

# Chronic liver disease in transfusion-dependent thalassaemia: hepatitis B virus marker studies

S DE VIRGILIIS, G FIORELLI,\* S FARGION,\* G CORNACCHIA, G SANNA,  
P COSSU, V MURGIA, AND A CAO

*From the 2nd Pediatric Clinic, University of Cagliari, Sardinia, and the \*3rd Clinic of Medicine, University of Milano, Italy*

**SUMMARY** The systematic screening of 253 children with transfusion-dependent homozygous  $\beta$ -thalassaemia revealed a high incidence of hepatitis B virus markers. The highest frequencies of hepatitis B surface antigen (HBsAg) and antibody to hepatitis B core antigen (anti-HBc) were found in the group of patients with the smallest number of transfusions, while the highest frequency of antibody to hepatitis B surface antigen (anti-HBs) was detected in the patients who had had the largest number of transfusions. Follow-up of these patients showed (a) a high incidence of acute hepatitis B, which was mainly subclinical; (b) normal hepatitis B surface antigen clearance and normal antibody to hepatitis B surface development; and (c) a high frequency of increased transaminase values for over six months. In all the subjects with persistently high transaminase, histological examination revealed chronic persistent hepatitis or chronic active hepatitis. Apart from two cases of chronic active hepatitis with no B virus markers, and two cases of chronic persistent hepatitis with HBsAg and anti-HBc in the serum, all these subjects were anti-HBs positive but HBsAg and anti-HBc negative.

Chronic hepatitis occurs very frequently in children with transfusion-dependent thalassaemia in the Mediterranean area, including Sardinia.<sup>1-6</sup> Viral agents implicated in transfusional hepatitis<sup>3 6 7</sup> and iron overload,<sup>8</sup> resulting from the high transfusion regimen are considered to be the main causes. Among the hepatitis viruses, a predominant role is ascribed to hepatitis B virus (HBV), while little, if any role, has been given to the A virus.<sup>7</sup>

In the United States, it has recently been shown that the non-A, non-B virus(es) seem to cause transfusional hepatitis even more frequently than HBV.<sup>9</sup> However, there are no data on the incidence of this viral infection in the Mediterranean area.

To elucidate the role of HBV in the development of chronic liver disease in 253 children with transfusion-dependent thalassaemia major, we carried out: (a) a survey of the prevalence of HBV markers; (b) a two-year follow-up of HBV markers and liver function tests; and (c) liver biopsy studies in patients with persistently increased transaminase levels.

## Patients and methods

This study concerns 253 Sardinian children aged 1 to 14 years with homozygous  $\beta^0$ -thalassaemia, who were followed in the 2nd Paediatric Department of Cagliari University from September 1975 to November 1977. The diagnosis of homozygous  $\beta^0$ -thalassaemia was made on the basis of haemoglobin electrophoresis and globin chain synthesis analysis.<sup>10</sup>

For the past three years these patients have been regularly transfused with packed red cells every three to four weeks in order to keep their Hb pretransfusion levels above 9 g/dl. They had chelation therapy with intramuscular desferrioxamine-B (750 mg/day at age 3-9 years and 1 g/day at 9+ years for six days a week). Previously, they received sporadic transfusions with whole blood, and chelation therapy was administered in low doses at irregular intervals.

Hepatitis B surface antigen (HBsAg) testing in blood donors was performed by passive haemagglutination.

In all patients, liver function tests were carried out at regular intervals for two years. Serum aspartate and alanine aminotransferase (SGPT and SGOT)

were determined monthly; alkaline phosphatase,  $\gamma$ -glutamyl-transpeptidase, pseudocholinesterase, serum protein electrophoresis, and prothrombin time (normotest) were performed every three months. The enzymatic tests were carried out with standard kits (Biochemia, Boehringer, Mannheim). Serum protein electrophoresis was carried out on gelatinised cellulose acetate strips (Chemetron, Milano). Normotest, which is a standardised coagulation test sensitive to clotting factors II, VII, and X, was performed according to the manufacturer's instructions (Nycegard, Oslo).

HBsAg and antibody to hepatitis B surface antigen (anti-HBs) were determined every three months by a solid-phase radioimmunoassay (RIA) (Ausria II-125 and Ausab, Abbott). Patients found to be HBsAg positive were subsequently tested monthly unless they became HBsAg negative. In 40 randomly selected subjects, these determinations were performed monthly.

Antibody to hepatitis B core antigen (anti-HBc), determined by indirect immunofluorescence on a core-rich human liver with no fixed anti-HBc (supplied by Professor Desmet), was performed as a screening test in all patients. Successive control tests were carried out in HBsAg positive patients and in those with clinical or biochemical signs of liver disease. One hundred and fifty-six Sardinian children of the same age and from similar income levels acted as controls.

