

Incidence of Inhibitor Development in a Group of Young Hemophilia A Patients Treated Exclusively With Lyophilized Cryoprecipitate

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The incidence of neutralizing isoantibody formation to infused factor VIII in a cohort of 67 hemophilia A patients, born between January 1, 1971 and April 30, 1990, who had been treated exclusively with lyophilized cryoprecipitate, was 6% (5.3 per 1,000 patient years of observation). The age-dependent cumulative risk was 4.6% at 4 years of age and 6.7% at 8 years of age. Recent reports in patients treated with a variety of more pure concentrates show a

much higher incidence of inhibitor formation and tend to be used as a reference when new concentrates are introduced. We believe that a patient group, such as the one studied here, is a more suitable reference population because these patients have been exclusively treated with a single factor VIII preparation.

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THE DEVELOPMENT of neutralizing antibodies (inhibitors) to infused factor VIII (FVIII) remains a major problem in the treatment of patients with hemophilia A. Previously untreated patients (PUPS) treated with new monoclonal antibody (MoAb) purified FVIII concentrates or recombinant FVIII concentrates seem to have a higher incidence of isoantibody formation than patients treated with less pure products.^{1,2} Published data on cumulative incidence of inhibitors in patients with hemophilia A are very divergent, ranging from 3.6% to 52%.³⁻¹⁰ The highest incidences are reported in the more recent publications. Ehrenforth et al⁸ describe inhibitor development in 24% (15 of 63) of all hemophilia A patients and even in 52% (14 of 27) of those with severe hemophilia, whereas Ljung et al⁹ find 16% (16 of 101) and 21% (16 of 77), respectively. Incidences are not always calculated in the same way; some investigators use all known hemophilia patients as the denominator for their calculations, whereas others restrict the denominator to all patients receiving transfusions or to patients with severe hemophilia A. The incidence rates are also influenced by frequency of testing because a 3-month inhibitor assay will detect some of the very transient inhibitors that would be overlooked by yearly or less frequent testing. Furthermore, most studies are not suited to evaluate the propensity of individual concentrates to induce antibodies because the majority of patients have used a mixture of FVIII concentrates. In Belgium, patients with hemophilia A have been treated exclusively with lyophilized cryoprecipitate from local donors from 1971 till April 30, 1990, when more pure concentrates were introduced. Most patients attending the Leuven hemophilia center have been screened yearly for inhibitor formation. They thus provide a suitable control population for the incidence of inhibitor formation

in patients treated with a crude FVIII preparation. We analyzed a cohort of 67 patients born after January 1, 1971 and before April 30, 1990, and calculated the age-dependent cumulative incidence of inhibitor development in these previously untreated patients with hemophilia A.

MATERIALS AND METHODS

Patients. All patients with hemophilia A born between January 1, 1971 and April 30, 1990 and attending the Leuven hemophilia center were included in the analysis. During this time period, patients have been treated exclusively with a lyophilized cryoprecipitate¹¹ distributed by the Belgian Red Cross, which was dry-heat treated from 1986 onwards. This cryoprecipitate was manufactured exclusively from pooled plasma of unpaid, volunteer Belgian donors. Most patients attending our hemophilia center were supplied by the Regional Blood Transfusion Center of Leuven, which collected plasma through a plasmapheresis program involving a limited number ($\pm 6,000$) of local donors. Patients were invited yearly to a multidisciplinary hemophilia clinic and at each visit a FVIII inhibitor determination was performed. Additional FVIII inhibitor assays were performed whenever the therapeutic response to transfusions appeared inadequate. Most patients were enrolled in home treatment programs; transfusions were administered either at the first sign of a hemorrhage or prophylactically three times a week. All patients were Caucasian.

Coagulation studies. FVIII was measured using a two-stage assay¹² and, more recently, a one-stage assay¹³ adapted to an automated coagulometer ACL-810 (IL, Milan, Italy) using severe hemophilia A plasma and a micronized silica aPTT reagent (IL). FVIII inhibitor levels were measured according to the Bethesda method¹⁴ from 1975 onwards. Patients were classified as inhibitor patient if the inhibitor level exceeded 1 Bethesda Unit (BU)/mL on two separate occasions. Before 1975, FVIII inhibitor was measured according to Biggs and Bidwell.¹⁵

Statistical analysis. The age-dependent cumulative risk of developing an anti-FVIII inhibitor was calculated by a Kaplan-Meier life table.¹⁶ Incidences were calculated as the ratio of the number of inhibitors over the patient-years of follow-up. Confidence intervals (CI95) for the cumulative risk were calculated under the assumption of a binomial distribution and for the incidence rates a Poisson distribution was assumed.

