Original Article

Title

Natural history and clinical characteristics of inhibitors in previously treated haemophilia A patients: A case series

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Abstract

Background

Development of inhibitors is the most serious complication in haemophilia A treatment. The assessment of risk for inhibitor formation in new or modified factor concentrates is traditionally performed in previously treated patients (PTPs). However, evidence on risk factors for and natural history of inhibitors has been generated mostly in previously untreated patients (PUPs). The purpose of this study was to examine cases of de novo inhibitors in PTPs reported in the scientific literature and to the EUropean HAemophilia Safety Surveillance (EUHASS) program, and explore determinants and course of inhibitor development.

Methods

We used a case-series study design and developed a case report form to collect patient level data; including detection, inhibitor course, treatment, factor VIII products used, and events that may trigger inhibitor development (surgery, vaccination, immune disorders, malignancy, product switch).

Results

We identified 18 publications that reported 43 inhibitor cases and 45 cases from 31 EUHASS centres. Individual patient data was collected for 55/88 (63%) inhibitor cases out of 12,330 patients. The median (range) peak inhibitor titer was 4.4 (0.5 – 135.0), the proportion of transient inhibitors was 29% and only two cases of ten undergoing immune tolerance induction failed this treatment. In the two months before inhibitor development, surgery was reported in 9 (16%) cases, and high intensity treatment periods reported in 7 (13%) cases.

Conclusions

By studying the largest cohort of inhibitor development in PTPs assembled to date, we showed that inhibitor development in PTPs, is on average, a milder event than in PUPs.

Keywords

haemophilia A, factor VIII inhibitors, previously treated patients

Background

The development of inhibitors, or neutralizing alloantibodies, continues to be the most serious challenge in the treatment of haemophilia A. High titre inhibitors interfere with factor VIll replacement therapy, which often becomes completely ineffective, and are associated with high morbidity and mortality.[1] The highest risk of developing inhibitors in persons with haemophilia A occurs within the first 50 exposures days (ED) to factor VIII; a substantially lower risk has been observed in patients treated for more than 150 ED, who are commonly called previously treated patients (PTPs);[2] indeed, the rate of inhibitor development in PTPs has been estimated about three events (95% CI = 2-4) per thousand patient years.[3] Due to this very low event rate in PTPs, our knowledge about risk factors for inhibitor development is mostly based on studies in previously untreated patients (PUPs), variably defined as patients with < 50 to 150 ED,[4,5] who are mostly young children with severe haemophilia A. On the other hand, current International Society on Thrombosis and Haemostasis (ISTH) and European Medicines Agency (EMA) / Food and Drug Administration (FDA) recommendations for assessment of the immunogenicity of new clotting factor concentrates indicate PTPs as the most suitable population.[4,6–8] The concept behind this recommendation is that persons with haemophilia A previously tolerized to factor VIII will maintain tolerance to sufficiently similar new molecules, while they would react to those presenting important neo-antigens.

For these reasons, many published reports reporting rates of inhibitors in PTPs are available only as part of phase III or IV studies, or as clinical observation reports. The main focus of these publications is to report, discuss and sometimes even compare (though comparisons are of course largely underpowered) rate of inhibitors with different molecules.[9–11] Much

less is known about the natural history of inhibitors development in PTPs or about the triggering risk factors at play, which would be clinically important considering that the life expectancy of patients with haemophilia has doubled since the 1960s from less than 30 to more than 60 years of age,[12] and there is mounting evidence suggesting a higher incidence of inhibitors in PTPs aged 60 to 69 years.[13,14]

To respond to this unmet clinical need, we have examined all cases of new inhibitors in PTPs identified from a systematic review of the literature and two international haemophilia registries.

