Regular Review

Specific serological diagnosis of viral hepatitis

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For many years at least two distinct types of acute viral hepatitis have been recognised in man, differing in their incubation periods and their primary modes of transmission. Hepatitis A (infectious hepatitis) is usually spread by the faecal-oral route, has an incubation period of three to five weeks, and is predominantly seen in epidemics. Nevertheless, though hepatitis A remains common in the more industrialised communities, the infection occurs mainly in small clusters, often with only one or two identified cases. Hepatitis B (serum hepatitis) is more sporadic and has a widely varying incubation period of perhaps 14 to 180 days. It is transmitted predominantly by the parenteral route, but there is also evidence of spread by non-parenteral routes.

The clinical range of hepatitis varies from inapparent or subclinical infections, mild non-specific gastrointestinal symptoms, the anicteric form of infection, acute icteric illness, fulminant hepatitis, and in some patients progression to chronic liver disease, which may be severe.

The discovery of Australia antigen in the circulation in 1965, and later its association with hepatitis type B, stimulated a spectacular rate of technological advance, which led to a bewildering array of tests for the specific diagnosis of hepatitis B and later hepatitis A. More recently widespread use of these tests showed that there must be another, unrelated form of viral hepatitis referred to as non-A non-B hepatitis.^{1 2}

Hepatitis B—During the incubation period infection with hepatitis B virus is associated with the appearance in the plasma of a specific antigen, hepatitis B surface antigen (originally referred to as Australia antigen) from two to eight weeks before biochemical evidence of liver damage or the onset of jaundice. The antigen persists during the acute illness, but it is usually cleared from the circulation during convalescence. Next to appear in the circulation is DNA polymerase associated with the core or nucleocapsid of the virus and e antigen, again preceding any rise in concentration of serum aminotransferase. Antibody to the core is found in the serum two to four weeks after the appearance of the surface antigen, and it is usually detectable during the early acute phase of the illness, persisting after recovery. The next antibody to appear in the circulation is directed against the e antigen. Antibody to the surface component is the last to appear late during convalescence.³ More recently, the presence of precipitating antibodies reacting with specificities on the complete virus particle has been described.⁴ These antibodies may be relevant to the clearance of circulating hepatitis B virions and the termination of acute infection, and their absence in all but one of the patients with chronic active hepatitis examined might explain why the infection persists in such patients. In addition to the complex serological events

which take place during the course of uncomplicated infection, cell-mediated immune responses to hepatitis B antigens have been described.^{5 6}

Some patients move on to become carriers of hepatitis B virus, which may be life-long. Such a carrier state may be associated with liver damage ranging in severity from minor changes in the nucleus of hepatocytes to chronic active hepatitis and cirrhosis. Among risk factors that have been identified as increasing the chances that an individual will become a carrier are male sex, infection acquired in childhood, and natural or acquired immune deficiencies.² Seroepidemiological surveys have shown a large reservoir of persistent carriers of hepatitis B virus, estimated worldwide to number about 180m.⁷ The carrier state is characterised serologically by persistence of hepatitis B surface antigen and absence of surface antibody. Core antibody is present, often in high titre, and there are reports that core antibody of the IgM class remains detectable. In some carriers hepatitis B DNA polymerase activity remains raised, not infrequently fluctuating in titre, and e antigen persists; in others, by contrast, hepatitis B DNA polymerase is not detectable and anti-e is found. Hepatitis B e antigen has been reported to be commoner in young than in adult carriers, while the prevalence of anti-e appears to increase with age.^{2 3}

Laboratory tests—Numerous laboratory methods of varying sensitivity and specificity are available for detecting the surface antigen and its antibody.^{1 2 8} The two dimensional micro-Ouchterlony immunodiffusion test, which was the first method employed, allows precipitin lines to be compared directly and is the simplest technique for detecting the surface antigen and the surface antibody, but it is also the least sensitive. Somewhat more sensitive techniques include counterimmunoelectrophoresis, complement fixation, rheophoresis, passive haemagglutination inhibition (for antigen), and immunofluorescence microscopy. Greater sensitivity for detection of antigens has been achieved by reverse passive haemagglutination, immune adherence haemagglutination, reverse passive latex agglutination, and immune electron microscopy. Passive haemagglutination is a sensitive technique for the assay of surface antibody. The most sensitive methods for detecting surface antigen and surface antibody are radioimmunoassays, including solid-phase radioimmunoassay and radioimmunoprecipitation. These two types of radioimmunoassay differ primarily in the techniques used for separating bound from free radiolabelled reagents. More recently, enzyme-linked immunosorbent assay (ELISA or enzyme immunoassay) has been adapted for detection of surface antigen and surface antibody.^{9 10} This technique has a sensitivity similar to that of radioimmunoassay. Reactions

