J. clin. Path., 1975, **28**, 620-624

Asymptomatic liver disease in haemophiliacs

P. M. MANNUCCI[1], ANNA CAPITANIO, E. DEL NINNO, M. COLOMBO, F. PARETI, AND Z. M. RUGGERI

From the Haemophilia and Thrombosis Centre Angelo Bianchi Bonomi, University of Milano; International Training Centre of the World Federation of Haemophilia; and Antonio Migliavacca Liver Unit, Institute of Clinical Medicine, University of Milano, Italy

SYNOPSIS The incidence of jaundice and of abnormal liver function tests has been assessed in 91 multitransfused patients with severe haemophilia A and B. Tests of hepatocyte function were within the normal range in the majority of patients. On the contrary, tests of biliary cell function, liver cell damage, and bromsulphthalein retention gave high rates of abnormal values, which tended to increase with age. Hepatitis B surface antigen was present in 8% and the corresponding antibody in 66% of the cases; 18% had a history of jaundice. All patients were asymptomatic and only a minority showed clinical signs of liver involvement. These data suggest that in haemophiliacs repeated and prolonged contact with the agent(s) responsible for post-transfusion hepatitis may cause chronic liver damage not associated with overt illness.

Haemophiliacs are the group of multitransfused patients exposed most frequently and for the longest period of time to the agent(s) implicated in post-transfusion hepatitis.

The rate of exposure has probably increased since the introduction in the replacement therapy of the highly purified freeze-dried concentrates of factor VIII and factor IX which carry a higher risk of contamination, having been manufactured by pools of plasma from a large number of donors.

Tests for the presence of hepatitis B surface antigen (HB_sAg) in plasma are now carried out routinely by blood bank officers or commercial manufacturers. However, the available methods for universal donor screening are unlikely to eliminate the risk of post-transfusion hepatitis because it has been clearly shown that exclusion of the HB_sAg positive samples reduces it by less than 25% (World Health Organization, 1970). The incidence of clinical illness associated with jaundice is surprisingly low in haemophiliacs (Kasper and Kipnis, 1972; Biggs, 1974; Lewis, Maxwell, and Brandon, 1974). These data, however, do not exclude the occurrence of anicteric hepatitis, which is particularly frequent in children, as well as the possibility that repeated and prolonged contact with the infective agent(s) may cause chronic liver damage not associated with overt illness. We therefore decided to assess the prevalence of abnormal liver function tests in these patients and to correlate the findings with the presumptive number of transfusions.

[1]Requests for reprints to P. M. Mannucci, Via Pace 15, 20122 Milano, Italy

Received for publication 10 February 1975.

Patients and Methods

Ninety-one patients (75 with haemophilia A and 16 with haemophilia B) regularly examined at the Haemophilia Centre were selected solely on the basis of their availability to take part in the study once informed consent had been obtained. At the moment of blood sampling, they had not been transfused for at least two weeks. All were severely affected (factor VIII or IX: less than 0·01 u/ml) and had been repeatedly exposed in the past to replacement therapy with cryoprecipitate, commercial freeze-dried concentrates (Kryobulin and Bebulin, Immuno, Vienna; Hemophil, Hyland, Brussels) and, in the older patients, with fresh-frozen plasma. Since a precise record of the total number of transfusions in the individual recipient was usually not available, age was thought to be a rough but reliable parameter to investigate any relationship between degree of transfusion exposure and abnormality of liver function tests. Three categories were thus arbitrarily established before starting the study—0-14, 15-30, and over 30 years. During physical examination of the patients at the Haemophilia Centre, they and

their relatives were asked about any previous occurrence of symptoms such as jaundice, pale stools, dark urine, nausea, and anorexia.

Blood samples were allowed to clot by adding thrombin (1 u/ml). Serum samples were tested for HB_sAg by counterimmunoelectrophoresis (CEP) (Gocke and Howe, 1970) and solid-phase radioimmunoassay (RIA) (Ling and Overby, 1972). A sample was considered positive when its radioactive counts were greater than 2·1 times the mean of the negative control and were specifically neutralized by unlabelled human anti-HB_s (Prince, Brotman, Jass, and Ikram, 1973). Antibody to hepatitis B surface antigen (anti-HB) was determined by an antigen neutralization radioimmunoassay. Two hundred microlitres of a mixture of equal volumes of serum samples and standard antigen were incubated for one hour at 37°C. The control antigen was used in the dilution which gave at least 2·5 times the average count rate of the negative controls. A sample was considered positive when the radioactive counts of the mixture were lower than 0·2 times the average count rate of the positive controls, corresponding to the complete neutralization of the antigen. The electrophoretic separation of serum proteins was carried out on gelatinized cellulose acetate strips (Chemetron, Milan); Normotest, which is a standardized coagulation test sensitive to clotting factors II, VII, and X, was performed according to the instructions of the manufacturers (Nyeegard, Oslo). Bromsulphthalein (BSP) retention at 45 minutes was measured by a standard colorimetric procedure.