Fifty-two patients, with three times normal transaminase values for over six months, had percutaneous liver biopsy performed with a Menghini needle. The liver tissue was processed by routine histological techniques. The diagnosis of chronic hepatitis was made on the basis of the criteria established by De Groote *et al.*<sup>11</sup> and subsequently modified by the International Group for the Study of Liver Diseases.<sup>12</sup>

## Results

### INCIDENCE AND FOLLOW-UP OF HBV MARKERS

The prevalence of HBV markers in the 253 regularly transfused thalassaemia major patients is shown in

Table 1. The patients are divided into three groups according to the units of blood received. The highest prevalence of HBsAg was found in the group of patients with the smallest number of transfusions, while the highest prevalence of anti-HBs is in those who had the largest number of blood transfusions. The behaviour of anti-HBc was similar to that of HBsAg. Anti-HBc was found in seven subjects: in four it was associated with HBsAg and in three it was the only viral marker detected.

On follow-up of the four patients with both anti-HBc and HBsAg, two, persistently positive for these markers, developed chronic persistent hepatitis (CPH), and two had clearance of both markers in 18 and 24 months, respectively, followed by the appearance of anti-HBs. The three cases showing anti-HBc as the only marker had clearance of this marker in six to eight months and developed anti-HBs positivity.

The HBsAg positive cases cleared in two to four months and developed anti-HBs, which was present throughout follow-up. Those with only anti-HBs positivity had persistence of this antibody for the two-year follow-up period.

After 12 months' follow-up one subject with persistent anti-HBs positivity developed both HBsAg and anti-HBc, persisting for six months. The anti-HBs positivity in this subject persisted for the whole two years. One month after the appearance of HBsAg and anti-HBc, clinical acute hepatitis with SGPT > 250 mU/ml developed. Another patient with persistent anti-HBs positivity developed HBsAg and anti-HBc while anti-HBs disappeared. During the six-month follow-up, HBsAg and anti-HBc remained positive while anti-HBs was persistently absent. This subject showed no clinical or biochemical manifestations of liver disease and, particularly, no signs of immunocomplex disorders. Unfortunately, HBsAg subtype characterisation was not performed.

### HBV MARKERS IN ACUTE CLINICAL OR SUBCLINICAL HEPATITIS

The monthly determination of HBV markers in a randomly selected group of subjects enabled us to follow up the development of these markers in 35

Table 1 *HBsAg, anti-HBs, and anti-HBc in 253 regularly transfused thalassaemic patients in relation to number of blood units received*

Blood transfused (units)	No. of cases	HBsAg	Anti-HBs	Anti-HBc
0-40	146	6 (5%)	41 (28%)	4 (2.7%)
41-80	73	3 (4%)	40 (54%)	2 (2.7%)
>80	34	1 (2.5%)	29 (85%)	1 (2.9%)
Controls	159	0	12 (7.5%)	0

This examination was made at the beginning of our study.

patients who showed clinical or subclinical hepatitis.

HBsAg was found in all cases examined from 30 days before to 30 days after the onset of clinical and/or biochemical signs of hepatitis. The number of positive cases decreased progressively in the following 7.5 months.

In general, anti-HBc appeared 30 days after the onset of clinical and/or biochemical signs and was detectable in the serum for six to eight months. In three patients, anti-HBc was the only marker determinable in serum between the fourth and fifth months after the onset of clinical or biochemical signs of hepatitis.

Anti-HBs began to be recognisable four months after the clinical and/or biochemical onset of hepatitis; the incidence increased progressively to 99% of cases in the seventh month. Successively it was present in almost all patients during the two-year observation period.

#### INCREASE IN SERUM TRANSAMINASES

As can be seen in Table 2, the monthly serum transaminase determination showed very high SGPT values (> 250 mU/ml) in 35 patients, who also had clinical signs of acute hepatitis; in 34 of them, HBsAg and/or anti-HBc, previously absent, was detected.

One hundred and fifty-six patients had serum transaminases ranging from 40 to 250 U; 135 of these showed the presence of at least one of the viral markers. None of these patients had clinical signs of acute hepatitis (Table 2). Serum transaminases of less than 40 were seen in 62 patients with no viral markers in the serum.

The incidence of raised transaminase levels for a shorter or longer period of time was higher in HBV

positive (approx 30%) than in HBV negative (approx 9%) subjects (Table 3).

Table 3 Follow-up of subjects with increased SGPT values in relation to HBV markers

HBV markers	No. of cases	Raised SGPT (3 times normal)	
		For <6 mth	For >6 mth
HBV-	22	2(9%)	2(9%)
HBV+	169	54(31%)	55(32%)

HBV- = no HBV markers.

