Treatment-related risk factors of inhibitor development in previously untreated patients with hemophilia A: the CANAL cohort study

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The CANAL Study (Concerted Action on Neutralizing Antibodies in severe hemophilia A) was designed to describe the relationship between treatment characteristics and inhibitor development in previously untreated patients with severe hemophilia A. This multicenter retrospective cohort study investigated 366 consecutive patients born between 1990 and 2000. The outcome was clinically relevant inhibitor development, defined as the occurrence of at least 2 positive inhibitor titers combined with a decreased recovery. Eighty-seven (24%) patients developed inhibitors (69 high titer [19%]). The incidence of inhibitors appeared to be associated with age at first treatment, decreasing from 41% for those treated within the first month of age to 18% in those treated after 18 months; after adjustment for treatment intensity, this association largely disappeared. Surgical procedures and peak treatment moments at start of treatment increased inhibitor risk (relative risk [RR], 3.7; 95% confidence interval [CI], 2.0-7.1; and RR, 3.3; CI, 2.1-5.3, respectively). Regular prophylaxis was associated with a 60% lower risk than on-demand treatment (RR, 0.4; CI, 0.2-0.8). Our findings suggest that the previously reported associated between an early age at first exposure and the risk of inhibitor development is largely explained by early, intensive treatment. The latter appears to be an independent risk factor for inhibitor development. In addition, early, regular prophylaxis may protect patients with hemophilia against the development of inhibitors. (Blood. 2007;109:4648-4654)

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Introduction

Hemophilia A is an X-linked inherited bleeding disorder characterized by a deficiency of functional clotting factor VIII. Nowadays, with prophylactic treatment, children with hemophilia look forward to a favorable orthopedic outcome and high level of health-related quality of life.[1] However, about 25% of the children with severe hemophilia A develop inhibitory antibodies against infused factor VIII.[2] Patients with high-titer inhibitors have a particularly increased risk of potential life-threatening bleeds and, furthermore, treatment of these patients is complex and costly.[3]

Several patient-related factors have been related to the risk of inhibitor development, such as ethnicity,[4,5] family history of inhibitors,[5-7] and factor VIII gene mutation type.[8] I, the HLA complex genotype[9-11] and polymorphisms in the promoter regions of the interleukin-10 gene and tumor necrosis factor-alpha have been suggested to play a role.[12,13] In addition to these genetic determinants, observations of discordant inhibitor status in monozygotic twins suggest that environmental risk factors also affect inhibitor development.[5,14]

At present, little is known about modifiable causes of inhibitor development. Several treatment characteristics have been associated with the risk of developing inhibitors. Some studies observed a higher risk in patients who were first treated with factor VIII at a younger age than those first treated at a later age,[8,15-18] but this was not confirmed in other studies.[19,20] Intensive treatment was suggested to be a risk factor in patients with mild hemophilia A, who appeared to develop inhibitors more frequently after surgery.[21] A protective effect has been suggested for prophylactic treatment,[18,22] but additional studies are required to confirm this association.[23,24]

Modifiable risk factors of inhibitors provide the key to predict and perhaps even prevent inhibitor development in hemophilia patients. Therefore, in collaboration with investigators from 13 European and 1 Canadian hemophilia treatment centers, we set up a study to examine whether age at first exposure, intensity of treatment, and regular prophylaxis are associated with clinically relevant inhibitor development in previously untreated patients with severe hemophilia A.

Patients and methods

The study population consisted of 376 patients with severe hemophilia A (residual factor VIII activity of < 0.02 IU/mL) born between 1990 and 2000, and treated in a single center from the first clotting factor administration onward. Ten patients were excluded: one because of an unknown baseline factor VIII activity, 2 because of treatment with a particular factor VIII product that was reported to be clearly associated with an increased risk of inhibitor development,[25] one because of treatment with desmopressin, and 6 because they were lost to follow-up before they received treatment with factor VIII on a total of 50 exposure days.

Measurements

We collected data from the medical records and patients' clotting factor infusions logbooks using standardized case report forms. The following data on patient characteristics were collected: date of birth, residual factor VIII activity level, ethnicity, family history of hemophilia and inhibitors, duration of breastfeeding, and factor VIII gene mutation. Additionally, from patients' infusion logbooks we recorded details on all clotting factor

infusions up to 50 exposure days or until inhibitor development, including dates of infusion, doses and types of factor VIII product, reasons of treatment, types of bleeds, and surgery. For a correct assessment of inhibitor development, we collected details on all performed inhibitor tests and recovery measurements, including dates, body weights, infused doses of clotting factor, preinfusion and postinfusion factor VIII activity levels, and time between infusion of clotting factor and blood sampling for postinfusion factor VIII activity level.

