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CLINICAL PRACTICE

Incidence of development of factor VIII and factor IX inhibitors in haemophiliacs

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The development of factor VIII:C inhibitors remains one of the most serious complications of repeated transfusion in patients with haemophilia A. The proportion of patients affected has been reported to range from 3·6% to 25%, but these figures have been derived mainly from retrospective data and from total numbers of known haemophiliacs instead of number at true risk. The assessment here is based on a prospective study, started in 1976, on the incidence of inhibitor development in haemophiliacs born after 1970 whose FVIII or FIX activity was 5% or less, and who had received replacement therapy at least once.

46 of 63 children with haemophilia A and 13 of 17 with haemophilia B fulfilled the enrolment criteria. Inhibitors developed only in haemophilia A patients who had previously been treated with FVIII products—inhibitor concentrations were high in 12 and low in 3. Inhibitors developed in 24% (15/63) of all haemophilia A patients, and in 52% (14/27) of those with severe disease. The incidence of inhibitor development for all haemophilia patients was 39·1 per 1000 patient-years of observation. All inhibitors were first detected when patients were aged 0·08–5·2 years. The cumulative risk was 33% at age 6 years.

The findings indicate that previous reports have underestimated the risk of acquiring FVIII inhibitors. Prospective, standardised studies, especially in children, are needed for the assessment of the true risk of this complication.

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Introduction

Despite improvements in the efficacy of treatment for bleeding, the development of factor VIII:C (factor VIII coagulant) inhibitors remains one of the most serious complications of repeated transfusion in patients with haemophilia A. It is not clear why the complication develops in only some haemophiliacs. The proportion in whom this complication occurs has been reported to range from 3·6% to 25%.[1-8] The frequency seems to be rising. The possibility that this rise is associated with amount of factor VIII infused and antigenicity of clotting factors given has been discussed.[7,9-11] In individual patients a direct relation between the formation of inhibitors and type of FVIII product used or the frequency of clotting factor substitution has not been proven. Inhibitors may develop after only 10 to 20 days' exposure to FVIII[5,8] or, occasionally, after 100 days or longer.[5,12,13] Although the development of FVIII:C inhibitors in haemophiliacs is a result of replacement therapy, exposure to FVIII concentrates was not a criterion for eligibility in most reported studies. Severity of deficiency should also be taken into account in assessment of role of inhibitor development—eg, inhibitors to FIX develop in only about 3% of all patients with haemophilia B, or 7–10% of those with severe disease.[1,2,14,15]

The aims of this study were to calculate the age-dependent risk and incidence of development of inhibitor in treated haemophiliacs; to find out whether there is a direct relation between type of purified FVIII product used and of inhibitor formation; and to define criteria of eligibility for patients for further inhibitor studies.

Patients and methods

Patients

Recruitment for a prospective study on inhibitor development in patients with severe or moderate haemophilia began in Jan, 1976. By Aug, 1991, 63 children with haemophilia A and 17 children with haemophilia B had been managed in our haemophilia outpatient clinic. For calculation of incidence and cumulative risk of acquiring an inhibitor in relation to age and FVIII or FIX exposure for patients at true risk, inclusion in the study was limited to those who were born after 1970, whose FVIII or FIX activity was 5% or less, and who had been exposed to FVIII or FIX concentrates at least once. 46 patients with haemophilia A (age range 1·1 to 20·1 years, median 8·5) satisfied the criteria for inclusion. 27 had severe haemophilia A (FVIII:C < 1%) and 19 moderate disease (FVIII:C activity 1–5%). 13 haemophilia B patients (age range 0·75 to 15·75 years, median 6·9) also fulfilled the inclusion criteria. 7 had severe haemophilia B (FIX < 1%) and 6 had moderate disease (FIX 1–5%).

Haemophilia A patients were treated with FVIII products from various manufacturers and of various purity—namely, cryoprecipitate, and commercial FVIII concentrates of intermediate and high purity, including monoclonal and recombinant FVIII products (table I).

Haemophilia B patients were treated with prothrombin complex concentrate (PCC) and commercial FIX concentrates of intermediate and high purity.

Coagulation studies

Factor VIII:C activity was assayed by a one-stage assay, which used FVIII-deficient plasma as substrate.[16] FIX activity was measured by one-stage assay in the same way.

