Laboratory diagnosis of EB virus infection in some cases presenting as hepatitis

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SUMMARY In 1975 and 1976, 7580 serum specimens were tested for HBsAg by the passive haemagglutination test, Hepanosticon. Serum from 38 people gave a positive result after absorption with the absorbent provided, but the presence of HBsAg could not be confirmed by other tests. Tests for current infection with EB virus, namely, the presence of heterophil antibody and EB virus specific IgM, were performed and were positive in 11 of the 38. In six of these the clinical picture was of hepatitis rather than infectious mononucleosis.

The Hepanosticon passive haemagglutination test for hepatitis B surface antigen (HBsAg) employs 'stabilised' sheep erythrocytes coated with sheep antibody to HBsAg (anti-HBs). These erythrocytes are 'agglutinated' by sera containing HBsAg due to reaction with the anti-HBs on the surface of the cells. The manufacturers warn that positive reactions can also occur due to the presence in the test serum of antibodies to the surface antigens of the sheep erythrocytes, such as Forssman or the heterophil antibodies present in the serum of cases of infectious mononucleosis. Certain antibodies including rheumatoid factor can also react with various sites on the globulin used to coat the erythrocytes. Nearly all such positive reactions can be removed by absorption with the absorbent provided, which consists of 'stabilised' sheep erythrocytes coated with a sheep gamma globulin not containing antibodies to HBsAg.

During a survey of the incidence of infectious mononucleosis and hepatitis B antigenaemia in university students (University Health Physicians and Public Health Laboratory Service Laboratories, 1971; Ruparelia and Edwards, 1976) two of 14 sera from cases of infectious mononucleosis gave positive haemagglutination tests for HBsAg in the Hepanosticon test after one absorption, although more sensitive tests for HBsAg gave negative results. This suggested that when high titres of heterophil antibody occur during recent infection with EB virus one absorption may be insufficient and this may

be one cause of 'false' positive results in the Hepanosticon test.

We have therefore looked for evidence of recent EB virus infection by testing for the presence of EB virus specific IgM and heterophil antibody in sera giving 'false' positive Hepanosticon results. This paper reports the result of this investigation and indicates that current or recent EB virus infection is one cause of false positive Hepanosticon reactions. Primary EB virus infection is strongly age-linked so that the proportion of false positives contributed by this cause will depend on the age distribution of the sera examined by the Hepanosticon test.

Material and methods

In 1975 and 1976 the Virus Reference Laboratory examined 7580 sera for the presence of HBsAg by the passive haemagglutination test (Hepanosticon by Organon Teknika), and for HBsAg and anti-HBs by immunoelectro-osmophoresis (IEOP) as described by Pesendorfer *et al.* (1970). If both tests for HBsAg were found to be positive the specimen was reported positive. If only the Hepanosticon test was positive an absorption was performed and the test repeated. If the test was still positive after this absorption, a radioimmunoassay (RIA, Ausria II) test was made to check the result.

The specimens selected for further study were those giving a positive Hepanosticon test after one absorption but negative results in the confirmatory tests. These specimens were sent to the Public Health

Laboratory and the Department of Microbiology at the Central Middlesex Hospital, London, where they were subjected to the full heterophil antibody test using horse erythrocytes and guinea pig and ox cell differential absorption (Davidsohn and Henry, 1969). A monospot test for infectious mononucleosis (Ortho Diagnostics Inc), which is a slide test also employing horse erythrocytes and the same absorbents, was substituted in testing nine sera because the remaining volume was inadequate for the full test. A simple sheep cell agglutination test was performed on all sera showing no agglutinins for horse erythrocytes.

EB viral capsid IgM antibodies were sought by the modified IgM test (Edwards and McSwiggan, 1974). This was essentially similar to the indirect immunofluorescence Henle test for IgG (Sumaya *et al.*, 1975) except that the HR-1K cell line was used for antigen, the patient's serum was allowed to react with the antigen for three hours, and, after washing, was stained by a specific anti-human IgM/fluorescein isothiocyanate conjugate made by Wellcome Research Laboratories. When the EB IgM test was found positive but the monospot and heterophil antibody tests were negative, the Rheuma-Wellco test for rheumatoid factor (RF) was used to check for specificity of the reaction. If the RF test was positive the EB IgM test was repeated using only the macroglobulin fraction of the serum after separation on a sucrose density gradient.

Results

Of the 7580 sera tested in 1975 and 1976, 38 (0.5%) gave a positive Hepanosticon test after one absorption but negative results in IEOP and RIA.

Table 1 shows the results of various tests on the 38 sera. Two of the nine sera tested by monospot, and nine of the 29 sera tested in the full heterophil antibody test were positive. All of these 11 positive sera were also positive in the EB IgM indirect immunofluorescence test. The EB IgM test was positive in six additional sera in which the monospot and heterophil antibody tests were negative. Five of these were

strongly positive in the test for rheumatoid factor which links with EB IgG to give a false positive EB IgM test in unfractionated serum. There was insufficient serum left to test the sixth. There was sufficient serum left in four of the five RF positive sera to repeat the EB IgM test on the macroglobulin fraction from a sucrose density gradient and these were all EB IgM negative. In these four sera the EB IgM tests were therefore nonspecific and there was no evidence of current EB virus infection. No conclusion can be reached about the other two sera on which tests could not be completed.