Simplified guide for the interpretation of serological markers of hepatitis B

Surface antigen	e antigen	Anti-e	DNA polymerase	Core antibody	Surface antibody	Clinical interpretation
+	+	-	+	-	-	Incubation period or early acute hepatitis
+	+	-	+	+	-	1 Acute hepatitis B
-	-	+	-	+	+	2 Persistent carrier state
+	-	+	-	+	+	Convalescence from acute hepatitis B
-	-	-	-	+	+	Persistent carrier state
-	-	-	-	+	-	Past infection
-	-	-	-	-	-	Infection with hepatitis B virus without detectable (excess) surface antigen
-	-	-	-	-	+	Immunisation without infection. Repeated exposure to surface antigen without infection

obtained with the highly sensitive techniques must be confirmed as specific by neutralisation or blocking with unlabelled surface antibody or surface antigen as appropriate.

Hepatitis B core antibody is a useful marker of infection with hepatitis B virus, and persisting high titres suggest continuing viral replication—though this antibody is known to remain detectable long after viral replication has ceased. Various techniques are available for detecting core antibody, ranging from immunofluorescence to radioimmunoassay. Core antibody may be found in the serum in the absence of other markers of hepatitis B virus,¹¹⁻¹⁵ and in some circumstances blood from such individuals may contain infective virus in the absence of excess production of surface antigen—a possibility currently under investigation. Reference has been made to the possible value of assay of IgM core antibody in testing for carrier states. Free core antigen has not been detected in the circulation.

Fluorescent antibody techniques may be used to distinguish the core antigen in the nuclei of hepatocytes. The presence of core antibody in the serum may also readily be shown by indirect immunofluorescence using as a substrate cryostat sections of liver obtained post mortem from patients with a large number of hepatocytes containing core antigen. This technique is widely used. Immune adherence haemagglutination for the assay of core antibody is used in some countries. Radioimmune procedures for detecting core antibody are being increasingly introduced into practice, and enzyme immunoassay is under development.

The presence of e antigen in the serum appears to correlate with the replication of hepatitis B virus in the host and, in general terms, with varying degrees of liver damage. There is also a highly significant correlation between e antigen, DNA polymerase activity, and the presence in the circulation of complete virus particles—and so of infectivity. Anti-e, on the other hand, usually signifies reduced or relatively low infectivity—but virus production has been reported in the presence of antibody to e antigen in some carriers. In general, the presence of anti-e has been found to be associated with relatively minor histological changes in the liver.

Several serological methods are now available for detecting e antigen and antibody. In order of increasing sensitivity these include immunodiffusion, rheophoresis, immune adherence haemagglutination, passive haemagglutination, and, more recently, enzyme immunoassay and solid-phase radioimmunoassay.¹⁶

A close correlation has been shown between the presence of a DNA polymerase with unique properties and hepatitis B virus particles.¹⁷ The detection of specific DNA polymerase activity is, therefore, a useful marker of virus replication. This enzyme activity is often found early in infection when large numbers of virus particles are present; it correlates with e antigen and it persists in some chronically infected people with continuing viral replication. Sometimes DNA polymerase may be the only, though transient, early marker of infection

with hepatitis B virus in the absence of surface antigen.^{18 19} This specialised assay, therefore, might complement other screening serological procedures for early diagnosis, especially where the risk of infection is high such as in haemodialysis units. Another application of the DNA polymerase reaction is that the assay may discriminate between complete and defective virus particles. The polymerase activity is also being used for evaluating several antiviral compounds for treating persistent hepatitis B infection, including leucocyte and fibroblast interferon and adenine arabinoside. Frequent fluctuations in serum concentrations of the enzyme are observed in serial samples from persistently infected patients, and such variations should be taken into account while monitoring the effect of treatment. Finally, because of the close association of the DNA polymerase with the core particles, the reaction has been used in preparing radiolabelled core antigen for use as reagents for assay of core antibody.²⁰⁻²²