FVIII and FIX inhibitors were determined by the Bethesda assay, where one unit of inhibitor (Bethesda unit) is defined as the activity in 1 ml test plasma which, when mixed with an equal volume of normal plasma for 2 h at 37°C, reduces the FVIII or FIX concentration by half.[17]

TABLE I—FACTOR VIII PRODUCTS GIVEN BEFORE INHIBITOR DEVELOPMENT AND TOTAL NUMBER OF EXPOSURE DAYS IN PATIENTS STILL AT RISK

Type of FVIII product*	Total no of patients treated	No of inhibitor patients	Non-inhibitor patients	
			No	Total no of exposure days
Crude and intermediate concentrates	7†	1	6	2691
Exclusively intermediate concentrates	35	13	22	10 746
Exclusively monoclonal purified concentrate	4‡	1	3	345

*Specific activity (U/mg) crude 0·1–0·9, intermediate purity 1–10, monoclonal (high-purity) > 10
†Given crude from 1970–1980, then switched to intermediate products 1980–83 and remained on intermediate products thereafter
‡2 received recombinant monoclonal products

All patients were tested for inhibitors every 20th exposure day (±2) and whenever the clinical or laboratory response to treatment was poorer than expected. An exposure day is a day on which at least one dose of FVIII or FIX was given.

Statistical analysis

The method of Kaplan and Meier was used to calculate the age-dependent cumulative risk of developing an inhibitor to FVIII or FIX. The cumulative risk of inhibitor development from time since first FVIII exposure was calculated in the same way. Subjects with inhibitor activities below 0·8 Bethesda units/ml, detected only once or twice were classed as "super-low" responders and excluded from the inhibitor group.

Results

Incidence and prevalence of inhibitor formation

During the observation period (0·08–15·25 years, median 8) FIX antibody formation was not observed in any of the 13 haemophilia B patients. By contrast, FVIII inhibitors developed in 15 (33%) of the 46 haemophilia A patients (14/27 with severe haemophilia, 1/19 with moderate haemophilia) and the incidence of inhibitor formation was 15 per 383 patient-years of observation (39·1 per 1000 patient-years of observation) for the haemophilia A patients included in the study. A factor VIII inhibitor developed in

TABLE II—SELECTED CHARACTERISTICS OF INHIBITOR PATIENTS

Patient number*/ age (yr) at Aug, 91	Age (yr) at discovery of inhibitor	Inhibitor response†	Inhibitor-titre (Bethesda units)			Exposure days
			Initial	Peak	Present	
1/5·90	0·60	HR	0·7	6·5	0	6
2/4·40	1·60	HR	2·3	12·0	0	16
3/12·1	5·16	LR	0·6	1·0	0	5
4/11·90	1·50	LR	3·7	4·3	0	7
5/9·40	4·25	HR	3	153	0	8
6/11·60	2·00	LR	0·2	2·4	0	11
7/13·70	5·00	HR	10	335	0	113
8/7·20	0·80	HR	420	530	0	14
9/2·00	0·16	HR	25	240	0	18
10/12·40	2·50	HR	11·8	11·8	0	8
11/16·80	4·60	HR	1070	1070	0	195
12/2·25	1·25	HR	12	1068	245	34
13/4·60	1·25	HR	232	1570	73	4
14/1·20	1·00	HR	20	83	83	10
15/2·16	0·08	HR	2	40	40	12

*All severe haemophiliacs (FVIII:C < 1%) except patient 3, who was moderate haemophiliac (FVIII C 1–5%).
†HR—high responder (FVIII inhibitor titre > 5 Bethesda units/ml), LR—low responder (FVIII titre < 5 Bethesda units/ml)

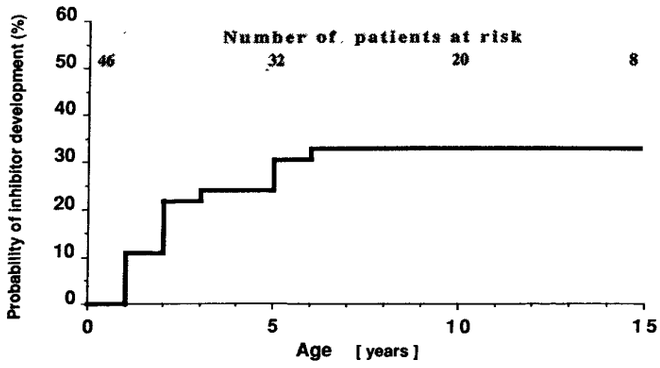

Fig 1—Age-dependent cumulative risk of FVIII inhibitor development in patients with severe and moderate haemophilia.

