Contrasting features and responses to treatment of severe chronic active liver disease with and without hepatitis B_s antigen¹

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SUMMARY To determine the clinical implications of HB_sAg in severe chronic active liver disease (CALD), patients with HB_sAg positive CALD were compared with those chosen by identical clinical, functional, and morphological criteria in whom this test and anti-HB_s were negative. HB_sAg positive patients were predominantly males over 40 years of age and more frequently failed to respond to conventional treatment programmes with prednisone. HB_sAg negative patients were more often female and younger, had a higher incidence of associated immunopathic disease and immunoserological markers in high titre, and more often responded to treatment with full remission of their disease. HB_sAg positive patients failing treatment with conventional doses of prednisone often improved with higher doses, but did not reach full remission of their disease. The benefit-risk ratio of both conventional and high doses of prednisone in HB_sAg positive severe CALD needs further clarification.

The discovery of HB_sAg as a serum marker of hepatitis B virus (Blumberg *et al.*, 1967) allows identification of patients in whom initiation and/or perpetuation of the liver disease is attributable to hepatitis B infection. However, the specific features of CALD associated with HB_sAg in CALD are poorly defined.

Several investigators (Bulkley *et al.*, 1970; Dudley *et al.*, 1972; Finlayson *et al.*, 1972; Reed *et al.*, 1973; Van Waes *et al.*, 1974) have compared findings in groups of HB_sAg positive and negative CALD, which were not selected by pre-established

criteria. Differences in sex, age, and incidence of non-organ specific immunoserological markers have been described, but only the predominance of males in HB_sAg positive CALD as opposed to the female predominance in HB_sAg negative cases has been a uniform finding. Chronic hepatitis associated with HB_sAg has been considered to have a milder course and better prognosis than when the antigen is absent (Sherlock, 1974). One retrospective report (Dudley *et al.*, 1973) indicates that prednisone therapy in HB_sAg positive chronic liver disease is followed by clinical and biochemical improvement, but fails to define a definite therapeutic role of corticosteroids, whereas another (Aronoff *et al.*, 1973) suggests that prednisone and/or azathioprine might have an adverse effect.

We have a treatment programme for patients with severe CALD who differ from those in other series by their homogeneity, particularly with regard to the severity of the liver disease, standardisation of therapies, and regularity of follow-up (Soloway *et al.*, 1972). The presence or absence of HB_sAg were not factors in selection or subsequent treatment. A comparison of HB_sAg positive and negative patients in this group has been made to

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investigate differences in responses to therapy with prednisone, as well as to clarify variations in initial clinical and immunoserological features.

Methods

Postpubertal patients were eligible for our treatment programme if they fulfilled the predetermined criteria of chronicity and activity and had features compatible with CALD on liver biopsy. Chronicity was defined as disease duration of 10 weeks without improvement, or the presence of cirrhosis on liver biopsy. Activity was defined as SGOT elevation $\times 10$ upper limit of normal (N 24 IU/l) or SGOT elevation $\times 5$ in combination with a two-fold or more increase in gamma (N 1.6 g/dl) (Geall *et al.*, 1968; Soloway *et al.*, 1972). Histological features included chronic active ('aggressive') hepatitis (de Groote *et al.*, 1968); subacute hepatitis, characterised by bridging or multilobular necrosis; or cirrhosis with active hepatitis (Baggenstoss *et al.*, 1972). Coded biopsies were interpreted by the same observer, who also recorded the presence and degree of features consistent with viral hepatitis (acidophilic cell necrosis, cell ballooning, lobular disarray, and Kupffer cell hyperplasia). Alcoholic liver disease, primary biliary cirrhosis, Wilson's disease, drug-induced liver disease, and other conditions were excluded by appropriate methods.

One hundred and thirty-six patients who were randomised to different therapies form the basis of the current report. Treatments (Soloway *et al.*, 1972; Summerskill *et al.*, 1975) comprised maintenance doses of prednisone (20 mg daily), prednisone (10 mg daily) together with azathioprine (50 mg daily) or prednisone given on alternate days in doses sufficient to suppress abnormal liver function tests (mean 20 mg on alternate days; range 10-50 mg). In all instances, higher doses of prednisone were given initially. In addition, some patients had been randomised to azathioprine (100 mg daily) or placebo until 1971, when these were discontinued because of inferior results (Soloway *et al.*, 1972).

