

## Non-A non-B hepatitis after transfusion of factor VIII in infrequently treated patients

M L FLETCHER, J M TROWELL, J CRASKE, K PAVIER, C R RIZZA

### Abstract

Thirty patients who had not previously received treatment with factor VIII concentrate or who had been treated only infrequently with factor VIII concentrate were studied after a transfusion of factor VIII. Tests of liver function were performed frequently. Four patients had evidence of chronic liver disease before transfusion. In 17 of the remaining 26 patients serum transaminase activities became raised and 10 patients developed jaundice. All of the nine patients who had not previously received factor VIII transfusion developed non-A non-B hepatitis. Four out of 10 patients followed up for a year had persisting abnormalities of liver function.

The pattern of illness suggests that more than one serotype of non-A non-B hepatitis virus may be transmitted by factor VIII concentrate prepared by the National Health Service from volunteer donors in the United Kingdom.

### Introduction

Treatment of haemophilia A with human factor VIII concentrate carries a risk of hepatitis.<sup>1,2</sup> Commercial factor VIII concentrate was first imported from the United States to the United Kingdom in 1972. A retrospective study in the United Kingdom of patients after treatment with one brand of American commercial factor VIII in 1974-5 showed that symptomatic hepatitis developed in 72 out of 371 patients known to have received transfusions.<sup>3</sup> Another retrospective survey of patients receiving factor VIII concentrates in the United Kingdom between 1974 and 1979 showed at least two types of hepatitis—namely, hepatitis B and non-A non-B hepatitis.<sup>4</sup> This study showed a greater risk of non-A non-B hepatitis developing in patients receiving factor VIII for the first time. The risk was also increased if the concentrate was a commercial product from the United States. This commercially produced factor VIII is manufactured from plasma obtained from paid donors. There is a higher incidence of hepatitis in patients receiving transfusions of blood obtained from paid donors compared with patients whose transfusions were obtained from volunteer donors.<sup>4</sup>

The commonest form of hepatitis after factor VIII trans-

fusion is reported as non-A non-B.<sup>4</sup> At least two types of non-A non-B hepatitis are associated with factor VIII transfusion in patients with haemophilia,<sup>5</sup> and this is supported by studies in chimpanzees.<sup>7-9</sup>

We carried out a prospective study to establish the incidence of hepatitis among infrequently treated patients with haemophilia in Oxford and to define the factors that influenced this. We report on the first 30 patients, who were followed up for at least six months, although some were followed up for more than one year.

### Patients and methods

#### DESIGN OF STUDY

The patients considered for this study were those attending the Oxford haemophilia centre who required treatment with factor VIII, were not suspected of having chronic liver disease, and had received not more than two transfusions of blood products in the previous year (table). None of the patients had received factor VIII in the six months before inclusion; all gave their informed consent. Patients were examined and liver function tests performed to establish those whose history or present health suggested pre-existing liver disease. Patients undergoing elective procedures were assessed before treatment, but when patients presented with trauma they were assessed for this study immediately after emergency treatment. Many of the patients were already known to the centre and had undergone tests of liver function previously. Thus we were able to class some of them as having chronic liver disease on admission to this study.

#### ASSESSMENT PROCEDURE

Patients were seen and blood samples obtained before treatment and then every two weeks for three months and thereafter monthly until six months and then at nine months and one year. When patients were admitted to the study blood samples were tested for hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (anti-HBs), hepatitis B core antibody (anti-HBc), and hepatitis A antibody (anti-HAV). Liver function tests were performed, which included measurement of bilirubin concentration; aspartate transaminase, alanine transaminase, and alkaline phosphatase activities; and total protein and serum albumin concentrations. Each time the patients were seen at follow up they were questioned for symptoms of hepatitis and any illness and drugs taken were recorded. Blood was taken for estimation of aspartate transaminase and alanine transaminase activities. If the patients became unwell or if transaminase activities were at least four times the upper limit of normal the patient underwent a full physical examination and was assessed by a physician specialising in liver disease (JMT). In those patients whose serum transaminase activities rose repeated samples were tested for hepatitis A and B, cytomegalovirus, and glandular fever (Epstein-Barr virus). To establish whether any patient had undergone seroconversion without abnormality in liver function samples from all patients were also tested for hepatitis A and B and cytomegalovirus at six months or later. Household contacts of patients were interviewed when the patients were admitted to the study and blood samples taken as soon as possible after the patient's treatment with factor VIII to eliminate the possibility of pre-existing hepatitis infection in the families and sexual partners. Subsequent testing of family contacts was performed as appropriate.