RESULTS

Seventy-two patients with hemophilia A born between January 1, 1971 and April 4, 1990 attended our hemophilia center. Forty-eight patients had severe hemophilia (FVIII:C, <1%), 10 had moderate hemophilia (FVIII:C between 1% and 5%), and 14 had mild hemophilia (FVIII:C

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between 5% and 40%). Five of these 72 patients (4 with mild and 1 with moderate hemophilia) never received transfusions and were excluded from further analysis. The mean FVIII consumption in the group with severe hemophilia was approximately 1,500 IU/kg body weight per year (range, 50 to 3,900 IU/kg/yr) and, on average, an inhibitor assay was determined every 2.7 ± 1.7 years (mean \pm SD) in this group. In the group with moderate and mild hemophilia, the median number of exposure days to cryoprecipitate was 6 (range, 2 to 200 days) and 1 patient had only a single exposure. Six patients seroconverted for human immunodeficiency virus (HIV). Two patients died during the observation period; one death was acquired immunodeficiency syndrome (AIDS)-related, the second was not related to hemophilia. Neither patient had an inhibitor to FVIII.

An FVIII inhibitor developed during the observation period in 4 of the 67 patients at risk (6%) and the incidence of inhibitor formation was 4 per 715 patient-years of observation (5.6 per 1,000 patient-years of observation; CI95, 1.52% to 14.3%). In the group with severe hemophilia, the incidence was 3 per 490 patient-years of observation (6.12 per 1,000 patient-years; CI95, 1.26% to 17.9%). Three of the inhibitors never exceeded 10 BU/mL despite repeated FVIII transfusions and were classified as low responders; the fourth peaked at 500 BU/mL and was classified as a high responder (Table 1). The three low responders were patients with severe hemophilia A; the high responder was a patient with mild hemophilia. Three of the four inhibitors developed within 5 exposure days to transfused FVIII (an exposure day being a day on which at least one FVIII transfusion was administered) and the age at which the inhibitor was first detected ranged from 1.7 to 7.6 years. The age-dependent cumulative incidence of developing an inhibitor was 4.6% (CI95, 1.5% to 13.7%) at age 4 and 6.7% (CI95, 2.5% to 17.1%) at age 8 (Fig 1). Restricting the analysis to the group with severe hemophilia, the age-dependent cumulative incidence was 4.3% (CI95, 1.1% to 16.0%) at age 4 and 7.2% (CI95, 2.3% to 20.9%) at age 8. None of the inhibitor patients had a family history of inhibitor formation or of hemophilia.

The prevalence of inhibitors in our total population of hemophilia A patients was 4.7% on April 30, 1990 (11 of 234). In the subgroup of patients with severe hemophilia A, the prevalence was 8% (10 of 126). These figures include as well patients with a current inhibitor as patients with a history of inhibitor.

DISCUSSION

The introduction of very pure FVIII concentrates (either MoAb-purified or recombinant) has raised a spirited debate concerning the "true incidence of inhibitors" in PUPS.^{1,2,17} Most available data, with the exception of data on MoAb-purified and recombinant concentrates, have been collected in less formally designed trials and proportions of patients affected range from 3.6% to 24%³⁻¹⁰ when all known hemophilia patients are considered, or from 15% to 52% of patients with severe hemophilia.^{5,8,9} The age-dependent cumulative risk of developing an inhibitor ranges from 20% (at 5 years of age) to 33% (at 6 years of age).^{4,5,8,10}

Most investigators exclude patients with mild disease from their analysis; however, in our patient group, the only patient who developed an antibody of the high responder type had mild disease; therefore, it seems more appropriate to include all patients who have been treated with FVIII in incidence studies. The risk of inhibitor development in our cohort of PUPS was low, ie, 4 of 67 patients (6%) or 5.6 per 1,000 patient-years of observation when all patients at risk are considered (3 of 48 [6.25%] in the group of patients with severe hemophilia A). The age-dependent cumulative risk was 6.7% at 8 years of age.

Some investigators report similar low incidences. McMillan et al⁶ found 8 per 1,000 patient-years of observation. In the Dutch inhibitor study, 3.9 per 1,000 patient-years was found over the period 1984 through 1989 and 4.4 per 1,000 patient-years over the period 1988 through 1990.¹⁸ However, these studies involved patients of all age groups and thus underscore the incidence in young children who are at the highest risk. If we restrict the analysis in our cohort to 0 to 10 years of age, an incidence of 7.5 per 1,000 patient-years of observation (4 per 531 patient-years) is found. Recalculating from McMillan et al⁶ the incidence in children who were less than 5 years of age at entry into the study (realizing that these were not necessarily PUPS), we found an incidence of 9 of 160 (5.6%) or 18.75 per 1,000 patient-years of observation. In the Dutch inhibitor study¹⁸ over the period 1988 through 1990, three inhibitors were found among 75 patients 0 to 10 years old (142 patient-years of follow-up), which yielded an incidence of 21 per 1,000 patient-years. The cumulative incidence at age 6 (as calculated by age-stratified life table analysis¹⁹) was 17.5% (2 of 15 in the age group of 1 to 2 years, and 1 of 21 in the age group of 5 to 6 years).

