LABORATORY DIAGNOSIS OF VIRAL HEPATITIS

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The development and refinement of techniques to detect markers of current or past infection by hepatitis A virus (HAV) and hepatitis B virus (HBV) now make available serological tests of considerable sensitivity and rapidity. Thus hepatitis B surface antigen (HBsAg) at levels as low as 1 ng/ml can ben detected by currently available radioimmunoassay techniques. Results are made available by this test in 4-18 hours. Many of the tests described below are now within the range of most clinical virology laboratories. Indeed, high quality commercially available reagents in the form of kits are available for the detection of HBV and HAV virus infection. The detection of serological markers is of value, not only in establishing the diagnosis, but also in monitoring the progress of disease, particularly when new antiviral agents are being assessed. Furthermore, detection of immune responses is of value in assessing hepatitis vaccines. Thus, results of numerous trials with hepatitis B vaccines prepared from heat or formalin inactivated purified HBsAg have recently appeared in the medical literature (Maupas *et al.*, 1981; Szmuness *et al.*, 1980); the propagation of HAV in cell cultures (Provost and Hilleman, 1979) makes it likely that trials with an HAV vaccine may shortly be carried out in humans.

In obstetric practice it is important to detect markers of HBV, not only to assess the risk of transmission of infection from patients to those who are in contact with them in hospital practice, but also to assess the risk of infection being transmitted to the infant perinatally in order that appropriate measures may be taken to reduce this hazard.

Hepatitis A Virus Infection

Infection by HAV has shown changing patterns in developing countries. This virus, which has the physico-chemical characteristics of an enterovirus, is transmitted from person to person via the faeco-oral route; in addition, explosive point source outbreaks may result from virus contamination of food, water, milk and shellfish. The incubation period is generally of the order of about 20-30 days. Viraemia is probably transient and of a low level and has not been demonstrated directly. This is probably why HAV is rarely, if ever, the cause of post-transfusion hepatitis (PTA) or outbreaks of hepatitis in haemodialysis units (Szmuness *et al.*, 1977).

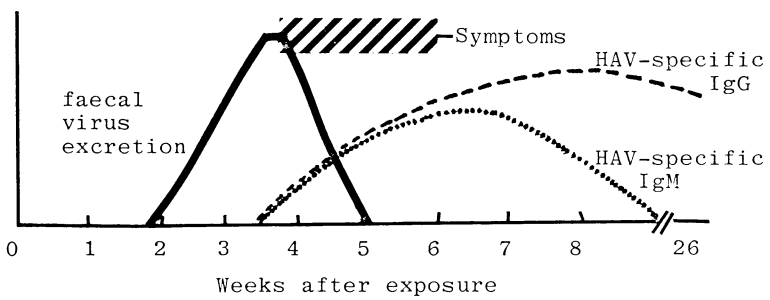

Fig. 1. — Clinical and Virological features of infection by hepatitis A virus.

Figure 1 illustrates the clinical and virological features of infection. Faecal virus excretion occurs from about 10-12 days before the onset of symptoms but only for a very short period after they have developed (5-10 days) (Dienstag *et al.*, 1975). Indeed, once jaundice develops, faecal virus excretion can only rarely be detected. Patients with severe clinical manifestations of infection generally experience prolonged virus excretion but those with milder or subclinical infection, including those whose clinical features have been suppressed by the administration of human immunoglobulin, may excrete only a very small amount of virus over a restricted period of about 48-72 hours. A persistent intestinal carrier state has not been reported and HAV does not appear to cause chronic liver damage (Dienstag *et al.*, 1978). Since it is difficult to detect virus once symptoms have developed, a diagnosis of HAV is usually made serologically. Although antibodies can be detected by such techniques as complement fixation, immune adherence haemagglutination, ELISA and RIA, antibodies may take a considerable time to rise significantly

Table 1.