In all instances the blood samples were centrifuged and separated as soon as possible and the plasma stored at 4°C overnight when necessary. Blood samples for virological examination were separated and the serum stored at -20°C before transfer to the Public Health Laboratory, Withington Hospital, Manchester.

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## LABORATORY METHODS

Liver function tests were performed at the John Radcliffe Hospital, Oxford, in the routine biochemistry laboratory. Aspartate transaminase activity, bilirubin concentration, and alkaline phosphatase activity were measured with a Vickers autoanalyser and alanine transaminase activity with a rate reaction analyser (LKB Ltd).<sup>10-13</sup>

HBAg was determined by reverse passive haemagglutination with Hepatest (Wellcome Laboratories) or by radioimmunoassay using the Blood Products Laboratory test.<sup>13</sup> Anti-HBs, anti-HBc, anti-HAV, and hepatitis A IgM antibody were determined using appropriate modifications of diagnostic radioimmunoassay kits (Abbott Laboratories). Cytomegalovirus was determined by complement fixation tests<sup>14</sup> and Epstein-Barr virus by using a Paul-Bunnell screening test (Monosticon; Organon Laboratories). Doubtful results were clarified by assay of antibody to Epstein-Barr virus viral capsid antigen by immunofluorescence.<sup>16</sup>

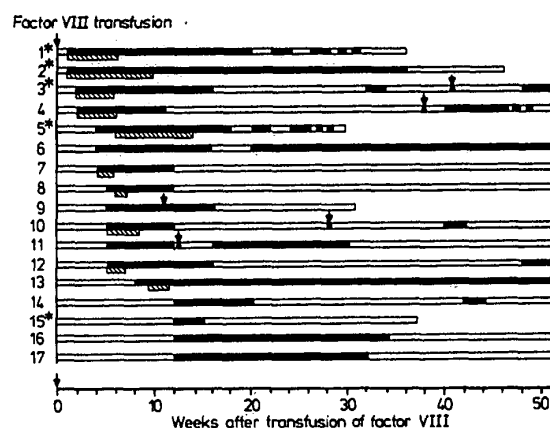
## HEPATITIS

During the study activities of aspartate transaminase and alanine transaminase of over 150 IU (at least four times the upper limit of normal) were taken to indicate hepatitis in those patients in whom pretreatment activity had been within the normal range (<35 IU). Non-A non-B hepatitis was diagnosed when markers indicating recent hepatitis A and hepatitis B infection were absent, tests for infection with cytomegalovirus and Epstein-Barr virus yielded negative results, and there was no clinical evidence of any other cause, including alcohol abuse, for raised serum transaminase activity. The importance of raised aspartate transaminase activity at the start of the study is discussed below.

## Results

The table shows the age and sex of the first 30 patients included in this study, and the diagnosis. Four patients had raised aspartate transaminase activity on entry to the study (cases 27, 28, 29, 30). All four had had aspartate transaminase activities of over 100 IU in the preceding year. All had received factor VIII in the past but not in the preceding six months; they continued to have raised aspartate transaminase activities throughout the study and were assumed to have chronic liver disease. They were therefore excluded from further analysis.

The remaining 26 patients had normal activities of aspartate and alanine transaminases on entry to the study. Of the 26 patients, 19 received only National Health Service (NHS) factor VIII, of whom 12 developed hepatitis (table); four (cases 2, 3, 5, 15) received only a commercial preparation of factor VIII from the United States (one Factorate, two Koate, and one Hemofil), and all four developed hepatitis; and one (case 1) received both Hemofil and NHS factor VIII and developed hepatitis. Two patients (cases 25, 26) received cryoprecipitate; neither developed hepatitis. All except two of the patients who developed hepatitis received treatment from only one batch of either NHS factor VIII or an American commercial factor VIII. Of those receiving treatment from more than one batch, one (case 7) received treatment from two batches of NHS factor VIII and the other (case 1) received treatment from one batch of Hemofil and one of NHS factor VIII.