Methods

We have designed the study as a case series, a design that has been recommended for studying rare adverse events. Indeed, this study design allows us to explore the characteristics of patients over a spectrum of cases, drawing loose inference from the underlying cohort and internal comparisons among cases with different characteristics. The design has high feasibility and is not resource intensive, and can be used as the first exploratory step in planning more robust future studies.[15,16]

Identification of inhibitor cases

Systematic Review

Methods for the systematic review have been published elsewhere.[3]

Haemophilia adverse events surveillance system

The European Haemophilia Safety Surveillance System (EUHASS) scheme collects information on adverse events related to haemophilia treatment, including the development of new inhibitors. For each inhibitor event, information is reported about the patient (age, gender, diagnosis, factor level) and the event (date, factor concentrate, additional blood products, assay, inhibitor levels, positive test cut-off). At the time of the study, EUHASS was in its fourth year. We identified cases of new inhibitors reported to EUHASS.

Case Report Form

We drafted the Case Report Form (CRF) based on current knowledge of development of inhibitors. The CRF was intended to gather additional data that was not often contained in published reports. The draft was circulated for review and feedback to the authors of the publications included in the study and the European Haemophilia Network (EUHANET) network coordinators. The CRF was revised and finalized based on reviewers' comments (Supplemental Table 1).

Data collection

We took a multi-stage approach for contacting study authors and directors of haemophilia treatment centres participating in the EUHASS network to complete a CRF for each PTP with inhibitors. We included in the CRF all the known risk factors for inhibitor development in previously untreated patients (PUPs), as detailed in the Table 1 (see also Supplemental Table 1). All respondents were invited to co-author the study report.

Definitions

Haemophilia was defined as severe for plasma factor VIII levels <0.01 IU/mL; moderate haemophilia, for 0.01 to 0.05 IU/mL level of factor VIII; and mild haemophilia, for 0.06 to 0.40 IU/mL of factor VIII. Previously treated persons (PTP) with haemophilia were defined as patients treated for 50 or more ED due to the lack of an accepted international definition for PTPs and variability in the definitions currently used to identify PUPs. However, we planned to report separately the number of cases falling into the following categories: 50-74, 75 to 149, and >=150 ED. High responders were defined as subjects with a peak titre >5.0 Bethesda Units (BU)/mL at diagnosis. Transient inhibitor was defined as an inhibitor that spontaneously resolved within six months without change in treatment regimen, i.e., without immune tolerance induction (ITI). As to the test used to diagnose inhibitors we accepted methods of Bethesda or its Nijmegen modification, and thresholds for negative values as reported by the authors or case contributors and the available information. Intense FVIII treatment period was as reported by the individual investigators who completed the CRF. Our guidance was that any treatment of 50 U/kg or more for three or more consecutive days would constitute an intense treatment period.

Statistical Analysis

We considered each of the cases for which we were provided CRF as one unit of a case series. We assumed data were missing at random both for inhibitors cases for which we did not get a CRF or information in the CRF was incomplete. Consequently, we described our cases series by calculating central tendencies as mean and standard deviation or median and range, or calculated proportions of cases with specific characteristics as appropriate. For each descriptive measure we reported the actual sample size.

Results

The Preferred Reporting Items for Systematic Reviews (PRISMA) flow diagram provided the details of the case identification and data-gathering process (Figure 1).

Systematic Review

Detailed results of the systematic review are published elsewhere.[3] In summary, we identified 19 publications that reported 38 new inhibitors in PTPs with haemophilia A.

Of the 38 identified inhibitors, we collected individual patient data for 29 (76%) inhibitor cases overall. The source population for the 29 inhibitors was 4,443 patients with haemophilia A (as calculated by summing up the number of patients included in the reports from which the 29 cases were obtained); thus the inhibitor rate was 6.5 per 1000 patients (29/4,443). The data originated specifically from:

- 13 (34%) CRFs completed by study authors for cases reported in nine publications [17–25]
- 16 (42%) CRFs completed by extracting patient level information available from eight published reports. [26–33]

For nine inhibitors (24%) reported in three publications,[26,34,35] data extraction was not possible because the relevant publications included only aggregated summary data and the study investigators were unable to provide individual level data. One inhibitor was also reported to EUHASS.

