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Inhibitor development in haemophilia according to concentrate: 4-year results from the European HAemophilia Safety Surveillance (EUHASS) project.

Fischer K[*,†], Lassila R[‡], Peyvandi F[§,¶], Calizzani G[**],Gatt A[††], Lambert T[‡‡], Windyga J[§§], Iorio A[¶¶,†††], Gilman EA[***] Makris M[†††] on behalf of the EUHASS participants.

[*]Julius Center for Health Sciences and Primary Care and [†]Van Creveldkliniek, University Medical Centre Utrecht, The Netherlands ;[‡]Department of Hematology, Unit of Coagulation Disorders, Helsinki University Central Hospital, Finland; [§]Angelo Bianchi Bonomi, Hemophilia and Thrombosis centre, IRCCS Ca' Granda Ospedale Maggiore Policlinico, and [¶]Department of pathophysiology and Transplantation, University of Milan, Milan, Italy; [**]European Haemophilia consortium and Italian National Blood Centre, Rome, Italy [††]Mater Dei Hospital, Tal-QRoqq, Msida, Malta; [‡‡]Hopital Bicetre, Paris, France; [§§]Institute for Hematology and Transfusion Medicine, Warsaw, Poland; [¶¶]Department of Clinical Epidemiology and Biostatistics, McMaster University, Hamilton, Canada; [†††]Department of Medicine, McMaster University, Hamilton, Canada [***]Department of Cardiovascular Science, University of Sheffield, Sheffield, UK; [†††]Sheffield Haemophilia and Thrombosis Centre, University of Sheffield, Sheffield, UK.

Correspondence:

Kathelijn Fischer MD PhD
Julius Center for Health Sciences and Primary Care and Van Creveldkliniek
University Medical Center Utrecht
Room C01.425
PO Box 85500
3508 GA Utrecht
The Netherlands
Tel +31 88 755 8450
Fax +31 88 755 5438
Email: k.fischer@umcutrecht.nl

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Abstract

Background:

Inhibitor development represents the most serious side effect of haemophilia treatment. Any difference in risk of inhibitor formation depending on the product used might be of clinical relevance.

Objective:

To assess inhibitor development according to clotting factor concentrate in severe haemophilia A and B.

Methods:

The European Haemophilia Safety Surveillance (EUHASS) was set up as a study monitoring adverse events overall and according to concentrate.

Since October 2008, inhibitors were reported at least quarterly. Number of treated patients was reported annually, specifying the number of patients completing 50 exposure days (PUPs) without inhibitor development. Cumulative incidence, incidence rates and 95% Confidence Intervals (CI) were calculated.

Results:

Data from Oct 1st, 2008 to Dec 31st, 2012 were analyzed for 68 centers that validated their data. Inhibitors developed in 108/417 (26%; CI 22-30%) PUPs with severe haemophilia A and 5/72 (7%; CI 2-16) PUPs with severe haemophilia B. For Previously Treated Patients (PTPs), 26 inhibitors developed in 17,667 treatment years (0.15/100 treatment years (CI 0.10-0.22)) for severe haemophilia A and 1/2836 (0.04/100;(CI 0.00-0.20) for severe haemophilia B. Differences between plasma-derived and recombinant concentrates, or among the different recombinant FVIII concentrates were investigated.

Conclusion:

While confirming the expected rates of inhibitors in PUPs and PTPs, no class or brand related differences were observed.