MAJOR SUBTYPES	*adw*, adr, *ayw*, ayr
BODY FLUIDS	*Serum*, semen, saliva, sweat, breast-milk
AMOUNT	Up to 500 μg/ml in serum (5-10% not detectable)
BLOOD DONORS	New 1:450; previously screened 1:20,000
TESTS	RIA (Ausria 2) 1 ng/ml (4-18 hours)
	ELISA 1-5 ng
	RPHA 10-100 ng/ml (40 minutes)
	* RPHA (Modified) 10-20 ng/ml

* Test modified by employing 0.1% rather than 1.0% anti-HBs coated turkey erythrocytes. (Barbara J. A. J., Harrison P. J., Howell D. R., Cleghorn T. E., Dane D. S., Briggs M., Cameron C. H.: *A sensitive single reverse passive haemagglutination test for detecting both HBsAg and anti-HBs.* J. Clin. Path., *32*, 1180, 1979).

although they are often already present when symptoms develop. A more rapid serological diagnosis may be made by demonstration of the presence of HAV specific IgM since the presence of specific IgM is indicative of a primary antigenic stimulus. HAV specific IgM responses are usually present for three months or sometimes even longer after the onset of symptoms (Locarnini *et al.*, 1977).

Acute Hepatitis B Virus Infection

Unlike hepatitis A virus, hepatitis B is a complex enveloped virus which produces a prolonged and sometimes persistent viraemia. Figure 2 shows the sequence of development of serological markers of HBV infection. Although the incubation period of HBV ranges from about 6 weeks to 6 months with a mean of 2-3 months, HBsAg may appear some 4-6 weeks before symptoms occur. Exceptionally, HBsAg has been reported as early as a week after exposure to HBV (Krugman *et al.*, 1979). The HBsAg response peaks at about the time that symptoms appear but within a short time after the onset of jaundice, it begins to decline, usually becoming undetectable some 8-20 weeks after its appearance.

Some of the features of HBsAg are shown in table 1. Serological analysis of HBsAg shows that it consists of distinct antigenic substructures. Thus, in addition to a group-specific antigen *a*, at least 2 sets of major virally coded mutually exclusive determinants have been described, *d*, and *y*, and *r*, and *w*

(reviewed by Sobeslavsky, 1978). Among carriers sub-types *adw* occur more commonly in the U.K., Northern Europe, U.S.A., Central America and East Africa, and *ayw* in Eastern and Southern Europe, the Eastern Mediterranean, Middle East, Pakistan, North and West Africa. Both *adw* and *ayw* may be found in Central Africa, Malaysia, India and Japan. HBsAg carrying the *r* determinant is rarely encountered, being confined to parts of South East Asia and the Far East. Outbreaks of HBV infection in dialysis units and syringe-transmitted HBV among drug addicts are almost exclusively associated with the *ayw* subtype. Although HBsAg has been detected in a number of different body fluids, only blood and blood stained secretions and excretions as well as semen are likely to transmit HBV to susceptible contacts. Analysis of data from London Regional Transfusion Centres suggests that approximately 1 : 450 new donors is likely to be an HBsAg carrier. However, many of these persons are likely to originate from those parts of the world where HBV is endemic. In contrast, only about 1 : 20,000 donors who have been previously screened HBsAg negative will be found positive when re-screened; such persons are likely to be in the incubation period of HBV (Barbara, personal communication).