Haemophilia adverse events surveillance systems

There were 45 cases of new inhibitors in PTPs with severe haemophilia A reported to EUHASS in 31 of 75 participating European treatment centres. Nineteen (61%) of centres reporting inhibitors in the study provided CRF for their 26 cases (60% of all those reported to EUHASS, Figure 1). The source population for the 26 inhibitors in EUHASS was estimated at 7,887 (based on 31,551 patient years of follow-up reported by the centres observing the 26 inhibitor cases – data obtained directly from the EUHASS registry); thus the inhibitor rate was 1.14 per 1,000 patient years. An approximate estimate comparable to the one calculated above from the published literature (based on the sum of the patients enrolled in each study) would be 3.3 per 1000 patients (26/7,887), not taking time into account.

Patient characteristics

In total, 55 cases were identified (29 in published literature, 26 reported to EUHASS). Severity of haemophilia A was available for 54 patients with inhibitors; the majority (48 of 54) had severe haemophilia; four patients had moderate haemophilia; and two patients had mild haemophilia. Thirty-six patients were reported to be White or Caucasian; one patient was Asian and another was Black. Information about ethnicity was missing for the other 18 patients.

Inhibitor characteristics

The inhibitor cases were diagnosed from 1998 (in the literature) to 2014 (reported to EUHASS). Forty-one of fifty-four cases were diagnosed using the Bethesda assay, while for the remaining the Nijmegen modification or a combination of the two tests was used. Forty of forty-three cases used a cut-off for inhibitor of 0.6 BU/ml or lower, and three used a cut off of 1.0 BU/ml.

ED at time of inhibitor detection was reported for 49 patients (of which, 43 had severe haemophilia A). Twenty-seven cases and (25 with severe haemophilia A) had 150 ED or more. Six patients were reported as PTPs by the hemophilia treatment centre to EUHASS (n = 2) or by the study authors in their publication (n = 4), but the numbers of EDs were not provided. Seventeen (15 with severe haemophilia) had reached 75 to 149 ED. Five patients had between 50 and 74 ED; three of these patients had severe haemophilia A, one had mild haemophilia A and the other patient's severity was not reported in the literature.

There were 24 high responders with severe haemophilia A. The peak titre levels for these patients ranged from 5.0 to 135 BU/mL (mean = 30.9). Fourteen of the cases were tested because of clinical signs and ten were clinically significant following diagnosis. The last known titre level of 10 high responders was more than 1 BU/mL. Further details regarding the inhibitors are reported in Tables 2-3.

Frequency of occurrence of known risk factors for inhibitors development

The age of the patient at first factor VIII (FVIII) exposure was known and reported for 31 patients; age at first exposure ranged from six weeks to 55 years (mean = 12.7; SD = 14.7). Eight PTPs had a known family history of inhibitors. Information about the factor FVIII product used before and at inhibitor detection was available for 54 patients with inhibitors (Table 4).

During their lifetime, 14 patients had a surgical procedure, 38 had switched FVIII products, 10 were vaccinated, five had an immune disorder, two had a malignancy, and 14 had a period of intense FVIII treatment (eight of these cases was associated to surgery; Supplemental Table 2)

Data on risk factors during the two months prior to inhibitor detection was provided for

41 cases. Eighteen patients had at least one risk factor during that time period; eight had two (1 had surgery and malignancy, 5 had surgery and intense FVIII treatment, 2 had surgery and switched FVIII products).