Keywords: Adverse Drug Reaction Reporting Systems

Antibodies, Neutralizing

Factor VIII

Haemophilia A

Haemophilia B

Introduction:

Haemophilia A and B are rare diseases affecting 1 in 10,000 and 1 in 50,000 individuals respectively (1). Since the development of cryoprecipitate in the 1960s(2) and clotting factor concentrates in the 1970s, a very effective replacement therapy is available for treatment of these patients. Overall treatment availability, together with the development of home-treatment and prophylaxis, has dramatically improved outcome and quality of life of haemophilia patients (3,4). Unfortunately, plasma-derived concentrates manufactured before the mid-1980s were contaminated with Hepatitis C and HIV virus, infecting many of the recipients. Since then, while viral inactivation has been implemented in the manufacturing of plasma derived concentrates (1), recombinant concentrates, virtually devoid of any transfusion infection risk, have been developed and became widely used in several European countries. Nowadays, the development of anti-factor VIII/IX inhibitors is the most frequent side effect of haemophilia treatment. This is most likely to occur during the initial 50 exposure days (EDs) to concentrate (in 25-32% of patients with haemophilia A and 5-10% of patients with haemophilia B (5-7)) but can also occur, in previously treated patients (PTPs), though much more rarely (1-5 per 1000 treatment years)(8). Treatment and prevention of bleeding in patients with inhibitors and inhibitor-eradication are the biggest challenges in haemophilia care today, making inhibitor prevention an important target.

In addition to endogenous risk factors, such as FVIII gene mutation, treatment related factors like intensive treatment are also important. In this context, many hypotheses have been proposed about the potential role of FVIII concentrate type in inhibitor development. Some have suggested that plasma derived FVIII concentrates are associated with less inhibitor development (9), others suggested that this finding could be due to confounding by study characteristics (10). Therefore, a randomized trial was initiated to address the issue in previously untreated patients (PUPs) (11).

Recently, the large multicentre observational study (RODIN) originating from the PedNet registry, reported that second generation recombinant FVIII concentrates were associated with more frequent inhibitor development in PUPs with severe haemophilia A (12). In addition, it has been suggested that

a B-domain deleted product (BDD rec FVIII) was associated with a higher inhibitor risk in previously treated patients (PTPs), a result which was not confirmed in a subsequent meta-analysis (8,13).

Early identification of risks of transmission of blood borne pathogens or inhibitor development are best addressed by prospective surveillance strategies or large cohort studies (12,14). Studies carried out by manufacturers as part of their licensing application typically involve highly selected small cohorts of usually less than 100 patients with a limited follow up. The European Haemophilia Safety Surveillance (EUHASS) commenced in 2008 to monitor treatment safety according to clotting factor concentrate brand and class in a large number of sentinel centres in Europe (15).

The present study reports the first four-year results of monitoring for inhibitor development in haemophilia in the EUHASS study.

Methods:

The design of the EUHASS study has already been reported (15,16). EUHASS is an international multicenter pharmacosurveillance system covering an open population. Briefly, data collection started on October 1st in 2008, or subsequent years for centres that joined EUHASS later. Subsequently, centres provided reports on all new inhibitors diagnosed at the centre every three months, using a secure web-based data-entry system. Inhibitors were defined as two consecutive tests above the local laboratory threshold; information on recovery tests was not collected, nor was central laboratory confirmation performed. For each patient with an inhibitor, anonymized data on age, type and severity of haemophilia, cumulative number of EDs to FVIII/FIX concentrate before inhibitor development (for each concentrate used), the date of the last negative inhibitor titre, the dates and titres of the first two positive inhibitor titres, the type of inhibitor test used, and the local threshold for positive inhibitor testing were collected. EDs were recorded up to 1000 EDs, and coded as >999 EDs for patients with 1000 EDs or more. Only new inhibitors with positive titres on two occasions were considered.

Data on the total number of patients at risk of inhibitor development are collected annually. The number of PUPs with severe haemophilia at risk for inhibitor development was established by collecting the number of PUPs reaching 50 EDs *without* developing an inhibitor in the previous year. These data were captured according to the concentrate used at the time of reaching 50 EDs including details of switching concentrate.

For PTPs, the number of treatment-years without inhibitor development was established by collecting the number of PTPs without inhibitor development who continued on a concentrate, as well as the number of PTPs switching to this concentrate during the year of observation.

