Hepatitis B antigen (HB_sAg) and/or antibodies (anti-HB_s and anti-HB_c) in fulminant hepatitis: pathogenic and prognostic significance¹

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SUMMARY Hepatitis B surface antigen (HB_sAg) and antibodies to both the surface and core antigens of the hepatitis B virus (anti-HB_s and anti-HB_c) have been studied in 64 consecutive cases of fulminant hepatitis. HB_sAg was detected by counter-electrophoresis in 23 (35.9%) but by radioimmunoassay in 38 (59.3%). Anti-HB_s was detected by passive haemagglutination in 26 (40.6%), coexisting HB_sAg and anti-HB_s were found in 16 cases (25%). Using an indirect immunofluorescence technique, anti-HB_c was found in all of the cases in whom either HB_sAg or anti-HB_s was present. The highest survival rate was observed in patients with no evidence of HBV infection (31.3%) and was lowest in those who had both HB_sAg and anti-HB_s detected simultaneously (6.2%). The prognosis of those who exhibited anti-HB_s only was no better than those with HB_sAg alone. In a further case, transient interruption of the asymptomatic chronic HB_sAg carrier state with seroconversion to anti-HB_s was associated with the development of a fulminant hepatitis syndrome. The results suggest that an unusually strong and rapid immune clearance of HB_sAg may be involved in the pathogenesis of fulminant hepatitis.

Fulminant hepatitis (FH) may occur in association with both types of viral hepatitis, halothane anaesthesia, and certain drugs and poisons (Rueff, Benhamou, 1973). There has been almost no data since the discovery of a specific surface antigen (HB_sAg) of hepatitis B virus (HBV) to indicate whether the type A or type B infection is predominantly responsible in cases of fulminant viral hepatitis. The pathogenesis of FH remains mysterious. The influence of virus strains, as well as both the role of cellular and humoral immunity, have been considered determining factors of the disease pattern (Almeida, Waterson, 1969; WHO report 1973).

In order to clarify the role of HBV and of the immune response in the pathogenesis of FH, HB_sAg, as well as antibodies to both hepatitis B surface and core antigens (respectively referred to as anti-HB_s and anti-HB_c), were determined in the present study of 64 cases of FH and the findings were correlated with survival.

Methods

PATIENTS STUDIED

Sera were obtained from 64 consecutive FH cases hospitalised in two intensive care units in Lyons, France. According to the criteria of the fulminant hepatic failure surveillance study (Trey, 1972), only patients who developed a syndrome of hepatic failure and coma within eight weeks of the onset of an illness and with no evidence of previous liver function abnormality were considered as fulminant hepatitis cases. Clinical and laboratory features and their prognostic values have already been reported in many of the patients (Robert *et al.*, 1974). Twenty-nine patients were male and 35 female and their ages ranged from 3 to 77 years (mean 36 years).

PROCEDURES

The sera were initially tested for HB_sAg and anti-HB_s in the Laboratory of Hygiene in Lyons by agar gel diffusion (AGD). The specificity of positive results was established by tests of identity with known reference reagents. All sera were further

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examined for HBsAg by counterelectrophoresis (CEP) (Pesendorfer *et al.*, 1970) and radioimmunoassay (RIA) using Ausria I kits, supplied by Abbott Laboratories. An aliquot of freshly separated serum was kept and shipped frozen in dry ice to the New York Blood Centre where it was retested for HBsAg by radioimmunoassay using the same method. The specificity of positive results obtained only by RIA was confirmed by repeating the test after neutralization with known human anti-HBs positive sera (Prince *et al.*, 1973). Anti-HBs was detected by passive haemagglutination (PHA) (Prince *et al.*, 1972), whereas anti-HBc was determined by a modification of the indirect immunofluorescence test of Brzosko *et al.* (1973). With the technique used (Trepo *et al.* in preparation) background nuclear fluorescence resulting from autologous anti-HBc bound *in vivo* was abolished by preincubating the substrate liver sections with goat anti-human gammaglobulin for 30 minutes, before applying the test serum.

In 17 cases more than one specimen was available for serial studies.

Results

The serological findings show that, at the time of admission to the intensive care unit, out of 64 cases, 16 were found to be positive for HBsAg by AGD (25%), 23 by CEP (35.9%), and 38 by RIA (59.3%). All the sera found to be positive by AGD or CEP were detected by RIA.

Anti-HBs was found in 26 cases (40.6%) by PHA, in titres ranging from 1:8 to 1:4096 (mean 1:330), but was detected in only six of these cases by AGD (9.3%). Either HBsAg or anti-HBs was found in 48 cases (75%) and both HBsAg and anti-HBs were present in 16 patients (25%). Anti-HBc was detected in all of the cases with either HBsAg or anti-HBs, but it was found in none of the 16 patients (25%) without detectable HBsAg or anti-HBs.