HBV+ = at least anti-HBs present.

#### LIVER HISTOLOGY

The relation between HBV markers and liver biopsy findings in 52 patients who had raised SGPT for over six months is shown in Table 4. On histological examination all the 52 patients were seen to have chronic liver disease, ie, chronic persistent hepatitis (CPH) in 20 and chronic active hepatitis (CAH) in 32.

HBsAg and anti-HBc were detectable in the serum of two patients with CPH. In the other patients, except two cases of CAH with no HBV markers, only anti-HBs was found. None had any clinical symptoms of liver disease.

#### Discussion

##### INCIDENCE AND FOLLOW-UP OF B VIRUS MARKERS

This study of thalassaemia major patients confirms previous results in thalassaemia<sup>1-6</sup> and haemophilia patients<sup>13-15</sup> showing a high incidence of HBV markers with frequencies of HBsAg-positive subjects

Table 2 Two-year follow-up of serum transaminase in 253 children with homozygous  $\beta^0$ -thalassaemia major in relation to HBV markers

No. of cases	GPT (mU/ml)*	No. of subjects		Clinical signs (jaundice, gastrointestinal disorders)
		HBV+	HBV-	
35	>250	34	1	+
156	40-250	135	21	-
62	40	—	62	-

\*Maximum value scored.

HBV+ = at least anti-HBs present. HBV- = No HBV markers.

Table 4 Histopathological findings in patients with raised SGPT for over six months in relation to HBV infection

Histopathological findings	No. of cases	HBsAg	anti-HBsAg	anti-HBc
CPH	20	2	19	2
CAH	32	—	30	—

CPH = chronic persistent hepatitis.

CAH = chronic active hepatitis.

decreasing and anti-HBs-positive subjects increasing with the number of transfusions received. Anti-HBc was always associated with the presence of HBsAg, except in three patients who showed anti-HBc as the only marker of HBV infection. The lower incidence of anti-HBc in this study than in previous reports<sup>16</sup> is probably related to the lower sensitivity of indirect immunofluorescence compared to RIA. Since anti-HBs may transiently develop without anti-HBc after immunisation with purified non-infectious HBsAg,<sup>16-21</sup> some of our patients, who had been transfused for many years with whole blood, could have developed anti-HBs in response to the weakly immunogenic activity of immune serum globulin containing low-titre HBsAg or HBsAg/anti-HBs complexes. However, the persistence of anti-HBs at the two-year follow-up examination is against this hypothesis. The development of HBsAg and anti-HBc in two subjects with persistent anti-HBs is surprising. One of these patients developed acute hepatitis with persisting anti-HBs; the other became a chronic carrier with disappearance of anti-HBs positivity. These findings may be explained assuming either passive anti-HBs transmission with blood transfusion or HBV infection by a subtype different from that of pre-existing antibody.

The monthly follow-up has shown a very high incidence of subjects with acute hepatitis B which was mainly subclinical. These patients had the highest transaminase values. The follow-up of hepatitis B virus markers has shown normal HBsAg clearance and an immune response as demonstrated by the development of anti-HBs. In our series, clinical or biochemical evidence of acute hepatitis not associated with the development of HBV markers was rarely observed.

#### CHRONIC LIVER DISEASE

The incidence of chronic liver disease following HBV infection was significantly higher in thalassaemia major patients (32%) than in non-thalassaemic subjects (roughly 10%) (unpublished results). On the other hand, in the HBV negative group there was a low incidence of chronic liver disease, which is probably due to non-A, non-B virus infection. We used increased transaminase values as a screening test, and this may have underestimated the frequency of liver abnormalities since 3 out of 21 patients with normal liver chemistry were found to have chronic hepatitis in a liver biopsy taken during splenectomy (unpublished results). The absence of HBsAg and anti-HBc in the serum in almost all our anti-HBs positive patients with chronic hepatitis has two alternative explanations: the disease may be due to non-A, non-B virus(es) or to the B virus, but there was no evidence of viral replication in the serum

probably due to low dose infection.

Assuming that chronic hepatitis in our patients resulted from hepatitis B virus infection, it remains to be clarified why there was chronic evolution in the presence of anti-HBs. Since HBsAg has been found to be heterotypic,<sup>22</sup> anti-HBs present in our patients could be specific for other HBsAg determinants. Alternatively, iron overload in the presence of low dose intact virus or viral products could be a factor in the development or perpetuation of chronic liver disease in anti-HBs positive patients.

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Requests for reprints to: Professor Antonio Cao, Clinica Pediatrica 2, Via Porcell, 1, 09100 Cagliari, Sardinia, Italy.