Patients were seen at six month intervals, when standardised clinical, biochemical, immunoserological, and histological evaluation was performed. Results of treatment comprised clinical and biochemical resolution; full (histological) remission; treatment failure and death (Soloway *et al.*, 1972). Clinical and biochemical resolution comprised absence of symptoms, return of the patient to customary activities, and normal results of standard liver function tests, apart from a two-fold elevation of SGOT. Full remission, in addition to these features, required disappearance from the liver biopsy of histological features of disease activity,

the appearances being normal or those of non-specific (identical with chronic persistent) hepatitis. Treatment failure was defined as the onset of hepatic coma, the development of ascites, increasing jaundice (serum bilirubin 66% above the lowest previous level and above 4 mg %) or a 66% increase in SGOT above the lowest previous value during treatment.

HB_sAg was sought in initial and serial six month serum samples (stored at -10°C) by radioimmunoassay (Ausria II Abbott) (Ling and Overby, 1972); the passive hemagglutination test (Vyas and Shulman, 1970) and, later, a radioimmunoassay (Ausab, Abbott) (Peterson *et al.*, 1973) were used for detection of anti-HB_s. These measurements, as well as other biochemical and immunoserological determinations, were made in laboratories unaware of the diagnosis.

Eighteen patients had HB_sAg detected in their serum on at least one occasion and six patients had anti-HB_s; 112 patients were repeatedly negative for HB_sAg and anti-HB_s. All patients except one were North American Caucasians without a history of drug addiction. HB_sAg positive patients were compared with HB_sAg and anti-HB_s negative patients for clinical, biochemical, immunoserological, and histological variables, and response to treatment. Statistical analyses were done by chi-square analysis or Fisher's exact test, for dichotomous variables, and by Wilcoxon rank sum tests for continuous variables. Because of possible interrelationships between the variables under study, linear discrimination methods were subsequently used to select that subset of variables which best contributed to discrimination between the HB_sAg positive and negative patients. The variables employed were age, sex, SGOT, bilirubin, prothrombin time, serum albumin, serum gamma globulin, presence of associated immunopathic disease, and immunoserology.

Survival curves were calculated by the product limit method (Kaplan and Meier, 1958). Since major differences in sex, age, and immunoserology were found between the groups, the responses to treatment in the HB_sAg positive group were also compared with that of HB_sAg negative groups of similar size, matched for histology, treatment group, sex, age, and immunoserology. Matching was done by an independent observer and by computer. Statistical evaluation was done by the use of Cochran's test for matched pairs (Cochran, 1950).

Results

ENTRY FEATURES: ALL PATIENTS (Table 1)
HB_sAg positive patients showed a predominance of

Table 1 Initial features in chronic active liver disease (all patients)

	HB _s Ag pos (18)	HB _s Ag neg (112)
Clinical features		
Sex, male	13 (89)	34 (30)*
Age, yr (median)	52	35*
Duration of symptoms > 12 mo	10 (56)	37 (33)
Ascites	2 (11)	15 (13)
Associated immunopathic diseases	1 (6)	31 (28)*
Biochemical features		
Bilirubin, mg/dl (median) N: <1.1	1.9	3.8*
SGOT IU/l (median) N ≤ 24	502	515
Prothrombin time, s (median) N ≤ 19	20	21
Albumin, g/dl (median) N ≤ 3.3	3.4	2.8*
Gamma globulin, g/dl (median) N ≤ 1.6	2.2	3.3*
Immunoserological features		
LE, ANA, SMA, and AMA, neg	10 (56)	7 (6)*
LE, ANA, or SMA ≥ 1:40 pos	1 (6)	85 (76)*
Histological features		
Chronic active hepatitis	4 (22)	29 (26)
Subacute hepatitis	9 (50)	59 (53)
Cirrhosis with active hepatitis	5 (28)	23 (21)
'Viral features' (in addition)	7 (39)	45 (40)

