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Early factor VIII exposure and subsequent inhibitor development in children with severe haemophilia A

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Summary. Recent reports have suggested that the incidence of inhibitors in haemophilia is the highest in those first exposed to factor VIII under 6 months of age. In this study, we investigated inhibitor development in children first exposed to FVIII as neonates and also examined the effect of other genetic and environmental variables. Three hundred and forty-eight children with severe haemophilia A were investigated. Inhibitors developed in 68 of 348 (20%), with 34 of 348 (10%) high titre inhibitors. The incidence in relation to initial FVIII exposure was: <1 month nine of 35 (26%), 1–6 months 13 of 51 (25%), 6–12 months 27 of 130 (21%), 12–18 months 13 of 66 (20%) and >18 months six of 66 (9%). While we observed a significant difference in inhibitor development and age at first exposure across all age groups ($P = 0.018$), no significant difference was observed in children treated at different time points during the first year of life ($P = 0.44$). Similar results were obtained for high titre inhibitors. There was also no difference in the incidence of inhibitors in relation to initial FVIII exposure in a subgroup of 144 children with the intron 22 mutation. Inhibitors developed more frequently in those initially treated with recombinant when compared with plasma-derived FVIII ($P = 0.006$) and in those with a major molecular defect ($P = 0.009$). In this study, exposure to FVIII during the neonatal period was not associated with a higher incidence of inhibitors than those treated later during the first year of life. Initial treatment with recombinant FVIII and the presence of a major molecular defect were the most important variables affecting inhibitor development.

Keywords: children, factor VIII, haemophilia, inhibitor

Introduction

The development of inhibitory antibodies against factor VIII remains a major complication in the management of haemophilia A and has a significant impact on both the success and cost of treatment [1,2]. Inhibitors are more common in severe haemophilia A than in mild or moderate disease and a number of other genetic and environmental factors have been identified as influencing inhibitor development [3]. Genetic factors include the type of mutation in the FVIII gene and it is now recognized that some genetic abnormalities carry a high risk of inhibitor development [4]. Less information is available regarding the effect of environmental factors on inhibitor development. Factors postulated to be of importance include the type of FVIII product used for replacement therapy, administration of FVIII concentrate by continuous infusion and frequent changes in FVIII product [5–7].

In addition, two recent publications from Spain and the Netherlands have suggested that early exposure to FVIII may be associated with a higher incidence of inhibitor development and have triggered further interest in the effect of initial treatment patterns on subsequent inhibitor development [8,9].

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In both these studies, exposure to FVIII during the first 6 months of life was associated with an increased incidence of inhibitors. Subsequent data from Italy have failed to demonstrate a clear association with early FVIII exposure and inhibitor development and has shown a potentially protective effect from prophylaxis suggesting that the effect of initial treatment on inhibitor development may be more complex [10].

Previous studies have defined early onset of FVIII treatment as <6 months of age. The aim of the current study was to examine the incidence of inhibitor development in children first exposed to FVIII at an earlier stage, during the first month of life, and also to examine the effect of other genetic and environmental factors in this cohort of children.

Patients and methods

Patients

This was a retrospective cohort study, in which data were collected from 348 eligible children attending eight Haemophilia Comprehensive Care Centres in the UK. Consecutive patients with severe haemophilia A (FVIII $\leq$1 IU mL^{-1}) who had commenced treatment between 1987 and 2003 were included. All children had received >50 exposures to FVIII or had developed an inhibitor prior to completing 50 exposures. Patients were treated with either a plasma-derived or recombinant FVIII (rFVIII) product and details of the individual product used for initial treatment were recorded. Plasma-derived FVIII included both high purity products and intermediate purity products containing von Willebrand factor (VWF).

Monitoring

Monitoring for inhibitory antibodies was performed on a regular basis at least every 3–6 months.

All laboratories used the Bethesda assay and a significant inhibitor titre was defined as being $\geq$1.0 BU mL^{-1} on at least two consecutive measurements. High titre inhibitors were defined as having a titre of >5 BU mL^{-1} at any time.

Data collection

Data were collected for each patient on ethnic origin, family history of inhibitor development, date of first exposure to FVIII, FVIII product used for initial treatment and the molecular defect in the FVIII gene. In those children who developed inhibitory antibodies, the age of the child at inhibitor development and the number of exposure days prior to inhibitor detection were also recorded.