Nine patients (of which 4 had > = 150 ED) had surgery. Six patients had severe haemophilia and had the following procedures: surgery for urinary cancer on the same day of inhibitor detection; total knee surgery nine days earlier; knee synovectomy on the same day; unspecified surgery 14 days earlier; dental surgery 21 days earlier; and prostatic adenoma and bladder polyps resection 43 days earlier. One patient had the inhibitor diagnosed on the day of surgery, which was complicated by sepsis; he had switched concentrate one month before surgery, and died on the day of surgery. The following details were reported for the other three non-severe PTPs: peripheral arterial occlusive disease bypass operation five days earlier; and prostate biopsies 20 days earlier (no details provided for one patient). Four severe PTPs and one with unknown severity switched FVIII (mean = 26 days, range = 1 to 60).

Two of these patients switched products 22 and 30 days prior to surgery (also counted above). One severe PTP was vaccinated, and one patient was diagnosed with an allergic reaction (urticaria). Three patients received the following diagnoses of malignancy: prostate cancer; lymphoproliferative disorder; and mesothelioma. Only the patient with prostate cancer also had surgery and was counted above. Seven patients had intense treatment with FVIII (2 had severed hemophilia A). The intense treatment was associated with surgery for five patients. One patient had severe ankle traumatic hemarthrosis and the other patient had a hip bleed following physical exercise.

FVIII genotype was reported for 26 patients, of which 24 had severe haemophilia A

(Table 5).

Clinical course

Sixteen of 48 inhibitors were reported as spontaneously disappearing after six months without treatment. This group included the four patients with moderate haemophilia and 12 patients with severe haemophilia. For these transient inhibitor cases, age varied from two to 61 years and peak titre level ranged from 0.5 to 30 BU/mL. One inhibitor spontaneously resolved after one year. Clinical events following the diagnosis of the inhibitor were reported for 17 of 40 patients, and included haemorrhage, decreased recovery, increased bleeding rate, and hemarthrosis.

Twenty-one of 40 patients required a bypassing agent (rFVIIa or APCC). Patient ages spanned from 1 to 72 years (mean = 38). Peak titres ranged from 2.0 to 135 BU/mL; 16 patients were high responders. Twenty had severe haemophilia and one had mild haemophilia.

Twelve of 40 patients were treated with immune tolerance induction. All had severe haemophilia A. Patients were aged 1 to 48 years (mean = 28), and all but the youngest patient had history of 150 ED or more. Nine patients were high responders with peak titres ranging from 7.0 to 135 BU/mL. For 10 of these 12 cases, ITI was successful.

Of the 55 inhibitor patients, 26 were still alive. Of the 26 inhibitors patients reported to EUHASS, 23 were alive, and were still followed in the reporting centre. Data on live status for cases reported in the literature were available for only six patients, three of which were reported as alive and being followed by the centre.

Discussion

This study reviewed a cohort of 55 cases of inhibitors which developed among approximately 12,000 previously treated patients with haemophilia A. To our knowledge, this is the largest ever cohort of inhibitor cases studied. By using a standardized case report form, we have been able to analyse the characteristics of these patients, the clinical course of their inhibitors and the role of risk factors. Inhibitor development is a complex multifactorial process. A number of risk factors have been identified in previously untreated patients, including non-modifiable risk factors, specifically related to genetics, and modifiable or environmental risks factors. [36] Many previously published papers assessed the inhibitor rate in previously treated patients enrolled in phase III or phase IV studies,[7,22,24,25,37] or presented meta-analyses of such studies.[3,11,38] The main focus of these publications was to report, discuss and sometimes compare rate of inhibitors observed with specific molecules, to define their immunogenicity. Almost no attempt has been made before this study to explore the natural history of inhibitor development in previously treated patients or the triggering risk factors at play.

In this study, most inhibitors developing in previously treated patients were of low titre, and disappeared spontaneously or after a course of immune tolerance induction. The risk conditions more frequently found shortly before inhibitor development were surgery and/or periods of intense treatment with factor VIII. Other conditions considered candidate risk factors for inhibitor development in previously untreated patients (product switching, vaccination, immune disorders, and malignancy) were found less frequently. We believe that the information about the frequency of occurrence of these characteristics is new, clinically relevant and confident it will trigger new research to explore causality.