Serum bilirubin, aspartate and alanine aminotransferases (SGPT and SGOT), alkaline phosphatase (AP), gamma glutamyl-transpeptidase (γ-GT) and pseudocholinesterase (ChE) were carried out by means of standard kits (Biochemica, Boehringer, Mannheim). The data were usually expressed as relative frequencies of the abnormal values, and differences were tested by the χ^2 test with Yates' correction.

Results

HISTORY

Table I shows that 16 patients (17·6%) had a history of jaundice (14 with haemophilia A; 2 with haemophilia B); the relative frequencies in the various groups increased with age. No patient admitted excessive alcohol ingestion. Before the introduction of concentrates for the routine management of bleeding episodes, the older patients (15-30 and over 30 years) had often used analgesic drugs such as paracetamol, codeine, and pentazocine. These drugs are practically unknown to younger haemophiliacs.

CLINICAL SYMPTOMS

All patients were asymptomatic. On physical examination, only five showed mild to moderate liver enlargement, and two had hepatosplenomegaly.

HEPATITIS B ANTIGEN AND ANTIBODY

HB_sAg was detected in seven haemophiliacs (8%) with a good correspondence between the two methods of assay employed in the present investigation. Anti-HB_s was present in 59 patients (66%).

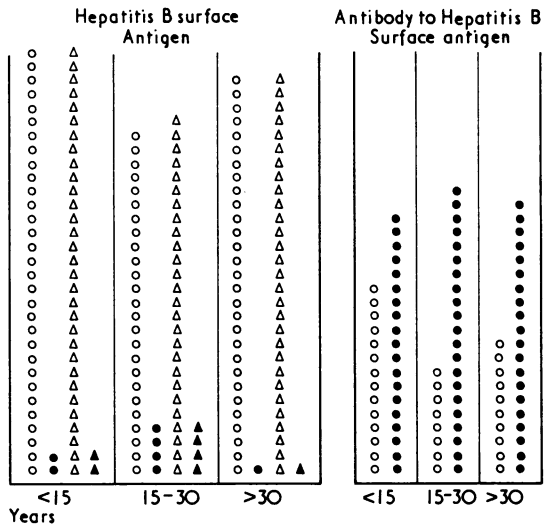

Fig 1 *Hepatitis B surface antigen and antibody in three age groups. Open circles and triangles—cases negative by RIA and CEP respectively; closed circles and triangles—positive cases.*

Age (yr)	*No.*	*Incidence %*
Total	91	17·6
<15	33	12·1
15-30	29	17·2
>30	29	24·1

Table I *Per cent incidence of jaundice in 91 patients with severe haemophilia*

Haemophiliacs	*HB_sAg*	*Anti-HB_s*
With history of jaundice (n = 16)	18·7%	75·0%
Without history of jaundice (n = 75)	6·3%	62·6%

Table II *Per cent incidence of HB_sAg and anti-HB_s in haemophiliacs in relation to history of jaundice*

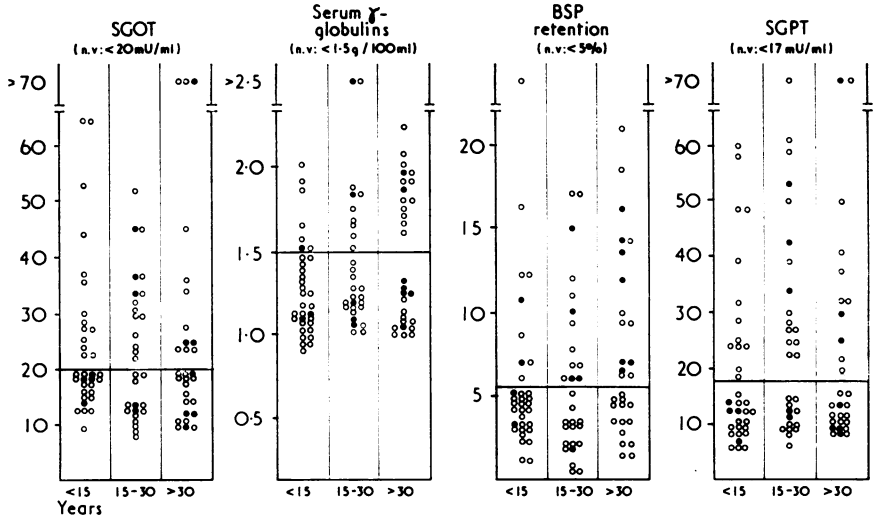

Fig 2 *SGPT, SGOT, BSP retention, and γ-globulins in three age groups. The upper limit of normal range is shown by the continuous horizontal line; closed circles—patients with a history of jaundice.*