Based on previously published data, molecular defects were classified into two groups [4]. Major defects (Group 1), were defined as those severe defects likely to be associated with a virtual absence of circulating FVIII and thus potentially with a higher risk of inhibitor development. This group included large deletions (>1 exon), the intron 1 and intron 22 inversions and stop mutations. Non-severe defects (Group 2) were those where some residual FVIII production was likely and included missense mutations, small deletions or insertions and splice site mutations.

Statistical analysis

Univariate analysis of the parameters of interest was performed using chi-squared testing. Following univariate analysis, the significant variables were included in a logistic regression model. All analyses were performed using MINITAB (version 14) using a significance level of 5%.

Results

Inhibitor development

A total of 348 children attending eight Haemophilia Comprehensive Care Centres in the UK were included in this study. All these children had haemophilia A and a FVIII level of $\leq$1 IU mL^{-1} and had commenced treatment between 1987 and 2003. The overall incidence of inhibitor development ($\geq$1.0 BU mL^{-1} on two consecutive occasions) in this cohort of patients was 68 of 348 (20%) of which 34 of 348 (10%) were defined as high titre inhibitors (>5 BU mL^{-1}). The median age at inhibitor development was 21 months (range: 35 days–12.5 years) and the median number of exposure days prior to inhibitor development was 15 days (range: 2–237).

Inhibitor development and age at first exposure to FVIII

In order to examine the relationship between age at first exposure to FVIII and subsequent inhibitor development, patients were divided into five groups. The children in the youngest group received their first treatment during the first month of life while subsequent groups were treated at 1–6, 6–12, 12–18 and >18 months of age. The percentages of

Table 1. Age at first exposure to FVIII and inhibitor development.

Age, months (days)	Number treated, n (%)	All inhibitors, *n* (%)	High titre inhibitors, *n* (%)
<1 (<30)	35 (10)	9 (26)	5 (14)
1–6 (30–180)	51 (15)	13 (25)	6 (12)
6–12 (180–360)	130 (37)	27 (21)	16 (12)
12–18 (360–540)	66 (19)	13 (20)	4 (6)
>18 (>540)	66 (19)	6 (9)	3 (5)
Total (with data)	348	68 (20)	34 (10)

children commencing treatment in each age group are shown in Table 1.

Within these time intervals the incidence of all inhibitors was: <1 month nine of 35 (26%), 1–6 months 13 of 51 (25%), 6–12 months 27 of 130 (21%), 12–18 months 13 of 66 (20%) and >18 months six of 66 (9%). The incidence of high titre inhibitors (>5 BU mL^{-1}) was: <1 month five of 35 (14%), 1–6 months six of 51 (12%), 6–12 months 16 of 130 (12%), 12–18 months four of 66 (6%) and >18 months three of 66 (5%) (Table 1).

These data demonstrate a significant inverse relationship between overall inhibitor development and age at first exposure to FVIII ($P = 0.018$). The relationship is also seen for the development of high titre inhibitors ($P = 0.04$). This relationship was not, however, observed in children treated at different time points during the first year of life, either in the cohort of all inhibitors ($P = 0.44$), or in those with high titre inhibitors ($P = 0.8$).

In 122 of 348 (35%) cases from this cohort the underlying molecular defect in the FVIII gene was the presence of the intron 22 inversion. A subgroup analysis was undertaken to examine the influence of age at first treatment on inhibitor development in this subgroup of children all of whom had the same molecular defect. The incidence of inhibitors in this subgroup according to age at first FVIII exposure is shown in Table 2. In this subgroup although there was a trend towards fewer inhibitors in those treated beyond 18 months of age, this was not statistically significant ($P = 0.93$).

Table 2. Intron 22 subgroup: age at first exposure to FVIII and inhibitor development.

Age, months (days)	Number treated	All inhibitors, *n* (%)
<1 (<30)	14	4 (29)
1–6 (30–180)	18	5 (28)
6–12 (180–360)	50	16 (32)
12–18 (360–540)	25	7 (28)
>18 (>540)	15	3 (20)
Total	122	35 (29)

Table 3. Distribution of molecular defects in the FVIII gene.