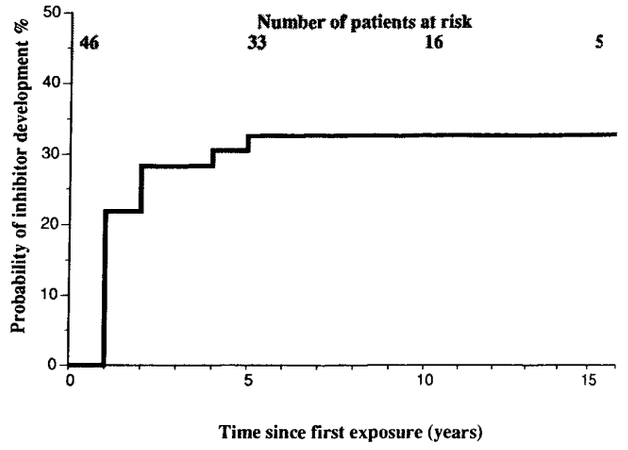

Fig 2—Cumulative risk of FVIII inhibitor development in patients with severe and moderate haemophilia from time of first exposure.

52% of patients with severe haemophilia. If patients with mild haemophilia (FVIII < 50%) were also taken into account, the proportion affected was 24% (15/63).

In all patients described above, inhibitor activity was detected repeatedly. 12 (80%) of the 15 patients with inhibitors had high titres and a typical anamnestic response each time FVIII was given; they were termed high responders. The highest concentration of inhibitor measured was 1570 Bethesda units/ml. In 3 (20%) patients, concentrations did not exceed 5 Bethesda units/ml despite subsequent transfusions with FVIII; they were therefore classified as low responders.

Inhibitors were first detected after 4 to 195 cumulative days of exposure to FVIII (table II). If the two longest intervals (113 and 195 days) are excluded, the mean number of exposure days was 11·7.

The age of patient at first detection of inhibitor activity ranged from 0·08 to 5·2 years (median 2·0). Inhibitors developed by age 1 in 33% of the patients, by the age of 2·6 in 73%, and by the age of 5·2 in all. The risk of acquiring an inhibitor was thus highest in the first 2·6 years of life (11/15). The age-dependent risk of inhibitor development was 11% at the age of 1 year and 33% at the age of 6 years (fig 1). In no patient did an inhibitor first develop after age 6. The cumulative risk of an inhibitor developing was 22% at 1 year after first treatment with FVIII substitution therapy and 33% at 5 years after first exposure (fig 2).

The 15 patients with inhibitors had received FVIII products of various purity and from different manufacturers (table I). There was no evidence that one product was more likely than others to induce FVIII inhibitor formation. The total number of FVIII exposure days for all patients still at risk of development of an inhibitor is given in table I. No significant differences were detected between patients with high titres of inhibitors and those with low titres in age at which inhibitor activity was first detected, in number of exposure days, or in amount or source of FVIII given before detection of inhibitor.

There were six brother-pairs; in two, both the boys had inhibitors against FVIII; in another pair only 1 had inhibitors; and in the other three pairs, neither of the boys was affected.

Changes in inhibitor concentrations

Since 1979 we have tried to eliminate the inhibitor by inducing immunotolerance with the following treatment schedules:[18]

(a) High responders were given FVIII twice daily at a dose of 50–300 U/kg per day and activated PCC (100–200 U/kg per day). After elimination of the inhibitor, doses of FVIII and activated PCC were gradually reduced, and the latter was discontinued when FVIII recovery returned to normal.

(b) Low responders received 30–60 U/kg FVIII concentrate every second or third day.

Elimination of inhibitors and induction of immunotolerance was successful in 11/15 patients; 3 of the 15 are still under treatment and 1 has not yet been treated. The 11 who have been successfully treated have received FVIII concentrate prophylactically (two to three times a week) without reappearance of inhibitor (follow-up period after elimination up to 10 years). The current prevalence of high responders in the study group is thus 9% (4/46).

None of our patients with inhibitors had a human immunodeficiency virus infection, changes in inhibitor concentrations were not biased by this infection.

There have been no deaths due to bleeding in any of the patients since 1976. The only death was that due to the acquired immunodeficiency syndrome in a patient without inhibitors.

Discussion

There are several reasons for the conflicting data that have been published on the frequency of development of FVIII inhibitor. First, some estimates have been based on prevalence, whereas others have been based on incidence, of inhibitor development. Prevalence indicates only the proportion of patients with an inhibitor at a given time, so if mortality among patients with inhibitors is higher than that among those without,[7,19-22] rates based on prevalence data will be underestimates of the true risk of acquiring an inhibitor. Among haemophiliacs with inhibitors, incidence correlates well with prevalence among those who are young, but as those affected die or drop out of a study for other reasons, prevalence falls, whereas the incidence remains constant. Thus only calculations based on incidence can give true information on the frequency of development of inhibitors of haemophilia A.