Definition of inhibitor development

We defined inhibitor development in 2 ways. First, the development of "all clinically relevant inhibitors" was defined as the occurrence of at least 2 positive antibody titers combined with a decreased factor VIII recovery within the first 50 exposure days, and second, the development of "high-titer inhibitors" occurred when the peak inhibitor titer was at least 5 Bethesda units/mL. A positive inhibitor titer was defined according to the inhibitor assay used and the cutoff level of the respective laboratory. The factor VIII recovery was considered to be decreased, when it was less than 66% of the expected level. The expected level of factor VIII activity was calculated according to Lee et al.[26]

Data analyses

The aim of our analyses was to examine whether potential determinants were associated with inhibitor development. Half of all inhibitors occur before the 15th exposure day and the other half occur with a sharply decreasing incidence rate relatively early afterward. At 50 exposure days, the risk of developing inhibitors is decreased to less than 1%. This gradual decrease in risk needs to be taken into account if risk factors occurring at various time points during the early treatment with factor VIII are studied. To this aim, we used Cox proportional hazard models with time-dependent variables[27] and pooled logistic regression analyses.[28] In the Cox proportional hazards models, inhibitor development was the event, and the cumulative number of "exposure days" was the time variable. An exposure day was defined as a calendar day during which one or more infusions of factor VIII were given. In the analyses with "all clinically relevant inhibitors" as the outcome, censoring occurred only at exposure day 50, since all patients either developed inhibitors or were followed until they received factor VIII on at least 50 exposure days. In the analyses with "high-titer inhibitor development" as the outcome, censoring occurred both at exposure day 50 in noninhibitor patients and at the cumulative number of exposure days at inhibitor development in patients with low-titer inhibitors.

We used pooled logistic regression to study and to adjust for time-varying treatment characteristics. To this aim, observations over all exposure days of all patients were pooled into a single sample, and a logistic regression model was used to relate the risk factors to the occurrence of inhibitors. This method accounts for varying risks according to the cumulative number of exposure days, and is equivalent to Cox regression with exposure days as time-variable and time-dependent covariates. Relative hazard rates and odds ratios were interpreted as relative risks.

Potential determinants of inhibitor development

Fixed potential risk factors of inhibitor development were baseline factor VIII activity, ethnicity, family history of inhibitors, breastfeeding, factor VIII gene mutation type, age at first exposure to factor VIII, reason for first factor VIII treatment (prophylaxis, bleeds, or surgical procedures), and peak treatment moments at first treatment episode. We studied inhibitor incidences in high-risk mutations (large deletions [> 200 base pairs], nonsense mutations, intron 22 or 1 inversions) and in low-risk mutations (small deletions/insertions [< 200 base pairs], missense mutations, and other mutations [splice site defects or promoter mutations]).

Time-varying potential determinants were peak treatment moments, major peak treatment moments, major surgical procedures, duration between exposure days, dose of factor VIII product, and regular prophylaxis. "Peak treatment moment" was defined as an episode of treatment with factor VIII for a bleed or surgery on at least 3 consecutive days. "Major peak treatment moment" was defined as a peak treatment moment during

which treatment was given on at least 5 consecutive days. We studied "major surgical procedures" for which replacement therapy lasting at least 3 consecutive days was given. The corresponding time-dependent variables were defined as "after peak treatment moment," "after major peak treatment moment," or "after major surgical procedure." "Duration between exposure days" was our measure for frequency of exposures; it was defined as the time period between the current exposure day and the fifth exposure day prior to this exposure day (or in the first 4 exposure days, the time period was converted to a total of 5 exposure days). For example, if a patient had developed an inhibitor at the 20th exposure day, the time period between the days at which he had his 15th and 20th exposure day was his duration between exposure days at the 20th exposure day. This was calculated for all patients at all exposure days. "Dose of factor VIII product" was defined as the mean dose of factor VIII product per kilogram body weight of the last 5 exposure days prior to each exposure day (or in the first 4 exposure days, mean dose of all previous days). Since prophylaxis influences these variables to a large extent, we restricted the analyses of duration between exposure days and dose of factor VIII product to the subgroup of patients who never received regular prophylaxis. We defined "regular prophylaxis" as regular factor VIII infusions at least once a week aimed at preventing bleeds, as we expected that one exposure to factor VIII a week could have an immunologic effect. To illustrate the association of treatment regimen and the risk of inhibitor development, we plotted the cumulative incidences of inhibitor development in on-demand treatment and prophylactic treatment. All patients contributed were classified into the on-demand group until they started prophylaxis. Subsequently, they were classified into the prophylaxis group.[29]

The continuous variables age at first exposure, duration between exposure days, and dose of factor VIII product were categorized into groups, in order to also detect nonlinear associations. Categories were based on approximately equal numbers of observations and practical cut points.

