?

By following the same program used for RY. In the case of her husband, the greater risk of HCC in males might prompt more active follow up.

Case study 3

JR, 29, is admitted to hospital after a motor bike accident. Tests in intensive care revealed a raised ALT.

Which hepatitis viruses might be the cause in a previously well patient?

HBV and HCV are the most likely causes.

He consents to preoperative screening. What tests would you request?

HBsAg (which was negative) and anti-HCV (which was positive).

On questioning it becomes clear that JR used a variety of IV drugs while at university but not since settling into an engineering career in Newcastle. Are further investigations warranted?

Yes, is it important that the anti-HCV result be supplemented by two independent ELISA methods (and any doubts resolved by immunoblot) at this point. There are many reasons for raised ALT values in trauma cases and false-positive screening tests for anti-HCV occur at an appreciable rate of about 0.1% in uninfected people.

Are further HCV tests desirable once it has been established that the patient's result is truly reactive for anti-HCV?

Yes. Although HCV PCR is almost always posi-

tive in anti-HCV positive patients with raised liver function tests, in this case trauma could have affected the ALT result. In ordinary circumstances this expensive test is usually reserved for cases with normal liver function tests who have a 40% chance of being non-viraemic. Ideally the PCR test for HCV should be performed on a new serum sample obtained when he is convalescent from his accident. Observation for a further interval of 6-12 months would also be a suitable option but even if his liver enzyme levels return to normal, biopsy may reveal continuing inflammatory damage or cirrhosis.

JR requests early investigation and treatment.

How significant is the finding that his HCV belongs to genotype 1b and his HCV RNA level is low?

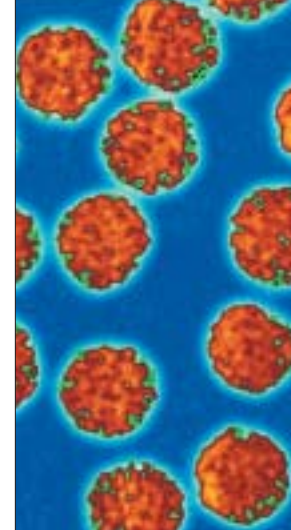
The genotype result does not bode well for viral clearance but the low level of viraemia points in a more favourable direction. His liver biopsy showing mild inflammation and little fibrosis is also favourable.

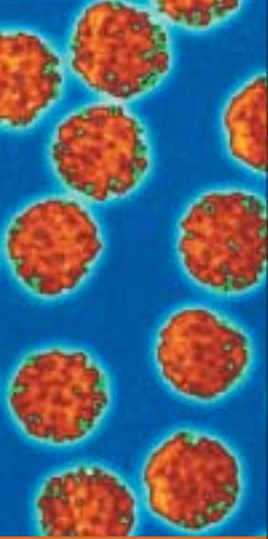
JR's fiancé now requests investigation for her hepatitis status — she has never used drugs but works in a residential school for mentally handicapped children and was vaccinated for hepatitis B when she joined the staff four years ago. What tests would you request?

Initially, HBsAg and anti-HCV for evidence of current infection and anti-HBs to check post-vaccination immunity. Her HBsAg test is negative and her anti-HBs level low at 40 IU/L. A booster dose of vaccine, while not strictly required, would provide long-lasting protective antibody levels.

Her anti-HCV test result is indeterminant (positive in one ELISA but negative in the other) but her ALT is at the upper level of normal. She is reluctant to wait one month for re-testing because she suspects she is pregnant. Her primary concern is the potential for infection of the baby. Are further tests of any value?

A Western blot will be useful to confirm the serological result. If it is positive, an HCV PCR is needed. A negative result is reassuring because non-viraemic anti-HCV positive mothers do not transmit the infection to their infants. In any case less than 10% of babies born to anti-HCV positive mothers become infected and approximately half of these clear the virus before their second birthday.





Case study 4

JM, 17, develops hepatitis with mild jaundice during the summer holidays. You have seen another case where you suspected hepatitis in a teenager a week ago, but the test results are not yet available.

Is this the start of an outbreak? What tests should you request for JM?

The statistical probability is that he will have hepatitis B as he had not travelled abroad recently, and hepatitis C rarely presents as an acute icteric illness. However, there have also been several recent hepatitis A outbreaks in Australia. Request an HBsAg test, as well as anti-HAV IgM. Anti-HCV is the third option.

The HBsAg test is positive, confirming hepatitis B, so the next issue is whether the other case is also hepatitis B and whether there is an epidemiological link between them. The other patient was HBsAg negative. Should you investigate further?

Requesting anti-HBs and anti-HBc will put it beyond doubt. If the other patient was already recovering by the time of testing, it is possible he had already cleared the virus and mounted an immune response. Both antibody tests will be positive. If only anti-HBc is present he is in the 'window' period where HBsAg has declined below

measurable levels but anti-HBs is not yet detectable.

Case 2 is indeed anti-HBc positive/anti-HBs negative. How will you proceed?

Repeating the antibody tests after an interval of 2-3 months should show that he has completed his immune response.

There is still the matter of epidemiological links between the two. Questioning reveals that several classmates, including both your cases, acquired tattoos at the same tattooist three months ago. Should the other boys be tested, and if so using what tests?

The circumstantial evidence of exposure is strong so testing for HBsAg now may reveal other anicteric cases, and possibly also a carrier who has been the source of infection in the tattooist's parlour. If these cases yield HBV DNA the strains can be subjected to sequence analysis to determine how closely they are related.

Practice point

Implementation of the NH&MRC recommendation for universal adolescent hepatitis B immunisation would have prevented these cases. Tattooing, ear and body piercing remain underestimated routes of transmission for blood-borne viruses.

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