Genetic defects ($n = 231$)	Number of cases (%)
Missense mutations	52 (23)
Small deletions/insertions	30 (13)
Splice site	8 (3)
Large deletions	5 (2)
Inversions	130 (56)
Stop mutations	6 (3)

Inhibitor development and molecular defect

The underlying molecular defect in the FVIII gene was recorded for 231 of 348 (66%) of patients. The distribution of molecular defects is shown in Table 3 and in keeping with other published data, inversions constitute the most common type of defect. Molecular defects were divided into two groups. One hundred and forty-one of 231 (61%; Group 1) had severe defects (large deletions, inversions, stop mutations) whereas 90 of 231 (39%; Group 2) were classified as having non-severe defects (missense mutations, small deletions/insertions, splice site mutations). Forty-one of 141 (29%) of children with severe genetic defect (Group 1) developed inhibitors compared with 12 of 90 (13%) in Group 2 ($P = 0.006$; Table 4). The incidence of high titre inhibitors was 26 of 141 (18%) and six of 90 (7%), respectively, (0.012; Table 4).

Molecular defect and onset of treatment

In order to examine how the underlying molecular defect might relate to the onset of treatment with FVIII, we analysed age at first exposure to FVIII in each of the two groups of molecular defects (Groups 1 and 2). In Group 1 120 of 138 (87%) of children commenced treatment within the first 18 months of life, compared with 66 of 89 (74%) of children in Group 2. Therefore, at 18 months of age only 18 of 138 (13%) children in Group 1 had not commenced treatment when compared with 21 of 89 (24%) in Group 2 indicating that there is a relationship between later onset of treatment in those with less severe defects ($P = 0.048$).

Inhibitor development and initial FVIII treatment

Information on the FVIII product used for initial management was available for 304 of 348 (87%) patients. One hundred and seventy-two of 304 children were initially treated with a rFVIII product and 132 of 348 (43%) were managed with a plasma-derived FVIII product. In children treated initially

	Number treated	All inhibitors, *n* (%)	*P*-value	High titre inhibitors, *n* (%)	*P*-value
Ethnic origin ($n = 324$)					
Non-caucasian	62	18 (29)	0.161	11 (18)	0.249
Caucasian	262	53 (20)		32 (12)	
Family history ($n = 309$)					
Positive	31	9 (29)	0.189	6 (19)	0.182
Negative	278	52 (19)		31 (11)	
Initial FVIII product ($n = 304$)					
Recombinant	172	47 (27)	0.009	26 (15)	0.173
Plasma derived	132	18 (14)		13 (10)	
Molecular defect ($n = 231$)					
Group 1	141	41 (29)	0.006	26 (18)	0.012
Group 2	90	12 (13)		6 (7)	

Table 4. Factors affecting inhibitor development.

with a recombinant product the overall incidence of inhibitor development was 47 of 172 (27%), with 26 of 172 (15%) developing high titre inhibitors (Table 4). In those who received a plasma-derived product, the incidence of all inhibitors was significantly lower at 18 of 132 (14%; $P = 0.009$) with 13 of 132 (10%) developing high titre inhibitors (Table 4).

Inhibitor development and ethnic origin

Data on ethnic origin were recorded for 324 of 348 (93%) patients. Although information was available on the specific ethnic origin of these patients, due to the relatively small numbers in each ethnic group, patients were divided into two subgroups for statistical analysis. Two hundred and sixty-two of 324 (81%) were of caucasian origin while 62 of 324 (19%) were non-caucasian. Within the non-caucasian subgroup 64% were of Asian or Arab origin and only 18% were of African or Hispanic descent. Inhibitor development was analysed for patients in each ethnic group. The overall incidence of inhibitor development in caucasian patients was 53 of 262 (20%) when compared with 18 of 62 (29%) in non-caucasians (Table 4). High titre inhibitors were recorded in 32 of 262 (12%) of caucasians and 11 of 62 (18%) of non-caucasians (Table 4). Although there was a trend towards a higher incidence of inhibitor development in non-caucasians, this did not reach statistical significance ($P = 0.16$).

Inhibitor development and family history

Data on family history of inhibitor development were available for 309 of 348 (89%) patients. Thirty-one of 309 (10%) of patients had a positive family history of inhibitor development in at least one affected male relative. Of those with a positive family history, the overall incidence of inhibitor development was nine of 31 (29%) when compared with 52 of 278 (19%) in those with a negative family history (Table 4). High titre inhibitors were recorded in six of 31 (19%) of those with a positive family history when compared with 31 of 278 (11%) in those with no family history (Table 4). These figures demonstrated a trend towards a higher incidence of inhibitor development in those with a positive family history but this was not significant ($P = 0.18$; Table 4).

Risk factors for inhibitor development and multivariate analysis

Odds ratios were calculated for the variables potentially likely to influence inhibitor development including age at treatment onset (<1 month; <18 months), ethnicity, family history of inhibitor development, initial FVIII product and molecular defect (Table 5).