Whether a study is prospective or retrospective is also important. Variables such as patient's age at first development of inhibitor and patient's FVIII:C activity and amount of FVIII infused before inhibitor development are difficult to assess retrospectively. In particular, retrospective data might indicate age at which the inhibitor produced a clinical effect, whereas low inhibitor concentrations might have been present for several years. The reliance of previous estimates on retrospective data could be one explanation for the low risk that has been reported.

Severity of the haemophilia in a study group can also affect estimates. More than 97% of patients who acquire inhibitors are those whose FVIII:C activity before inhibitor development was less than 3% (<0·03 U/ml).[23] Few patients with mild haemophilia A have been reported to have acquired inhibitors.[24] Nevertheless the total number of all haemophiliacs known has been taken as the denominator in most papers. Some papers have given frequencies for patients with severe haemophilia A only. For example, Stenbjerg et al[25] reported a prevalence of inhibitor development of 9·8% among their total number of haemophiliacs and 20·6% among their severe haemophiliacs; and Schwarzinger[7] observed a prevalence of 8·6% among all haemophiliacs, and 17·5% among severe and moderate haemophiliacs. We found new inhibitor formation in 24% of all haemophiliacs, 33% of those with FVIII levels 5% or less, and 52% of severe haemophiliacs.

With few exceptions, patients with classic haemophilia do not develop inhibitors unless they have received FVIII replacement therapy. In 75% of patients with high titres of inhibitors these antagonists have developed before 50 days of exposure to FVIII, and all patients with inhibitors have been identified before 250 days of exposure.[26] Strauss[8] reported a median number of 35 exposure days before the inhibitor development in his paediatric study group, Schwarzinger[7] reported a median of 25 days in patients aged up to 25 years, and we found a mean number of 11·7 days (excluding the two longest intervals of 113 and 195 days).

Another factor that influences reported frequency is age of study group. Inclusion of all age groups, as has occurred in most reported studies, leads to underestimates. In well over 50% of cases, inhibitor development starts before the age of 10 years, in another 20% it starts at ages 10–20 years, and in the remainder it starts in adult life.[5,7,8,14,27] In young age-groups (up to 25 years) the reported prevalence rates have been 21%[8] and 18%.[7] The age-related cumulative risk of acquiring an inhibitor has been as high as 24% by age 25 years[7] or 22% by age 10.[27] Our finding of a risk of 33% by the age of 6 years is higher than that previously reported. According to inhibitor surveys among children,[6-8] very rarely inhibitors develop after the age of 11, and the greatest risk of inhibitor formation was before the age of 5, as confirmed by our findings. Published reports have put incidence of inhibitor formation at 8 per 1000 patient-years[26] or 10·3 per 1000 patient-years,[27] whereas we found it to be 39·1 per 1000 patient-years. Our high figure may be accounted for by our inclusion only of patients with severe or moderate haemophilia, who had been treated at least once, who were within the age-group at greatest risk of development of an inhibitor, who were followed up in a major haemophilia centre, and who were tested frequently for presence of inhibitor.

There were 8 super-low responders; their inhibitor activity was identified only once or twice, during a follow-up visit, but was subsequently undetectable despite repeated treatment with FVIII. Maximum inhibitor concentrations in these patients were between 0·3 and 0·8 Bethesda units/ml, and the inhibitors were detected before the patients reached age 15. Highly transient presence of antibodies to FVIII:C has been previously noted only by McMillan,[26] who reported that new inhibitor formation in 31 of 1306 patients and that in 7 of the 31 the antibodies were detected on only one occasion (1·0–4·3 Bethesda units/ml). The importance of such inhibitors is difficult to assess, so patients with these antibodies ought to be followed-up carefully to determine whether persistent inhibitors develop later. It is likely that such patients have been overlooked in retrospective analyses.