In the multivariate analyses, we adjusted for other possible determinants that could have confounded the specific association studied, independent of their statistical significance in univariate analyses. We specified in the table footnotes for which variables we adjusted each association. All associations were adjusted for factor VIII gene mutation type categorized as high-risk and low-risk mutation types as specified earlier. All associations were also adjusted for the type of factor VIII product, categorized as recombinant factor VIII products, monoclonal antibody purified plasma-derived products, and other plasma-derived products.

Sensitivity analyses

We repeated all analyses (1) in patients with a baseline factor VIII activity level of less than 0.01 IU/mL, (2) in patients with high-inhibitor risk factor VIII gene mutations (ie, patients with large deletions, nonsense mutations, or inversions in the gene for factor VIII), and (3) in patients who were tested for the presence of inhibitors on at least 2 occasions during the first 50 exposure days.

Missing data

In 319 patients (87%), complete data were available. In 32 patients, only limited data on treatment characteristics in addition to patient characteristics were available, and in 15 patients, only data on patient characteristics were available.

In the univariate analyses, we excluded the patients who had no value of the variable that was studied (complete case analysis). The missing values of the variables that were included in the multivariate models were imputed using multiple linear regression methods. As expected, family history of inhibitors was not available for more than half of the patients because they did not have relatives with hemophilia. Thus, it was not reliable to impute the missing values on this variable,[30] implying that we could not adjust our analyses for family history of inhibitors. As not all patients' body weights were available at all exposure days, we assigned patients' body weights using the Dutch growth reference data.[31]

Results

Patient characteristics

Table 1 presents the characteristics of all 366 patients. Eighty-seven patients (24%) developed clinically relevant inhibitors, of whom 69 (19%) had high-titer inhibitors and 18 (5%) had low-titer inhibitors (Figure 1). Seventeen patients with marginally positive inhibitor titers between 0.6 and 1.0 Bethesda units/mL (BU/mL) on only one occasion with normal factor VIII recoveries did not meet the definition of clinically relevant inhibitor. The characteristics of the patients with clinically relevant inhibitors are described in Table 2. Patients developed inhibitors after a median of 14 exposure days (interquartile range [IQR], 8-21 days) at a median age of 15 months (IQR, 10-22 months). The median duration between the first exposure to factor VIII and inhibitor development

Table 1. Baseline characteristics

|	Data
No. patients	366
Baseline FVIII activity, no. (%)	
Less than 0.01 IU/mL	332 (91)
0.01 to 0.02 IU/mL	34 (9)
Ethnicity, no.(%)	
White	327 (90)
African	8 (2)
Asian	15 (4)
Other	15 (4)
Family history hemophilia, no. (%)	
Positive	168 (47)
Negative	191 (53)
Family history inhibitors, no. (%)	
Positive	24 (15)
Negative	138 (85)
FVIII gene mutation type, no. (%)	
Large deletions	10 (3)
Nonsense mutations	30 (10)
Inversions	172 (55)
Small deletions/insertions	41 (13)
Missense mutations	49 (16)
Other	10 (3)
Breastfeeding, no. (%)	
Yes	91 (69)
No	41 (31)
Median age at first exposure day, mo (IQR)	11 (6-15)
Median age at 50th exposure day, mo* (IQR)	31 (21-46)
Duration first and 50th exposure day, mo* (IQR)	19 (12-32)
All peak treatment moments, no. (%)	
Yes	251 (78)
No	71 (22)
Major peak treatment moments, no. (%)	
Yes	176 (55)
No	146 (45)
Surgery during first 50 exposure d, no. (%)	
Yes	80 (25)
No	242 (75)
Treatment regimen, no. (%)	
Prophylaxis	196 (55)
On demand	162 (45)
Median age at start of prophylaxis, mo (IQR)	20 (14-35)
Median no. exposure d at start of prophylaxis (IQR)	16 (8-28)
Reason for first exposure to factor VIII, no. (%)	
Bleed	286 (84)
Prophylaxis	37 (11)
Surgery	17 (5)

*In noninhibitor patients.
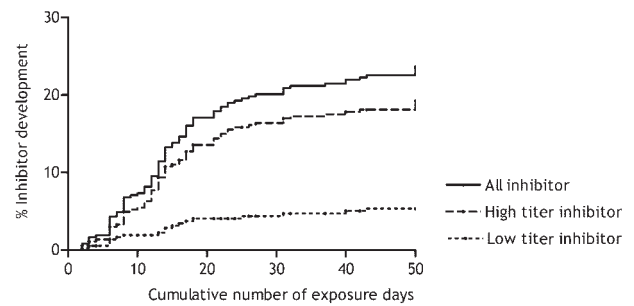
Figure 1. Cumulative incidence of inhibitor development: all inhibitors, and high- and low-titer inhibitors.

was 6 months (IQR, 2-12 months). The median number of inhibitor assays during the first 50 exposure days was 4 (IQR, 2-6) in noninhibitor patients. There were no clinical signs of inhibitors in any of the patients with fewer than 2 inhibitor tests.