Antibody to HBsAg (anti-HBs) may begin to develop when HBsAg first becomes undetectable, although usually there is a period varying from a few days to 2-3 months before the anti-HBs response appears. Anti-HBs is protective and exhibits long-term persistence, being indicative of immunity to HBV. However, some 10-15% of patients fail to develop an anti-HBs response. Three other HBV markers may be detected during the acute phase of the disease, these being HBeAg, specific DNA polymerase and antibody to the core antigen (anti-HBc). These markers correlate closely and are indicative of virus replication. Specialised biochemical techniques are required to detect DNA polymerase (DNAP) and therefore tests to detect DNAP are not generally carried out in most clinical virology laboratories. However, HBeAg is an important marker of infectivity (Alter *et al.*, 1976; Grady, 1978) which is particularly useful in assessing risks of transmission of HBV to susceptible hosts from patients who are HBsAg carriers. HBeAg exhibits a more transient response than HBsAg and if it persists for longer than 10-12 weeks, this suggests that the patient is likely to develop a prolonged HBsAg carrier state. The anti-HBe response develops as HBeAg is declining and its appearance is indicative of recovery and reduced infectivity. HBeAg is present in the blood of patients who have transmitted infection to susceptible contacts as a result of blood transfusion or needle-stick exposure, and is usually

present in haemodialysis patients who are HBsAg positive. A high proportion of HBsAg positive male homosexuals are also HBeAg positive (Simmons *et al.*, 1977). Mothers who have this marker almost invariably transmit infection perinatally to their offspring. Perinatal transmission appears also to be associated with ethnic factors. Thus 10-15% of women in certain parts of S.E. Asia are HBsAg positive and of these some 60-70% are also HBeAg positive (Skinhøj *et al.*, 1981). In contrast, 80% of Caucasian women who are HBsAg carriers are anti-HBe positive; perinatal transmission of virus is rarely encountered in this group. Core antibody (anti-HBc) may persist for 2 years or even

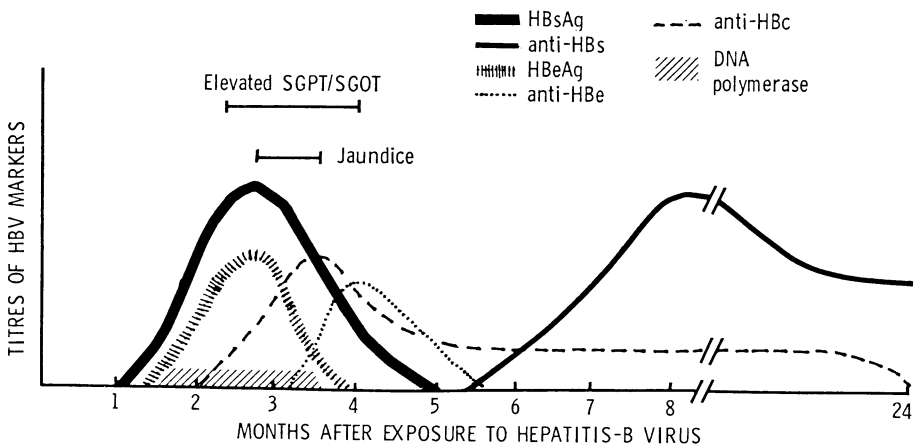

Fig. 2. — Serological markers during acute hepatitis B virus infection.

more (Krugman *et al.*, 1979) after infection. Between the time at which the HBsAg response has disappeared but the anti-HBs response has yet to appear, it may be the only marker of HBV infection ("window" effect). The bulk of this antibody during the acute phase of the infection resides in the IgM class of immunoglobulins and the presence of core IgM is therefore indicative of current infection. Although almost invariably present in the acute phase of disease, it is undetectable or present at only a low level among patients who are HBsAg carriers who have minimal or no histological changes in the liver. Subclinical HBV infection may occur in up to 40% of patients (Hoofnagle *et al.*, 1978); such cases may exhibit an undetectable or transient HBsAg response, this being followed by a high anti-HBs response. Low levels of anti-HBc and anti-HBe may be present over a limited period.