*P < 0.05. Percentages in parentheses.

males, in contrast with the female preponderance in HB_sAg negative patients, and the age of HB_sAg positive patients was greater. HB_sAg negative patients had a higher incidence of associated immunopathic disease, comprising eight examples of ulcerative colitis, seven patients with thyroiditis, and the remainder with Sjogren's syndrome, scleroderma, cutaneous lupus erythematosus, rheumatoid arthritis, iritis, myasthenia gravis, idiopathic thrombocytopenic purpura, or pernicious anaemia. By contrast, diseases attributable to HB antigen-antibody complex deposition (Gocke *et al.*, 1970; Combes *et al.*, 1971) were limited to two HB_sAg positive patients with periarteritis nodosa and immune-complex glomerulonephritis respectively.

Abnormalities in serum bilirubin, albumin, and gamma globulin were less in patients with HB_sAg, but results of other liver function tests were similar. Differences were found in the incidence of so-called immunoserological markers. Lupus erythematosus (LE) cell, antinuclear antibody (ANA), smooth muscle antibody (SMA), and antimitochondrial antibody (AMA) were all negative in approximately 50% of HB_sAg positive patients, whereas one or more tests were positive in high titre (≥ 1:40) in 75% of the HB_sAg negative patients.

The extent and characteristics of the histological lesions in HB_sAg positive CALD resembled those in the negative group. In particular, histological features consistent with viral hepatitis were equally frequent in both.

By linear discrimination methods, the subset of variables making a significant contribution were those representing immunoserology, age, and sex, in

that order. The resulting discriminant function gave median values of 6.0 for the HB_sAg negative and 0.8 for the HB_sAg positive. The region of overlap between the groups (scores less than 4) contained all 18 HB_sAg positive patients and only 17% of the 112 negative patients. The total ranges of the scores was -1.4 to +3.85 for positive patients and -0.4 to +8.1 for those who were negative. Thus, differences in sex, age, and immunoserology each remained significant.

RESPONSES TO TREATMENT WITH PREDNISONE (Table 2)

Of the 18 HB_sAg positive patients, 13 treated with prednisone, with or without azathioprine, have reached an end-point (histological remission or treatment failure) or have been treated for longer than 12 months (average 24 months). HB_sAg positive patients reached clinical and biochemical resolution and histological remission significantly less frequently when compared with all 82 patients without HB_sAg who received prednisone treatment. Also, treatment failure and death were significantly more frequent in HB_sAg positive patients.

When these 13 HB_sAg positive patients were compared with the same number of matched HB_sAg negative patients, HB_sAg positive patients again reach histological remission less frequently and more often become treatment failures.

Initial responses of patients to higher (60 mg daily) doses of prednisone or of the combination (prednisone 30 mg and azathioprine 50 mg daily) were usually as favourable as in HB_sAg negative patients, although two HB_sAg positive patients died during the first month of treatment. However, when doses were reduced to our conventional maintenance levels by a standardised procedure (Soloway *et al.*, 1972), biochemical deterioration followed in six of 11 (54% surviving HB_sAg positive patients) but occurred in only one of 13 (8%) of HB_sAg negative patients (Fig. 1). Reinstitution of

Table 2 Response to prednisone therapy in HB_sAg positive and HB_sAg negative CALD†

	HB _s Ag neg (82)	HB _s Ag pos (13)	HB _s Ag neg† (13)
Clinical and biochemical resolution	66 (80)*	6 (46)	11 (85)
Histological remission	56 (68)*	2 (15)	10 (77)*
Treatment failure	13 (16)*	6 (46)	1 (8)*
Death, total	9 (11)*	4 (31)	1 (8)

*P < 0.05.

†Matched for morphology and treatment (type and duration), sex, age, and immunoserology. Percentages in parentheses.

Fig. 1 Effect of prednisone (with or without azathioprine) on SGOT concentration in HB_sAg positive and HB_sAg negative CALD treated with prednisone.