Inhibitor risk according to patient characteristics

Table 3 presents the cumulative incidences of inhibitor development and crude and adjusted relative risks of inhibitor development according to patient characteristics. Patients with a baseline factor VIII activity of less than 0.01 IU/mL had a more than doubled risk of developing inhibitors than patients with a baseline factor VIII activity between 0.01 and 0.02 IU/mL. Half of the patients (46%) had a positive family history of hemophilia. The inhibitor risk was similar in patients with positive and negative family histories of hemophilia. The risk of developing inhibitors was 3-fold higher in patients with a positive family history of inhibitors than in patients with a negative family history. We had data on breastfeeding in 132 patients (36%). Ninety-one patients had been breastfed, for a median of 13 (IQR, 8-26) weeks. The inhibitor risk was similar in patients with and without breastfeeding.

The type of factor VIII gene mutation was known in 312 patients (85%). The cumulative incidence of all inhibitors was 30% (3/10) in patients with large deletions, 37% (11/30) in patients with nonsense mutations, 31% (54/172) in patients with intron 1 and 22 inversions, 22% (9/41) in patients with small deletions/insertions, 8% (4/49) in patients with missense mutations, and 0% (0/10) in patients with other (splice site or promoter) mutations. In high-risk mutations, the risk of developing inhibitors was 2.8 (95% confidence interval [CI], 1.5-5.0) times higher than in low-risk mutations.

Inhibitor risk according to treatment characteristics at the first exposure day

Table 4 presents the crude and adjusted relative risks of inhibitor development according to treatment characteristics.

Age at first exposure to factor VIII. Patients were first treated with factor VIII at a median age of 11 months (IQR, 6-15 months; range, 0-89 months). The cumulative incidence of clinically relevant inhibitor development was 41% (16/39) in patients first treated with factor VIII before the first month of age, 30% (13/43) in patients first treated between 1 and 6 months, 23% (30/130) in patients first treated between 6 and 12 months, 20% (16/82) in patients first treated between 12 and 18 months, and 18% (10/57) in patients first treated after 18 months (*P* for trend = .005). The association between age at first exposure and risk of inhibitor development that was present in the whole group largely disappeared after adjustment for confounding factors (Table 4).

Reason of first factor VIII treatment. Patients who were first treated for surgical procedures had a markedly higher risk of inhibitor development (65%) than patients who were first treated for bleeds or prophylactically (23% and 22%, respectively).

Table 2. Characteristics of all patients with clinically relevant inhibitors

	All inhibitors	Range	High-titer* inhibitors	Range	Low-titer inhibitors	Range
No. patients (%)	87 (24)	NA	69 (79)	NA	18 (21)	NA
Median no. exposure days at inhibitor development (IQR)	14 (8-21)	2-50	14 (8-22)	2-50	14 (6-20)	2-43
Median age at inhibitor development, mo (IQR)	15 (10-22)	0.3-79	15 (10-22)	0.3-79	17 (10-26)	10-47
Median duration between first exposure day and inhibitor development, mo (IQR)	5 (2-12)	0.2-60	5 (2-11)	0.2-60	8 (4-18)	1-47

NA indicates not applicable

*High-titer inhibitor was defined as clinically relevant inhibitor with a peak titer of at least 5 BU/mL.

Peak treatment moment at first treatment episode. Patients who received factor VIII for a bleed or surgery on at least 5 consecutive days (major peak treatment moment) immediately at the first treatment episode had a 3.3-fold (CI, 2.1-5.3) higher risk of inhibitor development than patients who received treatment on a single day or on 2 consecutive days.

Inhibitor risk according to treatment characteristics during the first 50 exposure days

Peak treatment moments. Similar to the observations at the first exposure, major peak treatment moments at any exposure day were associated with an increased risk of inhibitor development (RR, 2.0; CI, 1.3-3.1).

Major surgical procedures. Eighty patients (25%) underwent a total of 84 major surgical procedures (63 portacath implantations; 21 other surgical procedures). Major surgeries at any exposure day were associated with an increased, but less pronounced, risk of developing inhibitors (RR, 1.4; CI, 0.8-2.5) than surgical procedures at the first treatment episode. In addition, we investigated portacath implantations and other surgical procedures separately. We found that the relative risk after portacath implantations was 1.2 (CI, 0.6-2.3) (adjusted RR, 1.4; CI 0.7-2.7), and the relative risk

after other surgical procedures was 2.0 (CI, 0.9-4.7) (adjusted RR, 1.3; CI, 0.5-3.0).