Optimism about the value of the new highly purified FVIII concentrates has been tempered by the observation that their use may be associated with a high incidence of FVIII antibodies. The highest frequency reported was 29%[6] and occurred among children who had received a recombinant FVIII product (the inhibitor developed in 6 of 21 children, for 20 of whom the infusion of recombinant factor VIII was their first exposure to FVIII and for the other, the second). The high frequency is not surprising since that study was prospective and was restricted to patients with severe disease who had been exposed to factor VIII and who were mostly aged under 5 years; moreover, the patients were closely monitored for development of inhibitors. By contrast, new inhibitors developed in 1 of 86 much older patients who had previously been treated with plasma-derived FVIII concentrates.[6]

Although there are now methods of helping to reduce inhibitor concentrations, the development of FVIII inhibitors remains a serious complication of the treatment of haemophilia A. Most reports are underestimates of the true risk of FVIII inhibitor development in haemophilia patients and do not emphasise the point that this complication occurs mainly in infants and childhood. On the basis of data from the few prospective studies so far reported, we agree with McMillan[26] and maintain that the relative risk of inhibitor formation is about four times higher for patients under age 5 than for older patients. What effect the new generation of FVIII:C products will have on the development of inhibitors remains unclear. Further studies on inhibitors should be conducted prospectively and according to standardised criteria.

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Hypocholesterolaemic effects of lovastatin in familial defective apolipoprotein B-100

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Familial defective apolipoprotein B-100 (FDB) is an autosomal dominant disorder associated with hypercholesterolaemia in which an aminoacid substitution in apoprotein B-100 leads to low-density lipoprotein (LDL) particles which have defective binding to the LDL receptor. All known patients are heterozygous, and their plasma contains normal and poorly binding LDL particles.

12 hypercholesterolaemic patients from 10 unrelated families with FDB were treated with lovastatin. In 6 patients treated with 20 mg lovastatin daily, LDL cholesterol decreased by 21·5% from 6·23 to 4·89 mmol/l (95% confidence interval 0·74, 1·96 mmol/l), whereas it fell by 32·1%, from 6·99 to 4·81 mmol/l (95% CI 1·55, 2·70 mmol/l), in 9 patients who received 40 mg daily.

These results indicate that the hypercholesterolaemia of FDB may respond to treatment with statins.

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Introduction

Familial defective apolipoprotein B-100 (FDB) is a disorder of lipoprotein metabolism in which substitution of the aminoacid glutamine for arginine at position 3500 in the apolipoprotein B molecule leads to a low-density lipoprotein (LDL) particle which binds poorly to high-affinity LDL receptors on hepatocyte and other cell membranes.[1] All reported patients are heterozygous for this mutation, and their plasma contains two populations of LDL particles—one with normal and the other with defective binding to LDL receptors. Although early reports indicated that this mutation was associated with moderate hypercholesterolaemia,[1,2] screening for FDB in patients with primary hypercholesterolaemia who attend lipid clinics has shown that FDB may cause clinical and biochemical features similar to those seen in patients with heterozygous familial hypercholesterolaemia, and that it is associated with premature atherosclerosis.[3,4]

The prevalence of FDB in the UK, Germany, and the USA appears to be similar to that of heterozygous familial hypercholesterolaemia, with an estimated frequency of 1 in 500–700. Thus FDB represents a relatively common cause of hypercholesterolaemia for which the increase in circulating LDL warrants drug therapy. In one report, 2 members of a family with FDB were found to be poorly responsive to simvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitor.[5] To assess whether or not this response is representative of patients with FDB, we examined the hypolipidaemic effects of lovastatin (the parent drug of simvastatin and until recently the only one of its class available for prescription use in the USA) in 12 patients with FDB from 10 unrelated families identified in Oregon.

Patients and methods

12 patients (8 women and 4 men aged 33–66 years [mean 50]) with FDB from 10 unrelated families have been identified from genomic DNA obtained from patients with primary type II hypercholesterolaemia who attend the Lipid Disorders Clinic at Oregon Health Sciences University. Genomic DNA was prepared from whole blood cells; after lysis, a segment of the apoB gene (nucleotides 10 823 to 10 799) was amplified by the polymerase chain reaction, essentially following the method of Hansen et al.[6] The 140 bp fragment spanned the 3500 mutation site and FDB subjects were identified by digestion of the fragment with *MspI*.[6]

10 patients had tendon xanthomas and 4 had clinical evidence of coronary artery disease, all had normal renal, hepatic, and thyroid function, and none had diabetes mellitus. All were below 120% ideal body weight and all had had dietary counselling from a lipid clinic dietitian. Blood samples were obtained under fasting conditions for measurement of plasma lipid and lipoprotein concentrations before the start of drug therapy and when lipid measurements had stabilised after at least 6 weeks' treatment with 20 or 40 mg lovastatin daily. 6 patients were initially treated with 20 mg lovastatin daily, in 3 of whom the dose was later doubled so that 9 patients received 40 mg lovastatin daily. Blood samples for lipid

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