The present analysis was based on data in patients with severe haemophilia A and B from Oct 1st 2008 to Dec 31st 2012.

Logical checks as well as checks for completeness of data are performed on each adverse event at the time of reporting. In the spring of 2013, all centres were asked to confirm accuracy and completeness of their data on adverse events and patients at risk entered up to Dec 31st 2012. Logical checks for

reported inhibitors included checking consistency of data, details provided in comments, age of patient, and check of inhibitor titre according to the local reference standard. Logical checks for non inhibitor patients included checks for consistency with other summary tables (e.g. total number of patients treated according to diagnosis with data on number of patients treated according to concentrate), consistency over the years and distributions of patient categories (e.g. proportion with haemophilia B, number of PUPs reaching 50 EDs relative to the total number of severe patients). Only data from centres with fully checked data and resolution of all queries were included in this analysis. Prior to study entry all centres approached their institutional review board for approval. Regulations in the 26 European Countries participating vary, and for the majority of centres no formal approval was required. If required, approval was obtained before study participation.

Statistical analysis:

The cumulative incidence of inhibitor development in PUPs according to product was calculated by dividing the number of patients who developed inhibitors by the sum of the number of inhibitor patients and the number of patients who reached 50 ED without inhibitor development (15).. In case of switching concentrates, patients with inhibitors were classified according to the last concentrate used. The validity of the calculation method was established by simulation studies. (16) The exact method (Wilson modification) was used to calculate 95% confidence intervals (CI) for data on PUPs (17), without adjusting for the unknown number of patients not yet reaching 50 ED, with the possible result that the reported confidence intervals might be larger than the actual ones. For PTPs, the inhibitor rate per 100 treatment-years was calculated for each concentrate. Patients who switched to this concentrate during the year of observation were assumed to have been using the concentrate for 6 months. The total observation period was 4.25 years, as the fourth year of the registry covered the period from Oct 1st 2011 to Dec 31st 2012. Confidence intervals for data on PTPs were calculated using the exact method (18).

All analyses were performed separately according to diagnosis (haemophilia A or B) and treatment history (before or after completing the first 50 EDs). Within these groups, inhibitor development was compared according to concentrate type (plasma derived vs recombinant), origin of recombinant concentrate (Baby Hamster Kidney cells or Chinese Hamster Ovary cells) as well as according to the different concentrates. For PUPs with severe haemophilia A, a sensitivity analysis was performed by excluding data collected during the first three years of EUHASS, for patients reported by centers participating in the RODIN study. The RODIN study included patients born up to Jan 1st 2010, collected data until May 1st 2011 (HM van den Berg, personal communication), and the median age at reaching 75 ED was 26 months (6). Year 4 of EUHASS started in Oct 2011, a minimum 22 months after the last inclusion in RODIN, so none of the inhibitors could be duplicated and most patients reaching 50 EDs during this year were likely to be born after Jan 1st 2010. All analyses were performed using SPSS version 20.

Results

Since the start of the EUHASS registry in 2008, the number of centres submitting data on inhibitor formation and number of patients at risk for PUPs increased from 40 to 60, and the number of centres reporting full data on PTPs increased from 49 to 74; 14 centres treat only adult patients. An overview of data submitted and inhibitors according to diagnosis for PUPs and PTPs is shown in Table 1. Failure to confirm submitted data resulted in exclusion of events and PUPs at risk from three centres that reported six inhibitors and 10 non-inhibitors, resulting in the inclusion of 95% of all inhibitors in PUPs. For PTPs, events and numbers at risk from six centres, including six inhibitors in 2290 treatment years, were excluded from the analysis resulting in the inclusion of 92% of centres and 82% of inhibitors in PTPs. Overall, centres analysed treated a median of 75 patients with severe haemophilia (interquartile range (IQR) 34-131; range 7-428).