Duration between exposure days. To examine whether the frequency of infusions or the dosing of factor VIII or both were primarily associated with inhibitor development, we studied these determinants separately. The relative risk of inhibitors was 1.9 (CI, 1.1-3.3) when the duration between 5 consecutive exposure days was fewer than 10 days and 0.8 (CI, 0.4-1.6) when the duration was 10 to 50 days, compared with a duration of more than 50 days (*P* for trend = .03). However, this observed trend was less pronounced after adjustment for confounders.

Dose of factor VIII. Compared to a mean dose of factor VIII of 5 consecutive exposure days of less than 35 IU/kg, the risk of developing clinically relevant inhibitors was 1.4 (CI, 0.7-3.0) times higher when the mean dose was between 35 and 50 IU/kg, and it was 3.3 (CI, 1.7-6.5) times higher when the mean dose was more than 50 IU/kg (*P* for trend < .001). This relationship did not change after adjustment for all potential confounders including patients' body weight at every exposure day (Table 4).

Regular prophylaxis. More than half of all patients started regular prophylaxis at least once a week during the first 50 exposure days. In these patients, prophylaxis was started at a

Table 3. Risk of inhibitor development according to patient characteristics

	All inhibitors					High-titer inhibitors*				
	Proportion with Inh (%)	Crude RR (CI)	P	Adjusted RR (CI)	P	No. of Inh (%)	Crude RR (CI)	P	Adjusted RR (CI)	P
Baseline factor VIII activity										
0.01 to 0.02 IU/mL	4/34 (12)	1.0		1.0†		2/34 (6)	1.0	.07	1.0†	
Less than 0.01 IU/mL	83/332 (25)	2.3 (0.9-6.3)	.10	4.1 (1.0-16.9)	.05	67/332 (20)	3.8 (0.9-15.3)		6.5 (0.9-47.5)	.06
Ethnicity										
White	78/327 (24)	1.0		1.0‡		63/327 (19)	1.0		1.0‡	
African	3/8 (38)	1.8 (0.6-5.8)	.31	1.9 (0.6-6.1)	.31	2/8 (25)	1.5 (0.4-6.2)	.57	1.4 (0.3-6.1)	.62
Asian	1/15 (7)	0.3 (0.04-1.9)	.19	0.3 (0.04-2.4)	.27	1/15 (7)	0.3 (0.05-2.4)	.27	0.4 (0.05-2.8)	.35
Other	5/15 (33)	1.5 (0.6-3.6)	.42	2.5 (1.0-6.7)	.06	3/15 (20)	1.1 (0.3-3.4)	.89	1.7 (0.5-5.7)	.41
Family history of hemophilia										
Negative	45/191 (24)	1.0		1.0‡		36/191 (19)	1.0		1.0‡	
Positive	42/168 (25)	1.1 (0.7-1.6)	.69	1.0 (0.7-1.6)	.88	33/168 (20)	1.1 (0.7-1.7)	.78	1.0 (0.6-1.7)	.85
Family history of inhibitors										
Negative	29/138 (21)	1.0		1.0‡		21/138 (15)	1.0		1.0‡	
Positive	13/24 (52)	3.1 (1.6-6.0)	.001	2.8 (1.3-6.1)	.007	12/24 (50)	4.0 (2.0-8.2)	< .001	3.5 (1.5-8.1)	.003
Breastfeeding										
No	12/41 (29)	1.0		1.0§		8/41 (20)	1.0		1.0§	
Yes	25/91 (27)	0.9 (0.5-1.9)	.88	1.3 (0.6-2.8)	.59	23/91 (25)	1.3 (0.6-1.9)	.51	2.1 (0.8-5.5)	.12
Factor VIII gene mutation type										
Low risk	13/100 (13)	1.0		1.0‖		7/100 (7)	1.0		1.0‖	
High risk	68/212 (32)	2.8 (1.5-5.0)	.001	2.3 (1.3-4.3)	.006	57/212 (27)	4.3 (2.0-9.5)	< .001	3.7 (1.7-8.3)	.001

Inh indicates inhibitors.

*High-titer inhibitor was defined as a clinically relevant inhibitor with inhibitor titers of at least 5 Bethesda units/mL at any time.

†Adjusted for ethnicity, factor VIII gene mutation type, age at first exposure, duration between exposure days, dose, prophylaxis, and product type.