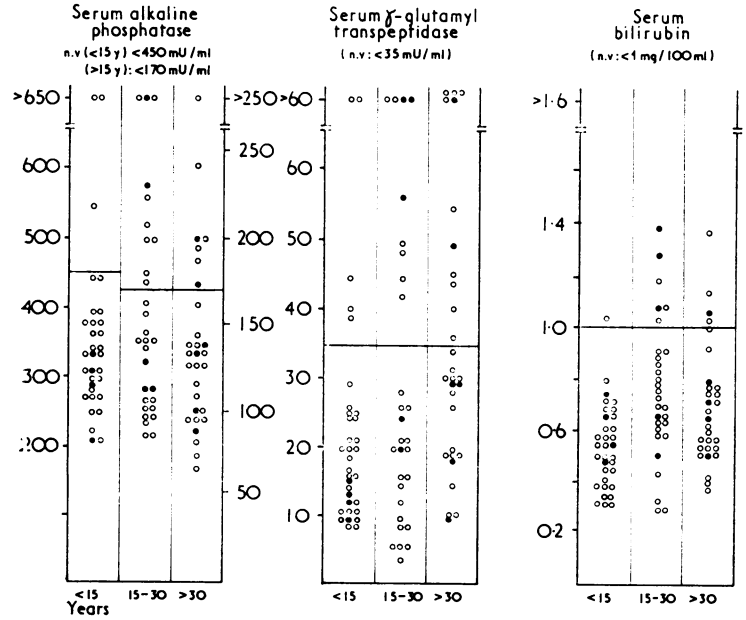

Fig 3 *Tests of biliary cell function in three age groups. The upper limit of normal range is shown by the continuous horizontal line; closed circles—patients with a history of jaundice.*

Age (yr)	*No.*	*SGPT*	*SGOT*	*BSP Retention*	*Gamma Globulins*	*Serum Bilirubin*	*AP*	*γ-GT*	*Normotest*	*Serum Albumin*	*ChE*	*Serum Proteins*
Total	91	45·0	45·0	40·6	34·0	12·8	23·1	27·2	0	13·1	8·7	4·4
<15	33	39·4	42·4	27·2	21·5	3·1	12·3	15·1	0	8·8	3·5	0
15-30	29	55·1	51·6	44·5	34·4	20·6	34·4	31·3	0	17·2	17·2	10·0
>30	29	41·3	41·3	51·7	48·2	17·2	24·1	37·8	0	16·6	13·7	34·0

Table III *Per cent incidence of abnormal liver function tests in 91 patients with severe haemophilia*

Differences between age groups have been analysed by the χ^2 test with Yates' correction. No value was significant at the 5% level

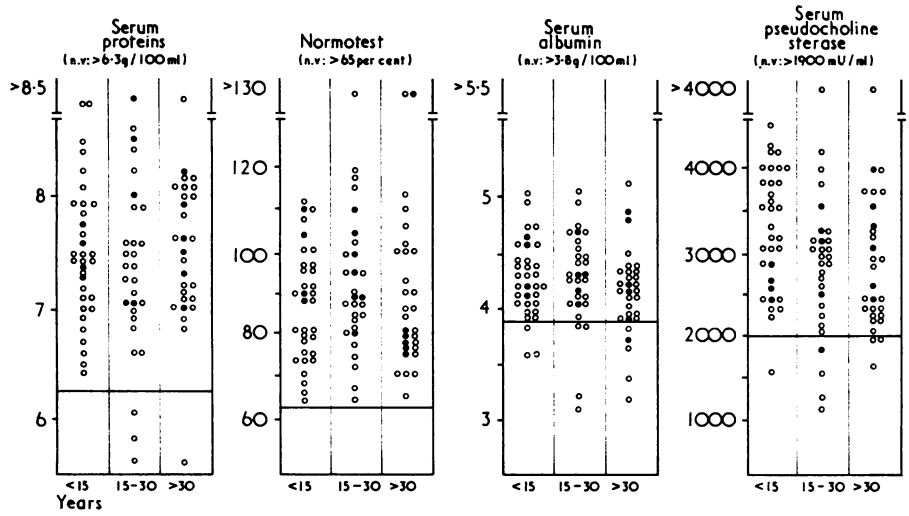

Fig 4 *Tests of hepatocyte function in three age groups. The lower limit of normal range is shown by the continuous horizontal line; closed circles—patients with a history of jaundice.*