Table 5. Odds ratios for inhibitor development.

	Univariate analysis		Multivariate analysis	
Variables	OR	95% CI	OR	95% CI
Onset of treatment <1 month	1.22	0.53–2.81	1.15	0.47–2.85
Non-caucasian origin	1.61	0.86–3.02	1.14	0.46–2.8
Positive family history	1.78	0.77–4.09	1.5	0.52–4.31
Onset of treatment <18 months	2.82	1.16–6.83	1.43	0.5–4.09
Recombinant FVIII product	2.24	1.24–4.04	1.83	0.9–3.72
Major genetic defect	2.67	1.31–5.41	3.34	1.45–7.71

Following logistic regression analysis using backward variable selection the presence of a major molecular defect remained the most important risk factor for inhibitor development (OR 3.34, 95% CI: 1.45–1.71; Table 5).

Discussion

In severe haemophilia A, inhibitors directed against FVIII usually develop during early childhood in association with the onset of FVIII replacement therapy. Whether the actual age at which treatment is commenced is important in determining inhibitor development has become a matter of debate following initial reports from Spain and the Netherlands that early exposure to FVIII during the first 6 months of life was associated with a higher incidence of inhibitor formation [8,9].

Whether early exposure to FVIII is related to inhibitor development has potentially important implications for the initial management of infants with haemophilia, particularly those who require treatment at a very young age. The option of delaying FVIII exposure in young infants was explored recently by Rivard *et al.* in a small pilot study in which the aim was to use rFVIIa in place of FVIII until the age of 2 years. Of the 11 infants in this study, six still required FVIII to control bleeding and four subsequently developed inhibitors, which suggests that this is unlikely to be a feasible approach [11].

Conversely, it has also been proposed that all neonates with severe haemophilia A should receive short-term prophylactic treatment following delivery in order to protect against the risk of cranial bleeding following delivery and it is therefore important to try to clarify whether exposure at this very early stage of life would adversely influence subsequent inhibitor development [12].

In this study, the effects of a number of variables including age at first exposure to FVIII were examined in relation to inhibitor development. In particular, we sought to determine whether treatment commenced during the first month of life was associated with a higher incidence of inhibitor development.

Most children with severe haemophilia A will commence treatment with FVIII at a relatively young age [13,14]. Consistent with previously published data, only a relatively small proportion of infants in this study (10%) required treatment with FVIII during the first month of life. The highest proportion of infants (37%) commenced treatment between 6 and 12 months of age, which coincides with the early stages of mobilization and more than two-thirds of children had been exposed to FVIII by the end of the first year of life.

In common with data published from Spain and the Netherlands [8,9] we did observe a relationship between age at first exposure and subsequent inhibitor development, with those treated at a younger age having a higher incidence of inhibitor development. However, in contrast to previous data we did not observe a stepwise decrease in inhibitor development with increasing age and indeed did not observe any significant difference in inhibitor development in children first exposed to FVIII at different time points during the first year of life. In particular, there was no excess inhibitor development in those first treated during the first month of life. These findings relate to both overall inhibitor development and the development of high titre inhibitors, although even in this relatively large cohort, it should be noted that the number of high titre inhibitors in each age group remained relatively small. The data do, however, support the conclusion that at least during the first year of life, the age of the child when first exposed to FVIII concentrate does not affect subsequent inhibitor development.

The nature of the underlying defect in the FVIII gene is recognized to be an important factor in determining inhibitor development; however, data on molecular defects has not been universally available in previous studies looking at the effect of initial treatment. In the present study, molecular data were available for a high proportion of cases and we were able, not only to examine the effect of the underlying molecular defect on inhibitor development, but also to examine the relationship between age at first exposure to FVIII and inhibitor development in a subgroup of children all of whom shared the same molecular defect.

Based on previously published data, patients were divided into two subgroups with respect to their underlying molecular defect [4]. Group 1 included those with severe molecular defects likely to be associated with negligible circulating FVIII, whereas in Group 2 the defects were less severe and might be expected to be associated with some residual circulating FVIII. Consistent with other published data, children in Group 1 with severe defects had a significantly higher incidence of inhibitor development. This applied to both overall inhibitor development and to the incidence of high titre inhibitors. In addition, in multivariate analysis, the underlying molecular defect in the FVIII gene was the most important predictor of inhibitor development. The relationship between major defects in the FVIII gene

and inhibitor development is postulated to reflect the absence of circulating FVIII, which may affect the development of immune tolerance to FVIII in these patients [15].