‡Adjusted for baseline factor VIII activity level, factor VIII gene mutation type, age at first exposure, duration between exposure days, dose, prophylaxis, and product type.

§Adjusted for baseline factor VIII activity level, ethnicity, factor VIII gene mutation type, age at first exposure, duration between exposure days, dose, prophylaxis, and product type.

‖Adjusted for baseline factor VIII activity level, ethnicity, age at first exposure, duration between exposure days, dose, prophylaxis, and product type.

Table 4. Risk of inhibitor development according to treatment characteristic

	All inhibitors					High-titer inhibitors*				
	Proportion of Inh (%)	Crude RR (CI)	P for trend	Adjusted RR (CI)	P for trend	Proportion of Inh (%)	Crude RR (CI)	P for trend	Adjusted RR (CI)	P for trend
At first factor VIII exposure										
Age at first exposure										
More than 18 mo	10/57 (18)	1.0	.005	1.0§	.21	9/57 (16)	1.0	.02	1.0§	.63
12 to 18 mo	16/82 (20)	1.1 (0.5-2.5)		1.2 (0.5-2.9)		13/82 (16)	1.0 (0.4-2.4)		1.0 (0.4-2.6)	
6 to 12 mo	30/130 (23)	1.3 (0.7-2.7)		1.5 (0.6-3.4)		23/130 (18)	1.1 (0.5-2.5)		1.1 (0.4-2.6)	
1 to 6 mo	13/43 (30)	1.9 (0.8-4.3)		1.8 (0.7-4.7)		10/43 (23)	1.6 (0.6-3.9)		1.3 (0.5-3.8)	
Less than 1 mo	16/39 (41)	2.7 (1.2-5.9)		1.6 (0.6-4.1)		13/39 (33)	2.4 (1.0-5.6)		1.1 (0.4-3.2)	
Reason for first factor VIII treatment										
Bleed	65/286 (23)	1.0		1.0‖		50/286 (17)	1.0		1.0‖	
Prophylaxis	8/37 (22)	1.0 (0.5-2.0)	.92	1.0 (0.5-2.2)	.95	7/37 (19)	1.1 (0.5-2.4)	.82	1.2 (0.5-2.8)	.63
Surgical procedure	11/17 (65)	3.7 (2.0-7.1)	< .001	2.6 (1.3-5.1)	.007	10/17 (59)	4.4 (2.2-8.7)	< .001	3.2 (1.6-6.7)	.002
Peak treatment moment at first treatment episode†										
None	44/229 (19)	1.0		1.0¶		31/229 (14)	1.0		1.0¶	
3 to 4 days	7/36 (19)	1.0 (0.5-2.3)	.98	1.1 (0.5-2.4)	.87	6/36 (17)	1.2 (0.5-2.9)	.66	1.3 (0.5-3.1)	.63
At least 5 days	32/57 (56)	3.3 (2.1-5.3)	< .001	3.1 (1.9-5.0)	< .001	30/57 (53)	4.3 (2.6-7.1)	< .001	4.1 (2.4-7.0)	< .001
During first 50 exposure days										
After peak treatment moment compared with before	NA	1.6 (1.0-2.7)	.06	1.5 (0.9-2.5)¶	.14	NA	1.7 (1.0-2.9)	.07	1.5 (0.8-2.6)¶	.18
After major peak treatment moment compared with before	NA	2.0 (1.3-3.1)	.002	1.6 (1.0-2.6)¶	.03	NA	2.3 (1.4-3.7)	.001	1.9 (1.1-3.1)¶	.02
After major surgical procedure compared with before	NA	1.4 (0.8-2.5)	.21	1.3 (0.8-2.3)¶	.32	NA	1.3 (0.7-2.5)	.43	1.2 (0.6-2.3)¶	.61
Duration between exposure days‡										
More than 50 d	NA	1.0	.03	1.0#	.26	NA	1.0	.06	1.0#	.62
10 to 50 d	NA	0.8 (0.4-1.6)		0.8 (0.4-1.6)		NA	0.6 (0.3-1.4)		0.6 (0.3-1.4)	
Fewer than 10 d	NA	1.9 (1.1-3.3)		1.5 (0.8-2.7)		NA	1.9 (1.1-3.4)		1.3 (0.7-2.5)	
Dose of factor VIII product‡										
Less than 35 IU/kg	NA	1.0	< .001	1.0**	.01	NA	1.0	< .001	1.0**	.01
35 to 50 IU/kg	NA	1.4 (0.7-3.0)		1.2 (0.6-2.6)		NA	1.7 (0.7-4.0)		1.5 (0.6-3.6)	
More than 50 IU/kg	NA	3.3 (1.7-6.5)		2.3 (1.2-4.7)		NA	4.2 (1.9-9.3)		3.0 (1.3-6.9)	
Regular prophylaxis	NA	0.4 (0.2-0.8)	.01	0.5 (0.2-0.9)††	.02	NA	0.5 (0.2-0.9)	.03	0.5 (0.2-1.0)††	.05

Inh indicates inhibitors; NA, not applicable.