The risk of inhibitor development in PUPS is not con-

Table 1. Selected Features of Hemophilia A Patients From a Cohort Born Between January 1, 1971 and April 30, 1990 Who Developed an Inhibitor

Year of Birth	Severity of Hemophilia	Age at Inhibitor Detection (yr)	Inhibitor Titer (BU/mL)			Exposure Days* to FVIII Before Inhibitor Detection
			Initial	Peak	April 30, 1990	
1973	Mild (8%-11%)	3.3	6.5	500	32	4
1975	Severe (<1%)	2.4	2.4	2.4	0	5
1981	Severe (<1%)	7.6	0.3	3	0	<100
1986	Severe (<1%)	1.7	5	5.5	0	4

* An exposure day is a day on which at least one FVIII transfusion was administered.

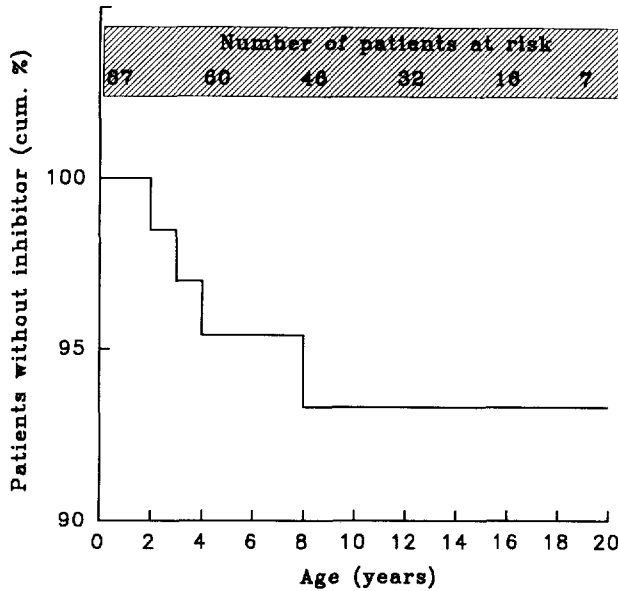


Fig 1. Cumulative survival without inhibitor in 67 patients with hemophilia A. The age-dependent cumulative incidence of inhibitor development is 4.6% at age 4 and 6.7% at age 8.

stant over age, because it steeply decreases to a very low risk after the first few transfusions. This finding implies that the incidence rate, calculated as the number of events over the patient-years of follow-up (which in fact is an average approximation of the instantaneous incidence rate), is highly dependent on the duration of follow-up. If a group of PUPS is followed-up from birth until age 12, the incidence rate will be half as high as when they were followed-up until age 6, because virtually all inhibitors will have developed by this age. Therefore, we are convinced that in PUP studies cumulative incidence should be reported instead of incidence rates. In the example, with all inhibitors occurring before age 6, this cumulative incidence will be the same at age 6 and at age 12, which will render comparisons between studies more meaningful.

In non-PUPS, the incidence seems to be low, but fairly constant over age. Therefore, in studies on these patients, the incidence rate should be preferred, because it is independent of the duration of follow-up, whereas the cumulative incidence is not.

Particularly in the more recent reports, the incidence of FVIII inhibitors is higher than in our study.^{5,8-10} Our data are more in agreement with older data,³ describing patients mainly treated with plasma or cryoprecipitate. Part of the increase in incidence has been ascribed to an increased proportion of treated patients,²⁰ whereas the recent observation of a higher than expected incidence of inhibitors related to the use of one particular FVIII concentrate^{18,21} has shown that differences in the production process (related to the method of purification or viral inactivation) can lead to increased immunogenicity. The low incidence described in our report cannot be due to a low consumption of FVIII because all described patients were enrolled in home treat-

ment and/or prophylaxis programs and patients who never received a transfusion were excluded from analysis. We feel that the most important difference between our patients and those reported in other studies is that our patients have been treated with only one FVIII preparation. It is not inconceivable that switching patients from one product to another entails a supplementary risk for inhibitor formation. In addition, the risk may also increase with higher product purity.

Because we measured inhibitors on a less than yearly basis, our study may have overlooked very low titer, very transient inhibitors, which are reported in the clinical studies concerning monoclonally purified and recombinant concentrates. The clinical relevance of these transient antibodies and the convenience to include them in incidence studies are not clear at this moment.

The very low incidence and prevalence of inhibitors in our population could also be partly influenced by the relatively limited number of donors to which our patients were exposed. Most patients attending our hemophilia center were provided with cryoprecipitate originating from plasma collected by the regional Blood Transfusion Center of Leuven through a plasmapheresis program including only a limited number ($\pm 6,000$) of local donors.

Evidence is currently accumulating that the incidence of inhibitor formation is in part product related and is connected with the complex process of purification and viral inactivation. Each newly introduced FVIII concentrate has to be evaluated separately on its propensity to induce neutralizing antibodies in comparison with a reliable reference population. We believe that a patient group, such as the one studied here, may be a suitable reference population because these patients have been exclusively treated with a single FVIII preparation. The information provided in this study could be used as background for further studies to design safe, virus-free products associated with a low incidence of inhibitors.

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