There were, in all, 11 cases of current EB virus infection confirmed by the presence of EB virus specific IgM and a positive monospot and/or heterophil antibody test. The ages and the clinical information available for these is given in Table 2. It will be seen that the majority were young people. Horse erythrocyte agglutinins absorbed out by both guinea pig kidney and ox cells were found in one other patient, and low titres of sheep cell agglutinins in two (Table 1). Agglutinins to sheep or horse erythrocytes were, therefore, shown to be present in a total of 14 of the 38 specimens. We still have no explanation for the positive reactions in 24 sera. These sera were almost all from patients with jaundice but only four were acutely ill. It is possible that anti-globulin antibodies are responsible in some.

Table 2 *Eleven cases of current EB virus infection*

Age (yr)	Symptoms or provisional diagnosis
16	Mild parenchymal hepatitis. Later diagnosed infectious mononucleosis
33	Infectious mononucleosis
60	No clinical details. Later presumed infectious mononucleosis because of positive heterophile antibody result
20	Guillain Barré syndrome
Over 30	Pyrexia of unknown origin occurring during chronic lymphatic leukaemia
8	Jaundice, ?hepatitis
15	
18	
Not known	
23	Jaundice with joint pains
28	

Table 1 *Tests on 38 Hepanosticon positive but Ausria negative serum samples*

Test	Results			
EB IgM	+	+	-	-
Full heterophile antibody or monospot	+	-	+	-
Number	11	6†	0	21*

*One positive horse cell agglutination but agglutinins absorbed out by both guinea pig kidney and ox cells.

† { 5/5 tested all positive in the RF test
4/4 tested all negative in the EB IgM test on the macroglobulin fraction

Discussion

About 8-10% of cases of infectious mononucleosis present initially with jaundice (Carter and Penman, 1969; Nye, 1977) and may be diagnosed clinically as acute hepatitis. When the Hepanosticon passive haemagglutination test is used for the detection of HBsAg a large proportion of tests are positive on screening but are negative when retested after one absorption. Chicot *et al.* (1975) found that in some sera agglutination could be removed by further

absorptions. Probably these were the sera containing high agglutinin titres to the erythrocyte themselves and therefore more than one absorption was required to remove it completely. Seven of the eight unabsorbed titres in our full heterophil antibody tests were 1 in 1792 or higher.

When sera give positive results after one absorption they are tested by RIA, electron microscopy, etc. When these confirmatory tests for HBsAg are negative the diagnosis becomes a problem. Quite frequently the clinician will have sent serum for hepatitis and heterophil antibody tests concurrently because the clinical differential diagnosis was between hepatitis and infectious mononucleosis. In other cases the heterophil antibody test will be requested later when an enlarged spleen, sore throat, or lymphadenopathy appears. However, it is clear from our series that EB virus infection can be missed altogether if jaundice and fever are predominant or accompanied only by joint pains. A request may never be made for heterophil antibody test and the diagnosis of hepatitis may remain final.

From studies of university students in this country (University Health Physicians and Public Health Laboratory Service Laboratories, 1971) and university students and military recruits in the United States of America (Niederman *et al.*, 1970; Hallee *et*

al., 1974) it has been found that there are many primary EB virus infections in the age group 18-25. Approximately one half of these develop classic infectious mononucleosis with a positive heterophil antibody test. In addition, some students whose sera were EB antibody negative initially and were found to have acquired EB antibody at a later testing could be shown to have heterophile antibody also at the time of seroconversion although they were asymptomatic.

Healthy young adults whose sera are screened for the presence of HBsAg for one reason or another may have had infectious mononucleosis recently or a recent asymptomatic EB virus infection. In our tests for HBsAg (Ruparelia and Edwards, 1976) on 627 sera from healthy university students, two were positive after one absorption in the Hepanosticon test and were also positive in the Wellcome Hepatest with no reaction with the control cells. Of these two students one was indeed a carrier of HBsAg. This was demonstrated by electron microscopic examination of the serum and a positive RIA test. The serum from the other student was negative in the RIA test but positive in the heterophile antibody and EB IgM tests, indicating current or recent EB virus infection. In such healthy people a positive haemagglutination test for HBsAg even in the absence of

Figure Flow diagram for further testing of Hepanosticon positive sera.

confirmation by other tests may cause administrative problems. If there is lingering doubt about the possibility of an HBsAg carrier state there might be unnecessary restriction of activity and repeated blood examinations. It may therefore be valuable to establish the diagnosis of current EB virus infection by performing a heterophile antibody test on sera showing positive Hapanosticon tests not confirmed by RIA (Figure).

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