Chronic HBV-Associated Liver Disease

Five to ten per cent of patients with acute HBV infection exhibit a persistent HBsAg response. Although most workers regard patients with an HBsAg response 6 months after the onset of symptoms as long-term carriers, some of these patients will eliminate HBsAg and develop an anti-HBs response at a variable interval after this. Patients who are persistent HBsAg carriers may have either an HBeAg or anti-HBe response; there is some evidence which suggests that the conversion of HBsAg to anti-HBs is associated with improved liver function. The core anibody IgM response in patients with chronic liver disease appears rather variabile. Some workers have detected it in as high a proportion as 90% of HBsAg positive patients with chronic active hepatitis (Roggendorf *et al.*, 1981), whereas others have detected it in only about 30% of patients with chronic HBsAg-associated liver disease (Tedder and Wilson-Croome, 1981). Differences in test sensitivity as well as differences as to where "cut off" levels are taken when such tests as RIA and ELISA are employed may be responsible for these discrepant results. Although only about 1-3 per 1000 apparently healthy blood donors in developed Western countries are likely to be HBsAg positive, in practice, about 1 : 450 donors in London regional blood transfusion centres are likely to be positive for HBsAg (Barbara, 1981). Many of those who are positive originate from countries which have a high endemicity for HBV infection. When donors who have previously been screened as HBsAg negative are re-tested before giving blood, only about 1 : 20,000 are found to be HBsAg positive, such persons usually being in the incubation period of HBV infection. Table 2 correlates serological markers of HBV infection with the various clinical phases of the disease.

Non-A, Non-B Hepatitis

Although there are probably a number of different viruses which cause sporadic, post-transfusion or even epidemic outbreaks in the community, there is yet no established laboratory marker which can readily identify one or more of these viruses. Considerable interest has been expressed recently in a non-A, non-B hepatitis which has a particularly high mortality (up to 20%) among pregnant women. This form of hepatitis has been described in developing countries and has many of the epidemiological features associated with HAV infection. Recently, a large water-borne outbreak occurred in Kashmir.

Table 2. — *Interpretation of serologic markers of hepatitis B virus (HBV) infection.*

Serologic Reactivity HBsAg	Anti-HBs	Anti-HBc	Interpretation
+	−	−	Early (Pre-symptomatic) acute type B hepatitis
+	−	+	a) Acute type B hepatitis, b) Chronic HBsAg carrier state.
−	+	+	Recovery from type B hepatitis
−	+	−	a) Long after HBV infection, b) Immunization with HBsAg.
−	−	+	a) Long after HBV infection,
		++	b) Immediate recovery from type B hepatitis,
			c) "Low levels" carrier state.

Modified from Hoofnagle J. H.: *Serologic markers of hepatitis B virus infection.* Ann. Rev. Med., 1981, *32*, Table 1, page. 8.

The incubation period ranged from 10-40 days (mean 15 days). Of 275 infected, 12 died, 8 of whom were female, of which 6 were pregnant (Khuroo, 1980).

Laboratory Tests

HAV specific IgM may be detected employing commercially available kits, results being available within 18 hours.

HBsAg may be detected by a variety of different techniques of which rapid PHA (RPHA) and RIA and ELISA are most frequently used, all of which are available commercially. A diagnosis may be obtained by RIA or ELISA within 4-18 hours. Such techniques are sufficiently sensitive to detect 1-2 ng/ml of HBsAg. Although slightly less sensitive, RPHA will detect 20-100 ng/ml, and has the advantage of rapidity, since a result can be obtained within 40 minutes of obtaining the specimen. The sensitivity of this technique may be considerably enhanced by employing a more dilute (0.1%) suspension of antibody coated erythrocytes (Barbara *et al.*, 1979). Employing this modification, HBsAg of the order of 10-20 ng/ml may be detected. Although the test is somewhat less sensitive, in practice this is rarely critical because patients with acute HBV infection have serum levels of HBsAg which approach 500 μg/ml. However, in addition, HBsAg titres can be determined easily by this method and it is therefore of prognostic value. Commercially available

preparations are also available for the detection of anti-HBs, HBeAg, anti-HBe and anti-HBc. Tests are usually set up overnight, results being available the next morning.

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