As a potential explanation for the observed results, we postulated that those with more severe molecular defects and therefore negligible circulating FVIII might be more likely to commence treatment at an early age than those with some residual circulating FVIII. In effect, could the relationship between age at first treatment and inhibitor incidence simply reflect the more severe genotype and therefore clinical phenotype in that patient?

We therefore examined the relationship between onset of treatment in those with severe and non-severe molecular defects. In this analysis we did observe that a higher proportion of children with severe molecular defects had commenced treatment during the first 18 months of life when compared to those with non-severe defects. This suggests that molecular defects may have some effect on early treatment requirements, which may at least partially account for the effect on subsequent inhibitor development.

Intron 22 inversions are the most common molecular defect in severe haemophilia A. In a subgroup analysis of 122 children, all of whom had an intron 22 inversion, we were able to examine the effect of age at first treatment on inhibitor incidence while controlling for the effect of the molecular defect in the FVIII gene. Importantly in this subgroup, there was no significant difference in the incidence of inhibitors in relation to the age at onset of treatment across any of the age groups. This again suggests that genetic factors may be a crucially important factor modifying the relationship between treatment onset and inhibitor development and highlights the important relationship between genetic and so-called environmental factors in this area.

Other genetic factors analysed in this study include the effects of ethnicity and family history of inhibitor development. In previous studies, the incidence of inhibitors has been observed to be higher in those of African and Hispanic origin [16]. Linkage of inhibitor risk to major histocompatibility genotypes has been postulated as an explanation for this observation but has not been convincingly demonstrated. In the present study, due to the relatively small numbers involved, it was not possible to subgroup those of non-caucasian origin into specific ethnic groups and this grouping is therefore somewhat heterogeneous. It was, however, apparent that there were fewer children of African or Hispanic descent (18%) when compared with those of Asian origin (64%). Despite this we did observe a trend towards a higher incidence of inhibitors in those of non-caucasian origin.

Family history of inhibitor development has also been observed to influence inhibitor development and is most marked in monozygotic twins where concordance is estimated at 90% [17]. Only 10% of cases in this study had a positive family history of inhibitor development and again there was only a trend towards a higher incidence of inhibitors in this subgroup. This may in part reflect the small numbers involved and also the inclusion of cases with a positive family history not just in a siblings, but also in a more distant relative where the effect is likely to be less marked.

There remains considerable debate regarding whether or not the type of factor replacement used in the management of haemophilia significantly affects the likelihood of inhibitor development [7,18–20]. In this study, we have observed a higher rate of inhibitor development in those treated initially with recombinant products. A number of previously untreated patient (PUP) studies have shown different rates of inhibitor development with different products, and in these studies there has also been a tendency to higher rates of inhibitor development in those treated with recombinant products [18]. It has been postulated that these differences may be explained by more intensive inhibitor monitoring in the rFVIII PUP studies when compared with older studies of plasma-derived FVIII. These differences in monitoring potentially make direct comparisons between individual studies misleading, particularly with regard to the incidence of low titre inhibitors. Nevertheless, data in this study are consistent with recently published data by Goudemand *et al.* who observed a higher incidence of inhibitors in those treated with a rFVIII product during the same time period as a second group receiving a plasma-derived FVIII containing VWF [7]. Whether this effect relates to the presence of VWF or other potentially immunomodulatory factors in plasma-derived FVIII products remains unclear at the present time [21].

Other treatment-related factors including the intensity and indications for early FVIII exposure have also been postulated to be important in subsequent inhibitor development but were not specifically addressed in this study [22]. These factors do, however, warrant further investigation in order to clarify the interaction between genetic factors and early treatment patterns in relation to inhibitor development.

Conclusion

The ability to predict which children with severe haemophilia might be most likely to go on to develop inhibitory antibodies has important implications for the management of this condition. In the present study, we have demonstrated a relationship between age at onset of treatment and inhibitor development but in contrast to other published data the effect was not observed during the first year of life and was also significantly influenced by the underlying molecular defect in the FVIII gene. We have also documented the likely higher incidence of inhibitors in those whose initial FVIII exposure is with a rFVIII product. These results highlight the complex influences affecting inhibitor development in haemophilia, which relates to both genetic and treatment variables. Further studies addressing initial treatment patterns are indicated in large groups of children with well-documented molecular defects to try to define which, if any, of these factors may be modified in future treatment protocols.

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