The only risk factor that has been explored to some extent has been switching factor concentrate, a concept closely related to the one of molecule immunogenicity. Indeed, some reports have discussed whether switching from one concentrate to another (regardless of the specific products) increases the risk of inhibitor development in previously treated patients, as a result of molecular differences[6,9,10,39,40]. However, few of the studies were comparative in nature and, most importantly, none took into account other risk factors concurrent with factor concentrate switching mostly due to insufficient power[40]. By contrast, in the analysis of our cohort, we considered factor concentrate switching as one of several candidate risk factors, and we rigorously adopted a standard and narrow time window around the switch itself; when doing so, switching did not appear to have any important role. Indeed, our analysis confirmed that switching in the two months prior to development of an inhibitor occurred only in 5/52 (10%) cases, of which only three (6%) had factor switching as a single candidate risk factor (the other two patients also had surgery during the two-month time period).

One compelling reason for interest in inhibitor development in previously treated patients stems from the evidence suggesting higher incidence in patients aged 60 to 69 years [13,14]. This is very important considering the increasing life expectancy of patients with haemophilia [12]. One might observe that only six of our cases fell in the above age range; the average age at inhibitor development in our case series was 36 years of age. This might cast doubts about the applicability of our findings to an older population; however, it must be noted that in our case series, mean (36 years) and median (35 years) almost overlapped, and the age range spanned from one to 72 years, suggesting that development of inhibitors in previously

treated patients is a random event, not correlated to age. The average age measured in our study likely overlaps with the average age of the underlying population at risk.

While the major strength of our study is the relatively large number of occurrences of a very rare event, its main limitation is the absence of a control group. We adopted, for convenience and economy, a case series design. This design has been recommended for studying rare adverse events and combines the power and simplicity of the cohort method and the economy of the case-control method, while reducing confounding caused by factors that vary between people. This design would also make it possible to provide richer and more comprehensive information that is usually gathered with randomized controlled trials [15]. We expect that the novelty of the evidence we have been able to produce will prompt the leveraging of resources and willingness to participate in a future matched case-control study, which is needed to confirm or deny the causality of the association we have suggested. We strongly recommend that performing such a study is seriously considered by organizations in the field and we will work with the EUHASS network to assess feasibility of a nested case-control study within their data collection framework. Other possible limitations of our study are the incompleteness of the case series, recall or detection biases. We have been able to gather data for 55 out of 87 cases (63%) reported in the literature and to EUHASS. While we acknowledge that the incompleteness of the case series might introduce bias, we have no specific reason to suspect that missing information is not random. Indeed, the authors and treatment centres tended to report either all or none of their patients. However, we found that the rate of inhibitors was about twice as high in the literature series as compared to the EUHASS data collection (6.5 versus 3.3 per 1000 patients). This difference can be explained by

either over-reporting due to recall bias in the literature series or underreporting of missed data in the EUHASS data collection. The former can introduce bias toward more or less severe cases being reported, the latter likely missing milder cases. In addition, the occurrence of events like surgery or need for intense treatment may have prompted more frequent inhibitor testing, thus increasing the chance of inhibitor detection and introducing potential bias. Finally, different thresholds for diagnosis of an inhibitor (Supplemental Table 3) and the process itself of estimating the denominator could be responsible for the observed difference. On average, we consider our estimates quite conservative, and a more efficient data collection would possibly show an even less severe impact of inhibitors in the natural history of inhibitors in previously treated patients. In regards to inhibitor testing, a minor limitation would also be the non-standardization of the clinical and laboratory cut-off for inhibitor diagnosis (Supplemental Table 3); however, this is less relevant when the inhibitors of interest are clinically significant. Finally, we could not explore the possible role of ethnicity as a risk factor for insufficiency of data and did not collect information about the success/failure criteria for ITI or its duration.