Inhibitor development in PUPs with severe haemophilia

Characteristics of all inhibitor patients reported to EUHASS and after exclusion of those reported in the RODIN study, are shown in Table 2. There were no missing data on inhibitor patients. Inhibitors developed very early, at a median age of 1.3 years and 13 EDs for PUPs with haemophilia A and 2.1 years and 11 EDs for PUPs with haemophilia B. Only the first two positive inhibitor titers were reported to EUHASS, taken with a median interval of 13 days (IQR 6-28). Exclusion of patients reported in the RODIN study (i.e. in years 1-3 of EUHASS for centres participating in both studies) resulted in a 35% reduction of reported inhibitors in PUPs with severe haemophilia A (from 108 to 70) and in a 29% reduction of the observed PUP population (from 417 to 297). Characteristics of all inhibitor patients reported and those reported to EUHASS only were similar.

Overall, 108 inhibitors were observed in 417 PUPs with severe haemophilia A (25.9%, CI 21.8-30.4) and five inhibitors in 72 PUPs with severe haemophilia B (6.9% CI 2.3-15.5). Inhibitor development in

PUPs according to concentrate type is shown in Table 3. Product switching occurred in only 4.3% of PUPs: in 2/108 inhibitor patients and in 16/309 patients without inhibitors. Inhibitor development was similar across plasma derived and recombinant FVIII concentrates, with 21.6% and 26.5% inhibitor incidence respectively, but the number of patients treated with plasma derived products was low (12% of all PUPS reported).

When comparing different recombinant FVIII concentrates according to product and cell-line, only marginal differences in inhibitor incidences were observed, with consistent large overlaps between the respective confidence intervals.

After exclusion of overlapping data from the RODIN study, the overall inhibitor development in PUPs with severe haemophilia A was similar at 23.2% (69/297, CI 18.6-28.6%). During these 3 years of overlap between the two studies, the relative risk of inhibitor development in Kogenate Bayer/Helixate NextGen compared to Advate in the overlapping subgroup of the sample was very similar to the HR observed in RODIN: 1.67 (CI 0.95-2.95), while no difference was seen in the not overlapping subgroup (0.95, CI 0.59 – 1.56; see Table 3 for number of events and patients at risk). Indeed, the cumulative incidence of inhibitors for Kogenate Bayer/Helixate NextGen in the participating RODIN centres appeared increased at 44.7% (CI 28.6-61.7) compared to 25.7 % (CI 18.3-34.8) in the remaining EUHASS centres (statistical significance not reached). Another difference between centres participating in RODIN and the remaining centres was the distribution of recFVIII concentrates. During the four years of observation, centres participating in RODIN used significantly more Advate (49.7% (CI 41.7-57.7) vs 30.0% (CI 23.8-36.7)) and slightly less Kogenate Bayer/Helixate NextGen (34.0% (CI 26.6-41.9) vs 43% (CI 36.2-50.0)) than those who did not participate in RODIN.

Only 11 inhibitors were observed in 51 PUPs treated with nine different plasma derived FVIII concentrates. None of inhibitor patients switched products, and 2/40 (5%) patients without inhibitor development switched products before reaching 50 EDs. Details on inhibitor development in PUPs with severe haemophilia A and B according to plasma derived concentrates are shown in Appendix A.

Inhibitor development in PTPs with severe haemophilia

Characteristics of inhibitors in patients with severe haemophilia occurring after the 50th ED are shown in Table 4, again there were no missing data. Inhibitors in PTPs with severe haemophilia A developed at a median age of 25 years and 294 EDs (IQR 95- >1000). Some of these inhibitors occurred quite early: 11/26 PTPs developed their inhibitor between 50 and 150 EDs, including five of these eleven 'early' inhibitors developing before the age of five years. Median first inhibitor titre was 4.6 BU, with only 54% of patients presenting with high titre inhibitors in the first two measurements. In PTPs with severe haemophilia B, only one inhibitor was diagnosed at age 1.9 years after 60 EDs.