One further case of FH, not included in this series of 64 cases, was studied. Fulminant hepatitis was associated with termination of the HBsAg carrier state and the appearance of anti-HBs in this additional case. This was a haemodialysed patient, previously recognized as an asymptomatic, chronic HBsAg carrier, who had repeatedly normal serum

transaminases values for more than a year before this episode. At the time FH developed, HBsAg was no longer detectable and only anti-HBs and anti-HBc could be demonstrated in the serum. The patient survived and HBsAg reappeared in the blood at the time of recovery, anti-HBs disappeared and anti-HBc remained present.

The relationship between the serological findings and survival is outlined in the Table. Thirteen of the 64 patients recovered (20.3%). Five of the 16 patients without HBsAg or anti-HBs survived (31.3%), in contrast with only eight out of 48 of those with HBsAg and/or anti-HBs (16.6%). Only one of the 16 patients with both surface antigen and antibody survived (6.2%), as compared with 2/10 (20%) of those with anti-HBs alone and 5/22 (22.8%) of those with HBsAg alone.

With the exception of a 77 year old woman, all patients over 40 died. If this exceptional case is omitted, the mean age was lower in the patients who survived (25.7 years) than in those who died (37.1 years).

Suitable follow-up specimens were available in 17 cases in whom no exchange transfusion was attempted. Three subjects who were repeatedly positive for HBsAg alone for up to seven, eight, and 12 days respectively, survived. Three patients had only anti-HBs detected repeatedly; one died. In 11 cases, persistence or decrease in HBsAg titre, with increasing anti-HBs titre, was followed by death in spite of total clearance of HBsAg and replacement by high titre anti-HBs in six. Clearance of HBsAg occurred between one and nine days (mean four days) after the patients were admitted to the intensive care unit; in four, HBsAg was cleared from the serum and replaced by high titre anti-HBs within 48 hours.

Discussion

Fifty-nine percent of these cases of FH were aetiologically related to the HBV as indicated by detection of HBsAg. If those 10 additional subjects who demonstrated anti-HBs and anti-HBc without HBsAg be included, this proportion would increase to 75%. Although previous exposure to HBV in these cases cannot be ruled out, the presence of anti-HBc in all of them would indicate that recent replication of

Outcome	Total no. of cases	No. cases with HBsAg only	No. cases with anti-HBsAg only	No. cases with HBsAg and anti-HBsAg	No. cases with either HBsAg or anti-HBsAg	No. cases with neither HBsAg nor anti-HBsAg
Alive	13	5	2	1	8	5
Dead	51	17	8	15	40	11
Total	64	22	10	16	48	16
Survival rate %	20.3	22.8	20	6.2	16.6	31.3

Table Relationship between HBsAg and/or anti-HBsAg positivity and survival in 64 fulminant hepatitis cases

HBV had occurred (Hoofnagle *et al.*, 1973). Several other reports have shown that HBV may be responsible for the majority of cases of FH (Boughton, 1968; Kassur *et al.*, 1974; Redeker, 1974).

Such a high prevalence of type B hepatitis in this series of FH could partially be explained by the frequency of hepatitis B antigen in non-fulminant cases in Lyons (47% by radioimmunoassay), as well as by the large proportion of cases that occur after transfusion or injections.

The titre of HBs antigenaemia appears to be unexpectedly low in FH. It was below the limit of sensitivity of CEP and could be detected only by RIA in 15 out of the 38 FH cases with HBs antigenaemia (39.5%). This is in marked contrast with the findings in 306 cases of non-fulminant acute, type B hepatitis, where only 37 (12%) were missed by CEP (Trepo *et al.*, 1973).

Serial blood samples were available from 17 patients with FH; nine of them cleared HBsAg from their blood. The mean duration of HBs antigenaemia as judged by RIA in these cases was 5.2 days, this being much shorter than the mean duration of HBs antigenaemia (67 days) that we have previously observed in non-fulminant hospitalized cases of acute, type B hepatitis, using the same RIA method (Trepo *et al.*, 1973). These findings support those of (Dudley *et al.* 1971) who reported that the titre of serum HBsAg was inversely proportional to the degree of liver damage present.

The apparent rapid disappearance of HBsAg can be explained by the early appearance of anti-HBs during the peak of liver damage in 26 out of 48 patients with supposedly HBV related FH (54.2%). Anti-HBs to titres sufficient to be detectable by AGD was observed in six of these 26 cases (9.3%). Again this pattern of anti-HBs response is strikingly different from that seen in non-fulminant, acute, type B hepatitis, when anti-HBs is generally detected during convalescence or even later (Barker *et al.*, 1973) and, when present, is detectable in only 0.5% by AGD, whereas it could be found in 12.5% of FH cases (Ashcavai and Peters 1971). Moreover, FH was observed in five out of 12 consecutive cases of acute hepatitis in which anti-HBs was detected by CEP within the first week of jaundice (Dragosics, Pesendorfer, and Wewalka, personal communication). The rapid immune clearance of HBsAg by anti-HBs observed in FH would explain the difficulty in detecting HBsAg and obviate the need to use radioimmunoassay.