Conclusions

The development of inhibitors in previously treated patients is a rare event, and we have now shown that it is usually milder than one might have predicted. Of course, each individual case deserves full support and care, and each case may be perceived as extremely severe for the patient, family and physicians experiencing the inhibitor. However, on a broader population perspective, the risk of development of inhibitors in previously treated patients might not be considered as relevant information for decisions about individual product

switches or tendering processes. Indeed, the benefits from the availability of new or cheaper products might outweigh the risk and impact of inhibitor development in previously treated patients.

List of Abbreviations

BU = Bethesda units

CHS = Canadian Hemophilia Society

CRF = Case Report Form

ED = Exposure days

EMA = European Medicines Agency

EUHASS = European Haemophilia Safety Surveillance System

EUHANET = European Haemophilia Network

FDA = Food and Drug Administration

FVIII = Factor VIII

ISTH = International Society of Thrombosis and Haemostasis

PTP = Previously treated patient

PUP = Previously untreated patient

PRISMA = Preferred Reporting Items for Systematic Reviews

Declarations

Ethics Approval and Consent to Participate

The study protocol was waived approval by the Hamilton Integrated Research Ethics Board. We recommended that EUHASS centres seek local ethical clearance.

Consent for Publication

Not applicable.

Availability of Data and Material

The dataset generated and analysed during the current study is available from the corresponding author on reasonable request.

Competing Interests

AI has received research funds as Principal Investigator from Bayer, Baxalta, Biogen Idec, NovoNordisk, Pfizer and as consultant from Bayer, NovoNordisk. All funds were paid to McMaster University and none received as personal honorarium. MM has acted as consultant to CSL Behring and NovoNordisk. He took part in an advisory panel organised by BPL and gave lectures for Baxter, Bayer, Biogen Idec, Biotest, Octapharma, Pfizer and SOBI. He received travel support from Baxter and Bayer. MS participated in clinical trials funded by Biotest, Baxter, Bayer, Novo Nordisk, and CSL Behring. VR was a full time employee of Baxter Healthcare, United States when the data analysis was conducted.

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410 *Author Contributions*

411 AI designed the study and wrote the first draft of the paper. AMB contributed to the study
412 design, collected and analysed the data, and co-wrote the first draft of the paper. The
413 remaining authors contributed by providing original data and participated in results
414 interpretation. All the authors approved the final version of the manuscript.