Pattern of hepatitis in 17 patients who developed raised transaminase activities after transfusions of factor VIII. Each horizontal bar represents results in one patient; the length of the line indicates the duration of follow up. Solid areas indicate transaminase activities raised above 100 IU. Hatched areas indicate jaundice. Arrows indicate transfusions of factor VIII. \*Patients received commercial factor VIII; all other patients received NHS factor VIII.

## Clinical details of patients studied

Case No	Sex	Age (years)	Diagnosis	Type of factor VIII given	Previous transfusions (No of batches)		Previous hepatitis	Serological evidence of past hepatitis B	Transaminase activity (IU)	
					NHS	Commercial			Before transfusion	Highest during study
1	M	61	Haemophilia 4%	NHS, commercial	10	1	+	+	16	736
2	M	35	Haemophilia 2%	Koate	0	0	+	+	39	1569
3	M	71	Haemophilia 5%	Factorate	2	1	+	-	9	352
4	M	38	Haemophilia 10%	NHS	Cryoprecipitate and plasma only	-	-	+	18	1136
5	F	59	Carrier 37%	Hemofil	0	0	-	-	10	635
6	M	45	Haemophilia 13%	NHS	1	0	-	-	21	1028
7	M	66	Haemophilia 5%	NHS	2	1	+	-	10	658
8	F	26	Carrier 31%	NHS	0	0	-	-	13	456
9	M	26	Haemophilia 0%	NHS	13	2	+	+	25	658
10	F	66	Acquired antibodies 0%	NHS	0	0	-	-	11	421
11	M	12	Haemophilia 11%	NHS	0	0	-	-	5	894
12	M	35	Haemophilia 3%	NHS	Cryoprecipitate and plasma only	-	-	-	21	750
13	M	48	Haemophilia 7%	NHS	2	0	-	-	10	478
14	M	41	Haemophilia 2%	NHS	2	0	-	-	50	309
15	M	68	Haemophilia 0%	Koate	13	6	-	+	32	152
16	M	62	Haemophilia 2%	NHS	0	0	+	-	35	360
17	F	60	von Willebrand's disease 14%	NHS	0	0	-	-	6	314
18	M	19	Haemophilia 10%	NHS	10	0	-	+	10	15
19	M	60	Haemophilia 1%	NHS	4	0	+	+	5	10
20	M	68	Haemophilia 6%	NHS	2	1	-	-	32	26
21	M	27	Haemophilia 1%	NHS	7	1	+	-	24	208
22	M	31	Haemophilia 2%	NHS	2	0	+	+	39	52
23	M	62	Haemophilia 2%	NHS	10	1	-	+	35	38
24	M	56	Haemophilia 0%	NHS	7	0	+	+	53	77
25	F	32	Carrier 39%	Cryoprecipitate	0	0	-	-	16	35
26	M	60	von Willebrand's disease 9%	Cryoprecipitate	0	0	-	+	22	23
27	M	29	Haemophilia 6%	NHS	2	1	-	+	70	216
28	M	21	Haemophilia 2%	NHS	4	6	-	-	148	288
29	M	42	Haemophilia 1%	NHS	2	1	-	-	86	256
30	M	25	Haemophilia 3%	NHS	15	1	-	-	119	229

NHS = National Health Service.

Sixteen out of the 17 patients who developed hepatitis had maximum recorded transaminase activities of at least 300 IU, the range being 301-1469 IU (table). The remaining patient (case 5) had transaminase activities of 152 IU in two samples taken four weeks apart. Ten patients developed jaundice, and six of these were acutely ill (figure). Three other patients (cases 6, 11, 17) though they did not develop jaundice, had classical symptoms of acute hepatitis. The symptoms included malaise, lethargy, "flu like" symptoms, nausea, vomiting, anorexia, diarrhoea, arthralgia, itching, and rashes. One other patient did not develop any new symptoms but was polysymptomatic with long standing indigestion and bowel disturbance (maximum recorded alanine transaminase activity 152 IU). Three patients (cases 9, 14, 16) had non-specific symptoms and felt tired and lethargic for between one and three months.