Both antigen and antibody occurred in one case. The occurrence of the hepatitis B virus markers did not appear to be age-related (fig 1) and tended to be higher in patients with a history of jaundice (table II).

LIVER FUNCTION TESTS

The most common observation was a moderate increase in both serum transaminases and BSP retention, which occurred in 45, 45, and 41% of cases respectively. Hypergammaglobulinaemia was detected in 34%, with values ranging between 1·6 and 2·5 g/dl (fig 2 and table III). Pathological values of tests of biliary cell function (serum bilirubin, alkaline phosphatase, and gamma-glutamyl-transpeptidase) were also detected in 13, 23, and 27% of patients (fig 3 and table III). The overall incidence of abnormality of the tests exploring the synthetic capacity of the liver was rather low. Normotest was normal in all patients. Serum proteins, albumin and pseudocholinesterase were abnormal in 4, 13, and 9% of patients (fig 4 and table III). Abnormal liver function tests tended to be more frequent in the group of older patients with the exception of transaminase, which was raised with a similar frequency in all age groups (table III). There was no obvious relationship between the occurrence of abnormal liver function tests and a history of jaundice (figs 2 to 4).

Discussion

The results of the present investigation carried out in a group of 91 severe haemophiliacs show a high incidence of abnormal liver function tests, which tends to increase with age and is not correlated to a history of jaundice. Although hypergammaglobulinaemia may be a non-specific effect of the continuous antigenic stimulation with blood, plasma and clotting-factor concentrates, the alterations of a sensitive test such as BSP retention and of the enzymatic activities demonstrate the frequent involvement of the liver in these patients. In a previous study, no significant variation of transaminase serum levels has been observed in 41 hospital patients with haemophilia A and B (Forbes, King, Prentice, and McNicol, 1972). The varied results may be explained by differences in case selection, age, severity of the disease, quantity and type of replacement material. In our patients, it is difficult to establish a reasonable correlation between the observed abnormalities of liver function tests and the source of plasma fractions, since all of them have been treated to a variable extent with cryoprecipitate from single donors, commercial freeze-dried concentrates and, in older patients, with plasma and whole blood.

The prevalence rate of antibody to hepatitis B surface antigen (anti-HB_S) is considered a reliable index of past exposure to hepatitis B virus (HBV). The incidence of positive cases, which is high in our study and compares well with that observed by Peterson *et al* (1973) and Burrell *et al* (1974) using an assay method of comparable sensitivity, indicates the frequent exposure of haemophiliacs to HBV. It is therefore surprising to find, in agreement with

other investigators (Kasper and Kipnis, 1972; Biggs, 1974; Lewis *et al*, 1974), a relatively low incidence of acute hepatitis, which contrasts with the observation of frequent abnormalities of liver function tests. Although anicteric hepatitis may account for a proportion of the cases without a history of jaundice, the frequent and repeated exposure of haemophiliacs to the agent involved in post-transfusion hepatitis is likely to be the most important cause of the observed abnormalities. This assumption is supported by the higher incidence of abnormal tests observed in the older patients, who have presumably been exposed to a greater number of plasma units.

The possibility that analgesic drugs may also have played a role cannot be entirely ruled out in those who have in the past made a large use of potentially hepatotoxic drugs for the relief of pain associated with bleeding episodes. However, since the introduction of early replacement therapy in the control of pain, these drugs have been practically abandoned and hence cannot be responsible for the pathological values observed in children.

The clinical and prognostic significance of the observed abnormalities is presently unknown, and the lack of liver biopsies renders the task of clarifying them rather difficult. The great majority of the patients were completely asymptomatic and free of physical signs of liver involvement. It is possible that constant exposure to the infective agent(s) induces a general immunological tolerance conditioning an attenuated pattern of chronic hepatitis (London, Difiglia, Sutnick, and Blumberg, 1969; Grady, 1974). It also seems reasonable to suggest that antibody to hepatitis B surface antigen occurring in haemophiliacs may offer protection (Hollinger, Werch, and Melnick, 1974).