*High-titer inhibitor was defined as a clinically relevant inhibitor with inhibitor titers of at least 5 Bethesda units/mL at any time.

†Only 2 of the 7 patients who developed inhibitors before the fifth exposure day received treatment with factor VIII on consecutive days (2); the others received treatment on single days before inhibitor development.

‡In patients who were treated on demand during the first 50 exposure days.

§Adjusted for baseline factor VIII activity level, ethnicity, factor VIII gene mutation type, duration between exposure days, dose, prophylaxis, peak treatment moment at first treatment, reason of first treatment, and product type.

‖Adjusted for baseline factor VIII activity level, ethnicity, factor VIII gene mutation type, age at first exposure, duration between exposure days, dose, prophylaxis, and product type.

¶Adjusted for baseline factor VIII activity level, ethnicity, factor VIII gene mutation type, age at first exposure, prophylaxis, and product type.

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**Adjusted for baseline factor VIII activity level, ethnicity, factor VIII gene mutation type, age at first exposure, duration between [AU12] exposure days, prophylaxis, product type, and body weight.

††Adjusted for baseline factor VIII activity level, ethnicity, factor VIII gene mutation type, age at first exposure, duration between exposure days, dose, and product type.

median age of 20 months (IQR, 14-35 months) and after a median number of 16 exposure days (IQR, 8-28 days). The majority of these patients (88%) received prophylaxis at least twice a week. Regular prophylaxis was associated with a 60% decreased risk of inhibitor development (RR, 0.4; CI, 0.2-0.8) compared with on-demand treatment (Figure 2).

Sensitivity analyses

This relationship between age at first exposure and risk for inhibitor development was particularly present in patients with low-risk mutations. Compared to patients who were treated for the first time when they were 18 months or older, the crude relative risk was 7.4 (CI, 0.8-71.4) in patients treated before the first month of age, 5.4 (CI, 0.6-51.9) in patients first treated between 1 and 6 months, 2.2 (CI, 0.2-19.9) in patients first treated between 6 and 12 months, and 1.6 (CI, 0.1-17.9) in patients first treated between 12 and 18 months; whereas, the association was less pronounced in patients with high-risk mutations: crude RR, 1.4 (CI, 0.6-3.4), 1.2 (CI, 0.5-3.0), 1.0 (CI, 0.4-2.1), 0.7 (CI, 0.3-1.8), respectively. All other findings were similar in subgroups of patients with a baseline factor VIII activity level of less than 0.01 IU/mL (332 patients, 91%), in patients with high-risk mutation type (ie, large deletions, nonsense mutations, or intron 22 and 1 inversions; 212 patients, 56%), and in patients who were tested for the presence of inhibitors on at least 2 occasions during the first 50 exposure days (265 patients, 72%) (tables presented in Appendices).
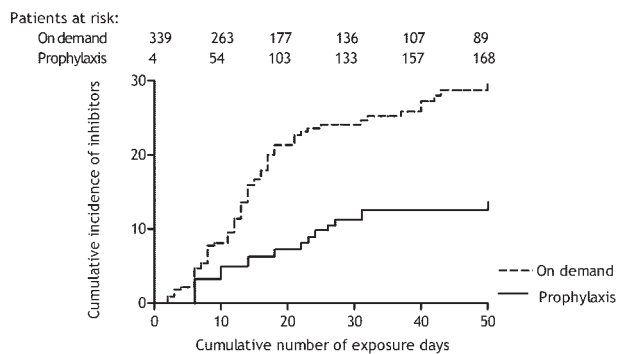
Patients at risk:
On demand   339   263   177   136   107   89
Prophylaxis   4   54   103   133   157   168

Figure 2. Cumulative incidence of inhibitor development according to treatment regimen: prophylaxis versus on demand.

Discussion

Summary

In this retrospective multicenter cohort study among 366 previously untreated patients with severe hemophilia A treated with factor VIII on at least 50 exposure days, a young age at first exposure to factor VIII was associated with an increased risk of inhibitor development. However, this association largely disappeared after adjustment for intensity of treatment. Additionally, intensive treatment (early surgical procedures, early major peak treatment moments, and high dosing of factor VIII) was related to a higher risk. Regular prophylaxis was related to a 60% lower risk of inhibitor development.