Fig. 2 Cumulative survival of HB_sAg positive and HB_sAg negative patients with severe CALD treated with prednisone, compared with control treatment group.

higher doses of prednisone resulted in biochemical improvement in all.

Survival curves (Fig. 2) indicate that HB_sAg positive patients treated with prednisone have a better prospect of survival than control patients, comprising three HB_sAg positive and 26 negative patients who earlier received azathioprine or placebo. Direct comparison of HB_sAg positive patients with or without prednisone was not feasible because of the small number of HB_sAg positive patients on control therapy; however, the three HB_sAg positive patients did not appear to have a

more benign course (two deaths within three years) than HB_sAg negative patients.

TREATMENT RESPONSE RELATED TO ANTIGENAEMIA

Ten HB_sAg positive patients have been followed up for more than one year and the antigen has persisted in eight, seven of whom have required continuing treatment. In two patients antigenaemia was transient. Both were under 40 years of age (whereas all but one of those with persistent antigenaemia were older), and one reached full remission and discontinued treatment two years ago.

Discussion

The frequency of HB_sAg in our patients with severe CALD was 14%. As prospective studies indicate that 50% of patients with HB_sAg positive acute viral hepatitis who later develop chronic hepatitis eliminate all detectable amounts of antigen from their serum (Giles *et al.*, 1969; Nielson *et al.*, 1971; Redeker, 1972), our HB_sAg and anti-HB_s negative patients may include some whose disease was initiated by hepatitis B infection. If so, our HB_sAg positive patients represent a subgroup characterised by failure to eliminate HB_sAg early in their course.

In controlled circumstances, we found that HB_sAg positive patients differ in several clinical and biochemical characteristics from HB_sAg, anti-HB_s negative patients. The predominance of males with HB_sAg positive disease found in previous studies (Bulkley *et al.*, 1970; Dudley *et al.*, 1972; Reed *et al.*, 1973) was confirmed. The higher age of patients with HB_sAg positive disease has also been described in a patient population not addicted to drugs (Dudley *et al.*, 1972) and our finding that patients eliminating the HB_s antigen were younger may suggest that older individuals do this less effectively.

The association between CALD and 'immunopathic' diseases mainly involves HB_sAg negative patients, whereas extrahepatic lesions attributable to HB antigen-antibody complexes typify those who are HB_sAg positive. Similarly, the presence of non-organ specific immunoserological markers in high titres is greater in HB_sAg negative patients, whereas their absence is commoner in those who were HB_sAg positive. These findings support differences suggested by others (Bulkley *et al.*, 1970; Finlayson *et al.*, 1972; Dudley *et al.*, 1973) but not universally confirmed (Reed *et al.*, 1973; Van Waes *et al.*, 1974). As the incidence of such 'autoantibodies' failed fully to differentiate between HB_sAg positive and negative patients, and as weakly positive results were not infrequent in both groups, it appears likely that positivity of these

tests represents alterations in host response, rather than specific aetiologies. Hence, the similar incidence of histological features associated with viral hepatitis in both groups is of interest.

Chronic hepatitis associated with HB_sAg has been considered to have a better prognosis than when the antigen is absent (Sherlock, 1974). However, this generalisation cannot be substantiated when, as in our series, patients selected by uniform clinical, biochemical, and morphological criteria of disease severity are evaluated. In these conditions, we found that HB_sAg positive patients with severe CALD enter remission significantly less frequently and fail therapy significantly more often than do negative patients when treated with prednisone, with or without azathioprine. This finding raises the question of the efficacy of treatment with steroids in HB_sAg positive patients. While the benefit of steroids in CALD has been established in controlled trials Cook *et al.*, 1971; (Soloway *et al.*, 1972; Murray-Lyon *et al.*, 1973), none of these has been restricted to HB_sAg positive patients. Since the number of HB_sAg positive patients in our control group was small, a valid comparison between prednisone treated and untreated patients with HB_sAg was not possible. Our data, however, suggest that therapy is indicated and that higher doses of medication given over longer periods of time may be required for some patients with HB_sAg, but the benefits and risks of such therapy await evaluation in a controlled trial.

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