The overall survival of cases of FH who had no evidence of exposure to HBV was 31.3%, whereas the survival rate of those with either HBsAg and/or anti-HBs was considerably lower (16.6%). Further breakdown of the survival rate of patients with the

type B infection showed that those in whom anti-HBs could be detected either alone or during and/or after HBs antigenaemia did no better than those in whom only HBsAg could be detected. In fact, 15 of 16 patients who were positive for both HBsAg and anti-HBs died.

In this context, it is relevant that FH coincided with transient replacement of HBsAg by anti-HBs in a previously asymptomatic HBsAg carrier and that recovery was associated with reappearance of HBsAg and loss of anti-HBs.

Our results suggest that development of anti-HBs is not associated with an improved prognosis in fulminant hepatitis. On the contrary, they suggest that an unusually dramatic immune clearance of HBsAg may be involved in its pathogenesis. These findings may explain the reported failure of attempts to treat HBsAg positive FH by infusion of anti-HBs (Acute Hepatic Failure Study Group, 1974; Dupuy *et al.*, 1975).

References

- Acute Hepatic Failure Study Group (1974). Treatment of fulminant hepatitis with hepatitis B immune globulin: a co-operative study. *Gastroenterology*, **66**, 752.
- Almeida, J. D., and Waterson, A. P. (1969). Immune complexes in hepatitis. *Lancet*, **2**, 983-986.
- Ashcavai, M., and Peters, R. L. (1971). Hepatitis-associated antigen: improved sensitivity in detection. *American Journal of Pathology*, **55**, 262-268.
- Barker, L. F., Peterson, M. R., Shulman, N., and Murray, R. (1973). Antibody responses in viral hepatitis, type B. *Journal of the American Medical Association*, **223**, 1005-1008.
- Boughton, C. R. (1968). Viral hepatitis in Sydney: a review of fatal illnesses in a hospital series. *Medical Journal of Australia*, **2**, 343-350.
- Brzosko, W. J., Madalinski, K., Krawczynski, K., and Nowoslawski, A. (1973). Duality of hepatitis B antigen and its antibody: 1, immunofluorescence studies. *Journal of Infectious Diseases*, **127**, 424-428.
- Dudley, F. J., Fox, R. A., and Sherlock, S. (1971). Relationship of hepatitis-associated antigen (H.A.A.) to acute and chronic liver injury. *Lancet*, **2**, 1-3.
- Dupuy, J. M., Frommel, D., and Alagille, D. (1975). Severe viral hepatitis type B in infancy. *Lancet*, **1**, 191-194.
- Hoofnagle, J. H., Gerety, R. J., and Barker, L. F. (1973). Antibody to hepatitis-B-virus core in man. *Lancet*, **2**, 869-873.
- Kassur, B., Nazariwicz, T., Morzycka, M., and Brzosko, W. (1974). HBsAg and anti-HBc in fatal acute hepatitis cases. *Proceedings of the VI International Congress of Infectious and Parasitic Diseases. Warsaw (Poland) 23-27 September 1974*, **3**, 233-234.
- Pesendorfer, F., Krassnitzky, O., and Wewalka, F. (1970). Immunelektrophoretischer Nachweis von Hepatitis-Associated-Antigen (Au/SH-Antigen). *Klinische Wochenschrift*, **48**, 58.
- Prince, A. M., Brotman, B., and Ikram, H. (1972). Hemagglutination assay: sub-typing by hemagglutination inhibition: an ultrasensitive identity test for HB antigen. In *Hepatitis and Blood Transfusion*, pp. 147-154. Edited by G. N. Vyas, H. A. Perkins, and R. Schmid. Grune and Stratton: New York.

- Prince, A. M., Brotman, B., Jass, D., and Irkam, H. (1973). Specificity of direct solid-phase radioimmunoassay for detection of hepatitis-B antigen. *Lancet*, **1**, 1346-1350.
- Redeker, A. G. (1974). Fulminant hepatitis. In *The Liver and its Diseases*, pp. 149-155. Edited by F. Schaffner, S. Sherlock, and C. M. Leevy. Intercontinental Medical Book Corp: New York.
- Robert, D., Caillat, B., Paliard, P., Motin, J., and Bolot, J. F. (1974). Les facteurs du pronostic de l'insuffisance hépatique aiguë grave. *Nouvelle Presse Médicale*, **3**, 1207-1210.
- Rueff, B., and Benhamou, J-P. (1973). Progress report: acute hepatic necrosis and fulminant hepatic failure. *Gut*, **14**, 805-815.
- Trepo, C., Trepo, D., Ortiz, J. P., Monier, J. C., and Sepetjian, M. (1973). L'antigène de l'hépatite B (AgHB ou Ag Australia), intérêt clinique de sa détection par un dosage radioimmunologique en phase solide. *Nouvelle Presse Médicale*, **2**, 2339-2340.
- Trey, C. (1972). The fulminant hepatic failure surveillance study. Brief review of the effects of presumed etiology and age on survival. *Canadian Medical Association Journal*, **106**, 525-526.
- World Health Organization, Scientific Group on Viral Hepatitis (1973). *Viral Hepatitis type B*. World Health Organisation Technical Report Series, No. 512, pp. 13-21. WHO: Geneva.