Two patients developed rashes, one (case 17) while she was taking co-trimoxazole prescribed for a urinary infection. At that time and fortnightly for the next six weeks her transaminase activities were normal, but after eight weeks they rose above 300 IU (figure). The second patient (case 8) with a rash was seen when her aspartate transaminase activity was 333 IU; it continued to rise to 459 IU two weeks later. She was not taking any drug treatment.

Ten patients gave a history of hepatitis, of whom six had a further episode of hepatitis during the study; four of these six had an acute symptomatic illness and were deeply jaundiced.

One patient (case 21) had aspartate transaminase activities below 42 IU in all samples obtained up to 12 months after his initial transfusion. The aspartate transaminase activity at 12 months was 208 IU, but as he had received further transfusions with cryoprecipitate he was excluded from the analysis.

#### VIRUS SEROLOGY

At the start of the study nine patients had serological evidence of past hepatitis A infection and 12 serological evidence of past hepatitis B infection (positive for anti-HBs or anti-HBc, or both) (table). No carriers of hepatitis B virus were detected. No cases of cytomegalovirus or Epstein-Barr virus infection were found. Subsequent blood samples tested from those patients who developed hepatitis confirmed that all episodes of hepatitis observed in this study were of the non-A non-B type. Repeated serological testing showed no evidence of virus infection during the study in any patients in whom results of liver function tests remained normal.

#### HISTORY OF TRANSFUSIONS

When the four patients with chronic liver disease, all of whom had had previous transfusions, were excluded, 15 patients had a history of transfusions of factor VIII. Of these, eight developed hepatitis (table). Of the seven patients who had had only NHS factor VIII in the past, three developed hepatitis. Eight patients had had both NHS and commercial factor VIII in the past, of whom five developed hepatitis. We were not able to show any statistically significant correlation between the development of hepatitis during our study and either the number of batches of factor VIII a patient had received in the past or the number of factor VIII units previously transfused. Hepatitis developed in all those nine patients, however, who had not previously received transfusions of factor VIII. Three patients who developed hepatitis had previously received 11, 15, and 19 batches of factor VIII. Many of these transfusions, however, had been performed before 1978, when the pool size of NHS factor VIII used was about 300 donations, in comparison with the more recent pool size of 1413-2504 (mean 1732) plasma donations.

#### INCUBATION PERIOD OF HEPATITIS

The pattern of illness differed according to the duration of the incubation period of hepatitis (figure), patients in whom the incubation period was relatively short having a more acute symptomatic illness. In 12 of the 17 patients who developed hepatitis the illness had an incubation period of under five weeks; in the remaining patients, the incubation period was eight weeks (one patient) and 12 weeks (four).

Five of the patients who developed hepatitis had received commercial factor VIII, and four of these patients had the shortest

incubation periods; three patients had raised alanine and aspartate transaminase activities two weeks after transfusion of factor VIII, and all four had severe jaundice and anorexia and suffered from nausea and malaise. None of the patients whose aspartate transaminase activities were raised at two weeks had been anaesthetised with halothane, and we have no reason to conclude that the liver dysfunction was related to their operation or immediate treatment. Their alanine and aspartate transaminase activities remained raised for at least six months. Eight of the 12 patients who received NHS factor VIII developed hepatitis after incubation periods of three to five weeks, and although their transaminase activities had returned to normal at 16 weeks, seven of these eight patients had high transaminase activities intermittently later in the study. In five patients some of these raised alanine transaminase activities followed further transfusions of factor VIII (figure). One patient (case 15), who developed hepatitis after a 12 week incubation period, had received a transfusion of commercial factor VIII. His transaminase activities remained raised (alanine transaminase activity 152 IU) for four weeks, and during this time had had no new symptoms (see above) (figure). Three other patients who received NHS factor VIII developed hepatitis after a 12 week incubation period; all had some symptoms—namely, malaise, anorexia, nausea, and lethargy. Among the 10 patients who developed jaundice the incubation period was under five weeks in nine and eight weeks in one. No patient in whom the incubation period was 12 weeks developed jaundice (figure).