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421

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Tables and Figures

558 **Figure 1: PRISMA Flow Diagram**

559

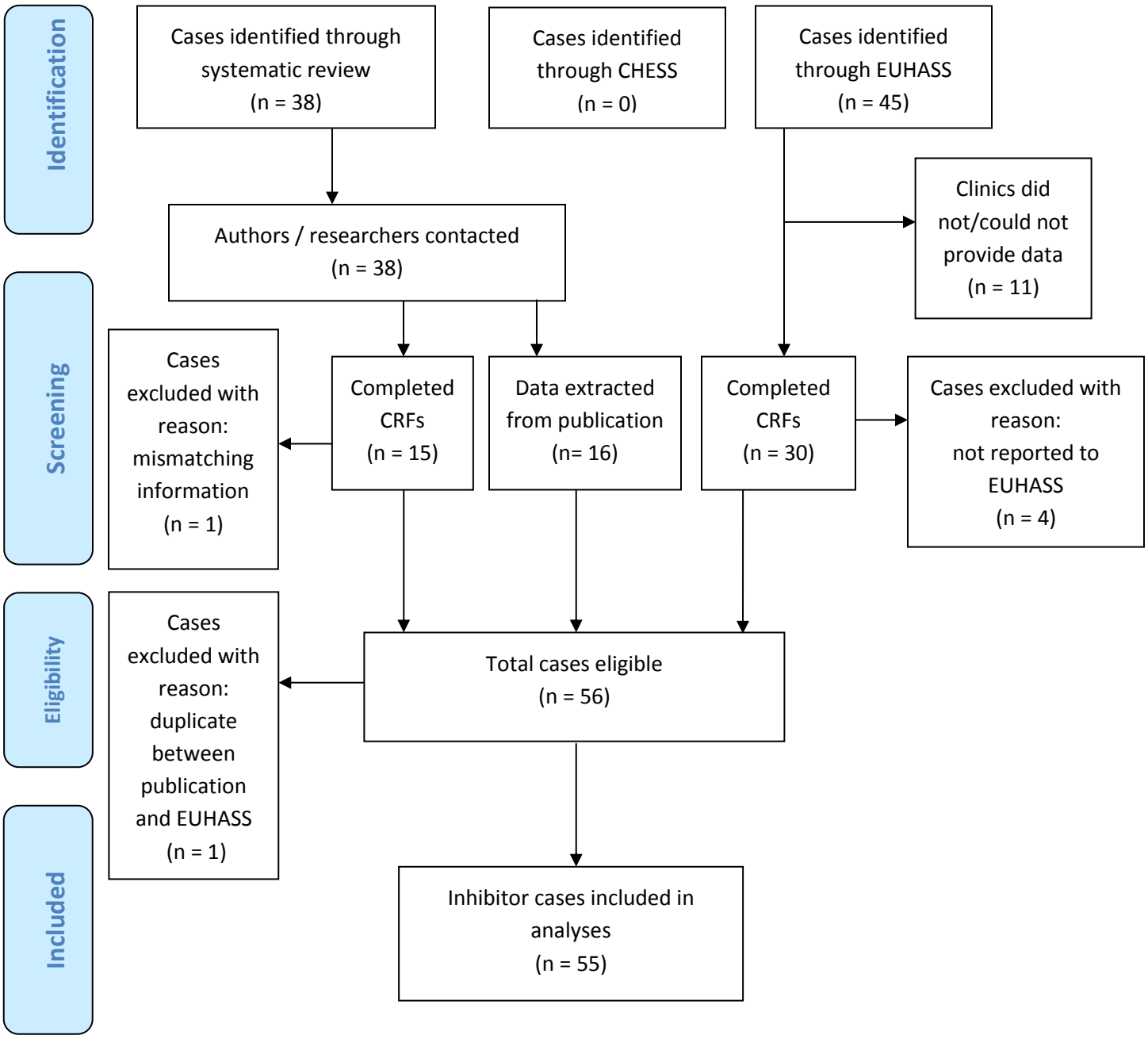

Table 1: **Risk factors for inhibitor formation**

Modifiable: Treatment • Factor VIII concentrate • Regimen (prophylaxis or on demand, dosage, interval) • Age of first exposure to FVlll concentrate
Modifiable: Trigger events or inflammatory responses • Surgery • Vaccination • Intense FVIII treatment periods • Infection or immunologic challenge • Switch in Factor VIII concentrate
Non-modifiable: Genetics • Ethnicity • Family history of inhibitors • Genotype • FVIII mutation

563 **Table 2: Inhibitor characteristics of *all* patients by data source**

Characteristic	N[1]	Mean (SD[2])	Median	Range
Age at inhibitor diagnosis (years)				
Published literature	23	44 (18)	50	2 - 67
EUHASS registry	26	29 (18)	32	1.1 - 72
All	49	36 (19)	35	1.1 - 72
Exposure days (ED) at diagnosis[3]				
Published literature	25	150 (76)	120	50 - 363
EUHASS registry	24	280 (372)	150	55 - 1850
All	49	215 (273)	150	50 - 1850
Titre level at first assessment, BU/mL[4]				
Published literature	28	4.4 (8.4)	1.2	0.4 – 34.0
EUHASS registry	26	9.0 (14.2)	3.1	0.6 – 54.0
All	54	6.6 (11.6)	1.6	0.4 – 54.0
Peak titre level, BU/mL				
Published literature	25	11.1 (18.6)	2.4	0.5 – 75.0
EUHASS registry	26	20.0 (30.9)	7.5	0.8 – 135.0
All	51	15.7 (25.8)	4.4	0.5 – 135.0
Last known titre level, BU/mL				
Published literature	15	1.5 (2.6)	0.4	0.0 – 10.4
EUHASS registry	26	3.4 (8.6)	0.5	0.0 – 41.0
All	41	2.7 (7.0)	0.4	0 – 41.0
Patient follow-up after inhibitor diagnosis, months[5]				
Published literature	10	62 (59)	40.5	1 - 143
EUHASS registry	22	43.6 (42)	29.5	1 -166
All	32	49.3 (48.6)	29.5	1 - 166