However, the evidence accumulated with the investigation of asymptomatic carriers of HB_sAg suggests that these humoral abnormalities are not entirely benign, since they may be associated with structural changes of the liver similar to those occurring in patients with chronic hepatitis (Feinman, Berris, Sinclair, Wrobel, Alter, and Holland, 1973; Simon and Patel, 1974; Woolf, Boyes, Jones, Whittaker, Tapp, MacSween, Renton, Stratton, and Dymock, 1974). In haemophiliacs, an answer to these problems can be given only by a long-term prospective evaluation of any possible relationship between the observed abnormalities and the development of overt hepatic dysfunction. We recommend, therefore, that complete liver function tests should be carried out at regular intervals for early detection of any abnormal evolution, the latter requiring the establishment of a therapeutic programme which appears unjustified in the great majoritv of these patients at the present stage of our knowledge.

This work was supported in part by a grant of the Fondazione Angelo Bianchi Bonomi.

References

Biggs, R. (1974). Jaundice and antibodies directed against factor VIII and IX in patients treated for haemophilia or Christmas disease in the United Kingdom. *Brit. J. Haemat.*, **26**, 313-329.

Burrell, C. J., Parker, A. C., Ramsay, D. M., and Proudfoot, E. (1974). Antibody to hepatitis B antigen in haemophiliacs and their household contacts. *J. clin. Path.*, **27**, 323-325.

Feinman, S. V., Berris, B., Sinclair, J. C., Wrobel, D. M., Alter, H. J., and Holland, P. V. (1973). Relation of hepatitis-B-antigen subtypes in symptom-free carriers to geographical origin and liver abnormalities. *Lancet*, **2**, 867-869.

Forbes, C. D., King, J., Prentice, C. R. M., and McNicol, G. P. (1972). Serum enzyme changes after intramuscular bleeding in patients with haemophilia and Christmas disease. *J. clin. Path.*, **25**, 1034-1037.

Gocke, D. J. and Howe, C. (1970). Rapid detection of Australia antigen by counter-immunoelectrophoresis. *J. Immunol.*, **104**, 1031-1034.

Grady, G. (1974). Tolerating hepatitis. *New Engl. J. Med.*, **291**, 96-97.

Hollinger, F. B., Werch, J., and Melnick, J. L. (1974). A prospective study indicating that double-antibody radioimmunoassay reduces the incidence of post-transfusion hepatitis B. *New Engl. J. Med.*, **290**, 1104-1109.

Kasper, C. K. and Kipnis, S. A. (1972). Hepatitis and clotting-factor concentrates. *J. Amer. med. Ass.*, **221**, 510. (Letter)

Lewis, J. H., Maxwell, N. G., and Brandon, J. M. (1974). Jaundice and hepatitis B antigen-antibody in hemophilia. *Transfusion*, **14**, 203-211.

Ling, C. M. and Overby, L. R. (1972). Prevalence of hepatitis B virus antigen as revealed by direct radioimmunoassay with ^{125}I-antibody. *J. Immunol.*, **109**, 834-841.

London, W. T., Difiglia, M., Sutnick, A. I., and Blumberg, B. S. (1969). An epidemic of hepatitis in a chronic-hemodialysis unit: Australia antigen and differences in host response. *New Engl. J. Med.*, **281**, 571-578.

Peterson, M. R., Barker, L. F., and Schade, D. S. (1973). Detection of antibody to hepatitis-associated antigen in hemophilia patients and in voluntary blood donors. *Vox Sang. (Basel)*, **24**, 66-75.

Prince, A. M., Brotman, B., Jass, D., and Ikram, H. (1973). Specificity of the direct solid-phase radioimmunoassay for detection of hepatitis-B antigen. *Lancet*, **1**, 1346-1350.

Simon, J. B. and Patel, S. K. (1974). Liver disease in asymptomatic carriers of hepatitis B antigen. *Gastroenterology*, **66**, 1020-1028.

Woolf, I. L., Boyes, B. E., Jones, D. M., Whittaker, J. S., Tapp, E., MacSween, R. N. M., Renton, P. H., Stratton, F., and Dymock, I. W. (1974). Asymptomatic liver disease in hepatitis B antigen carriers. *J. clin. Path.*, **27**, 348-352.

World Health Organization (1970). Viral hepatitis and tests for the Australia (hepatitis-associated) antigen and antibody. *Bull. WHO*, **42**, 957-992.