Age at first exposure

In line with 5 previous reports, we observed a higher incidence of inhibitors among boys who were treated with factor VIII at a young age.[15-18,32] These studies did not adjust for treatment intensity, which in our study accounted for a large part of the association. Other studies did not find any association between age at first exposure to factor VIII and inhibitor development.[19,20,33,34] Our findings suggest that age at first exposure is associated with inhibitor development, but that this association is explained by intensity of treatment.

Intensity of treatment

We found that surgical procedures and major peak treatment moments carried a markedly increased inhibitor risk. In a small study among mild hemophilia patients, intensive treatment was suggested to be a risk factor for inhibitors.[21] In a case-control study, no clear association was found between surgery and central nervous system bleeding and inhibitor development.[18] This discrepancy might have been caused by differences in design of the studies.

It seems biologically plausible that patients are more prone to inhibitor development during periods of intensive treatment, because major bleeds or surgeries cause tissue damage and inflammation. Danger signals released from injured cells activate antigen-presenting cells, which subsequently present factor VIII antigen with up-regulated costimulatory signals to T lymphocytes. These cells then enhance antibody formation in B lymphocytes.[35]

Regular prophylaxis

Additionally, factor VIII infusions in absence of immunologic danger signals, like in prophylactic treatment, may lead to inhibition of the immune response through peripheral anergy of factor VIII–specific T lymphocytes.[35] In our study, we indeed found a 60% decreased risk of inhibitor development in patients on regular prophylaxis. This clearly confirmed previous suggestions of a protective effect of prophylactic treatment.[18,22,32,33]

Patient characteristics

Furthermore, our study confirmed previous reports of an increased risk of inhibitor development in patients with a positive family history of inhibitors[5-7] and patients with large deletions, nonsense mutations, or intron 22 inversion in their factor VIII gene.[8,36,37] In addition, in agreement with previous observations, we did not find a relation between breastfeeding and inhibitor development.[18,38,39] Because the vast majority of patients were white, we could not investigate ethnicity as a risk factor for inhibitor development.

Strength and limitations

To reduce selection bias in the study population, we included all patients who were treated at a single center born between 1990 and 2000, including patients for whom limited treatment data were available. Since all centers were tertiary hemophilia treatment centers, we carefully excluded patients with an inhibitor who were referred from other centers. Furthermore, 98% of eligible patients were treated with factor VIII on at least 50 exposure days, thus there is little chance of bias due to patients being still at risk of developing inhibitors. Thus, these measures provided us with a valid estimate of the cumulative incidence of clinically relevant inhibitor development at 50 exposure days in previously untreated patients with severe hemophilia A.

A major asset of our study is that we performed survival analyses with time-dependent determinants. The absolute risk of developing inhibitors decreases with increasing exposure days. It is therefore important to compare patients with a similar number of exposure days with each other. Comparisons of patients with a different number of exposure days may lead to spurious associations.

We did not include a minimum frequency of inhibitor testing in the inclusion criteria, as we focused on inhibitors that were important in clinical practice. We expected that even if inhibitor testing is performed infrequently, clinically relevant inhibitors would be noticed by an absent or reduced response of treatment with factor VIII. Moreover, we observed similar results in the subpopulation of patients who had at least 2 inhibitor tests during the total follow-up.

We included patients with baseline factor VIII activities of less than 0.02 IU/mL, as we supposed that during the study period not every center's laboratory would have been capable of reliable factor VIII activity measurements less than 0.02 IU/mL. In order to ensure that the observed associations were not biased, we verified all analyses in patients with baseline factor VIII activities less than 0.01 IU/mL and in patients with high-risk mutations, who are theoretically incapable of producing any factor VIII protein, revealing similar findings.

Despite the fact that the intensity of treatment may be high in response to the presence of a yet-undetected inhibitor, the evident relation between surgery at the start of treatment and a higher incidence of subsequent inhibitors indicates that, at least in the case of surgery, intensive treatment preceded the development of the inhibitors.

Our observations suggest that it may be possible to predict and possibly decrease the risk of developing inhibitory antibodies against clotting factor VIII in previously untreated patients with severe hemophilia. Whether specific treatment regimens, such as avoiding early elective surgery or early regular prophylaxis, reduce the risk of developing inhibitors should first be evaluated in a study designed for this purpose.

Authorship

Contribution: All CANAL Study participants participated in designing the research, collected patient data, and approved the final report; S.C.G. coordinated data collection, analyzed and interpreted the data, and wrote the first draft of the paper; J.G.B. and H.M.B. conceived, designed, and supervised the research, and prepared the final version of the paper. J.G.B. analyzed and interpreted the data; H.M.B. obtained funding.

A complete list of the members of the CANAL Study group is available on the *Blood* website; see the Supplemental Materials link at the top of the online article.

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