564 [1]N=number of patients with available data
565 [2]SD=standard deviation
566 [3]5 patients had EDs of 50, 55, 59, 65 and 68 EDs at time of inhibitor detection; 17 patients had 75 to 143
567 EDs, and 27 had >= 150 ED; ED were not reported for 6 patients
568 [4]BU/mL=Bethesda units per millilitre
569 [5]4 patients followed up for less than 1 year.
570

571

572 **Table 3: Inhibitor characteristics of *severe* haemophilia A patients (n=48)**

Characteristic	N[1]	Mean (SD[2])	Median	Range
Age at inhibitor diagnosis, years	43	34 (19)	36	1.1 – 72.0
Exposure days (ED) at diagnosis[3]	43	227 (287)	150	55 – 1850
Titre level at first assessment, BU/mL[4]	48	6.8 (12)	1.6	0.39 – 54.0
Peak titre level, BU/mL	47	16.8 (26.3)	4.8	0.7 – 135.0
Last known titre level, BU/mL	38	2.9 (7.1)	0.5	0.0 – 41.0
Patient follow-up, months	30	50 (49)	30	1 - 166

573 [1]N=number of patients with available data
574 [2]SD=standard deviation
575 [3]3 patients with EDs of 55, 59 and 65 EDs at time of inhibitor detection; 15 patients had 75 to 143 EDs,
576 and 25 had 150 ED or more; ED was not reported for 5 patients
577 [4]BU/mL=Bethesda units per millilitre.
578

579

Table 4: FVIII use before and at inhibitor detection

Characteristic at inhibitor developent	All	Severe only	150 ED or more
Product used			
Recombinant[1], all	43[2]/54	37/48	21/27
Plasma-derived, all	11[3]/54	11/48	6/27
Treatment indication			
On demand	20/38	19/33	13/26
Prophylaxis	14/38)	12/33	8/26
Surgical prophylaxis	4/38	2/33	2/26

[1]Top recombinant products: Kogenate (n=11), Refacto AF/Zyntha (n=11), Advate (n=4), Helixate-Nexgen (n=3)

[2]Of the 43 patients, 14 were previously on another recombinant product, 10 were on a plasma-derived product and 1 patient switched from another unspecified product

[3]Of these 11 patients, 5 were previously on a different plasma-derived product, 2 were on a recombinant product, 2 never switched their product, 2 switched from other products, for which there are not available details

589 **Table 5: Reported details of known FVIII genotype for 26 patients**

FVIII genotype details	Severe only (n)	Non-severe (n)
Intron 22 inversion	11	0
Missense mutations, without further specifications	2	0
c.971>G, pTrp33Gly	1	0
Small inversion A 6960 6961	1	0
Stop codon in exon 16	1	0
Stop codon 1198 in exon 14	1	1
p.Arg2169His	0	1
p.Gly470Arg	0	1
p.ArgR1997TrpW	1	0
p. Val 253 Phe	1	0
pR1997W	1	0
p.Asn1460LysfsX2 (insertion of nucleotide A in a stretch of 9 A in exon 14; stop codon)	1	0
Arg3Gly	1	0
Deletion R1696 (A3 domain)	1	0
Complex gene rearrangement - not typical IVS 22	1	0

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