#### Discussion

This study shows a high incidence of non-A non-B hepatitis in patients treated with factor VIII who had either not received it previously or had received it only infrequently. All of those who received commercial concentrates developed hepatitis regardless of their transfusion history; those who received NHS factor VIII were less likely to develop hepatitis if they had been treated before. All nine patients who received NHS factor VIII for the first time developed hepatitis, while only eight out of the 15 who had received it previously did so. We attempted to relate the risk of hepatitis to the number of batches of factor VIII a patient had received before, and with knowledge of the pool size from which factor VIII was prepared we estimated "donor exposure." No correlation was found with either of these. It may be that the pool size of NHS concentrates has now increased to the point where the benefit conferred by using plasma from volunteer donors has been lost.

The pattern of illness in the patients in our prospective study may indicate that at least two types of hepatitis can be distinguished on the basis of shorter or longer incubation periods. This agrees with previous transmission studies in chimpanzees.<sup>7</sup> A further study of multiple episodes of hepatitis in individual patients with haemophilia is in progress and will be reported separately.

All patients, with one exception (case 15), had symptoms when their serum transaminase activities were raised, which is unlike the experience reported elsewhere.<sup>14</sup> As only 10 of our patients developed jaundice it is possible that the hepatitis would not have been diagnosed in the seven others if serum transaminase activities had not been assayed frequently as part of this study.

Eight patients still had raised transaminase activities six months after receiving the dose of factor VIII. Of the patients who had hepatitis, 12 were followed up for a year and four of these had evidence of continuing liver disease. Eight of the 17 patients who developed hepatitis showed intermittent abnormalities with fluctuating transaminase activities (figure). In three patients intermittent abnormalities persisted over 12 months without any further factor VIII transfusions. The remaining patients had not been followed up for a year, but regular review was continued to see whether they would show evidence of chronic liver disease.

Because of the high incidence of hepatitis alternative ways of raising and maintaining factor VIII activity, such as administration of desmopressin acetate, are used, but we have not described them here.

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# Perthes' disease of the hip in Liverpool

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## Abstract

A survey of Perthes' disease of the hip in three regions of England showed a higher incidence in the Mersey region compared with Trent or Wessex. To explore this further a case register was set up in Liverpool. Analysis of all new cases that occurred in Liverpool and adjacent parts of Knowsley and Sefton during 1976-81 showed a steep gradient with social class, ranging from 7.7/100 000 children in the higher classes to 2.6/100 000 in social class V. The inner city of Liverpool, which has been shown to be underprivileged, had the highest yearly incidence of the disease ever reported—21.1 cases/100 000 children aged 14 years and under.

The associations with poverty support the hypothesis that undernutrition is a causative factor in the disease.

## Introduction

The aetiology of Perthes' disease of the hip is unknown. A survey of its incidence in three regions of England during 1976 showed a yearly rate of 11.1 cases/100 000 children aged under 15 in Mersey compared with 7.6 in Trent and 5.5 in Wessex.<sup>1</sup> The incidences were higher within the conurbations of Merseyside and South Yorkshire than elsewhere in the Mersey and Trent regions. In order to explore further the apparent high incidence in Merseyside a register was set up in Liverpool. This paper describes an analysis of all new cases registered during 1976-81.

## Methods

The cases comprised inpatients and outpatients attending the Royal Liverpool Children's Hospital and the Alder Hey Hospital from 1 January 1976 to 31 December 1981. This analysis is confined to children resident in Liverpool City or in the adjacent areas of Knowsley and Sefton Districts, which at the outset of the survey were deemed to be within the exclusive catchment area of the two hospitals. Radiological criteria for the diagnosis of the disease were established at the inception of the register with the help of an independent observer. To ensure completeness of the register, which depended primarily on collaboration with orthopaedic surgeons, diagnostic registers in the medical records departments of the two hospitals, operating lists, and Hospital Activity Analysis data for the Mersey region were searched.

In 1982 a questionnaire was sent to the mothers of the children. The information requested included place of residence at the birth of the child and occupation of the father. When a mother failed to respond, usually because of change of address, she was visited at home.

Incidence rates for each of the 57 electoral wards were calculated using population data from the small area statistics of the 1981 Census of England and Wales. These statistics also provided data on

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