Hepatitis A—The identification in 1973 by immune electron microscopy of virus particles in extracts of faeces obtained during the early acute phase of illness in two out of four volunteers inoculated with hepatitis A virus provided the long-awaited lead to further studies of this infection.²³ The availability of viral antigen permitted in turn the identification of specific antibody, the development of serological tests for hepatitis A, and determination of susceptibility to infection in both man and non-human primates. Human hepatitis A has been transmitted to certain species of marmosets and to chimpanzees shown to be free of homologous antibody, thereby providing a model for experimental infection and also a source of reagents.^{1 2}

Many virus particles are detectable during the incubation period in chimpanzees, beginning as early as nine days after exposure and continuing, in general, until the rise in serum aminotransferase concentrations reaches its peak.²⁴ Similar observations have been made during natural infection in man; virus is also detected in faeces early during the acute phase of illness but relatively infrequently after the onset of clinical jaundice.²⁵ Interestingly, antibody is also detectable late in the incubation period, coinciding roughly with the onset of biochemical evidence of liver damage, and the antibody persists.

The serological tests available for hepatitis A²⁶ include immune electron microscopy, complement fixation, immune adherence haemagglutination, radioimmunoassay, and enzyme immunoassay. The complement-fixation test for hepatitis A antibody using as antigen liver extract of infected marmosets is specific, but anticomplementary activity is not infrequent. Immune adherence haemagglutination is more specific and more sensitive, and is widely used. Several methods of radioimmunoassay have been described, and of these a solid-phase competitive type of assay for detecting hepatitis A antigen and antibody is particularly convenient. Very sensitive enzyme immunoassay techniques have also been developed.

Only one serotype of hepatitis A virus has been identified

in volunteers infected experimentally with the MS-1 strain of hepatitis A virus, in patients from different outbreaks of hepatitis A in different geographical regions, in the sera of random cases of hepatitis A, and in naturally and experimentally infected non-human primates. Use of the various serological techniques has shown that there is no chronic excretion of hepatitis A virus; that hepatitis A is endemic and common in all parts of the world; that the infection is not transmitted by blood transfusion; and that there is no evidence of progression to chronic liver disease.

Serological tests for hepatitis A antibody are useful for epidemiological surveys, for screening of individuals to determine susceptibility to infection and the need for prophylaxis with normal immunoglobulin, and for titration of the content of hepatitis A antibody in immunoglobulin. Since antibody develops very early in the infection serological diagnosis can be established only by titrations of serial samples of serum, which is expensive. The most useful diagnostic procedure is assay for the IgM class of hepatitis A antibody, and several techniques have been developed,²⁶ including enzyme immunoassay and radioimmunoassay. IgM antibody remains detectable in serum by sensitive methods for 45 to 60 days after the onset of illness, whereas IgG tends to persist for many years. Another method for the diagnosis of recent infection is by detection of hepatitis A virus in faeces if appropriate early samples are available.

Non-A non-B hepatitis—The specific laboratory diagnosis

of hepatitis A and hepatitis B has shown the existence of an unrecognised form of hepatitis, clearly unrelated to either type, now termed non-A non-B hepatitis. This is the most common form of hepatitis occurring after blood transfusion and the administration of certain blood products in some areas of the world.^{2,3} The infection has also been transmitted to chimpanzees.²⁷⁻³⁰ Though specific laboratory tests for identifying this new type of hepatitis are not available, the infection seems not uncommon, and there is evidence that a carrier state exists and that in some patients the infection progresses to chronic liver disease. At least two different types of non-A non-B hepatitis have been identified. There is only one published report on the identification by double immunodiffusion and counterimmunoelectrophoresis of an antigen, designated hepatitis C antigen, in patients with non-A non-B post-transfusion hepatitis in Japan.³¹ Much effort is now directed in many laboratories towards identifying the viral antigens responsible for the new form or forms of hepatitis and developing sensitive and specific serological techniques.

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