Inhibitor development according to concentrate type for PTPs is shown in Table 5. For PTPs with severe haemophilia A, 26 inhibitors were observed in 17667 treatment years, resulting in an overall inhibitor rate of 0.15/100 treatment years (CI 0.10-0.22). When comparing plasma derived and recombinant FVIII concentrates, as well as different FVIII concentrate groups and individual recombinant concentrates, all inhibitor rates were similar with overlapping 95% confidence intervals. The only exception was ReFacto, represented by only 209 treatment years, which showed a higher inhibitor rate. Notably, the inhibitor rate reported for ReFacto was significantly higher than the inhibitor rate reported for its newer version ReFacto AF, represented by 2338 treatment years.

For PTPs with severe haemophilia B, only one inhibitor was observed in a total of 2836 treatment years, resulting in an inhibitor rate of 0.04/100 treatment years (CI 0.001-0.20). Due to these low numbers, it is impossible to reliably compare inhibitor rates between different concentrate types or individual FIX concentrates. Details on different plasma derived FVIII and FIX concentrates are shown in Appendix B.

Discussion:

Main findings

With data on 489 PUPs with severe haemophilia and 20,503 treatment years in PTPs with severe haemophilia, the present study reports on one of the largest dataset collected following a pre-specified protocol to date.

The cumulative incidence of inhibitors in PUPs with severe haemophilia A was 25.9% (CI 21.8-30.4) and 6.9% (CI 2.3-15.5) in PUPs with severe haemophilia B. Inhibitor development was similar across different FVIII concentrates. Inhibitor rates in PTPs with severe haemophilia A were 0.15/100 treatment years (CI 0.10- 0.22) and for haemophilia B 0.04/ 100 treatment years (CI 0.001-0.20). Patient numbers were too low to compare different FIX concentrates.

Study design

An important strength of the present study is the homogenous data collection following a prespecified protocol from a large number of centres throughout Europe, including centres of different size and countries using different treatment strategies. This variability increases the generalizability of the results and is in sharp contrast with national studies with more homogeneous treatment strategies, and international studies predominantly including large centres (5,12,19). If one of the concentrates is associated with a true risk of increased inhibitor development, this effect should be observed independent of the treatment strategy used, provided a sufficient number of patient and events is observed. The present study is the largest attempt to date of prospectively assembling a large sample of non-selected patients treated in parallel with different concentrates over the same period of time and in several centres , which is an important advantage in comparison to single-concentrate PUP-studies conducted by manufacturers. Due to the need for inclusion before the first exposure, these studies can almost exclusively include patients with a known family history of haemophilia, and are expected to include more patients with a negative family history of inhibitors.

The validity of the results of EUHASS is dependent on a constant use of the concentrates involved (15). The distribution of concentrates used remained stable across the four years of observation (supplemental data) and the validity of the results are confirmed by the observation of a slightly increased inhibitor risk for PUPs treated with Kogenate Bayer/Helixate NextGen in the data overlapping with RODIN. Weaknesses of the present study concern the level of detail of the data collected and the confirmation that all patients were included. Indeed, as in most pharmacosurveillance systems, non-differential misclassification bias could have take place, reducing the power to detect a difference where a difference was true. In fact, EUHASS was built with the main aim of accruing a large population sample, reason for which it was not possible to plan and perform source data monitoring (including confirmation that all patients were included), collect data on confounding factors or perform central testing of inhibitors. In this case, data control other than consistency checks was not included in the original protocol. This was acknowledged and corrected during the project: all reports of side effects are being checked for inconsistencies, and all centres were asked to review their data and answer queries for the present analysis. Centres were asked to report all inhibitors diagnosed, and all patients treated according to diagnosis and concentrate. The inclusion of all patients treated was checked by comparison with data on previous and subsequent years reported by the centre. Most centres answered our queries, resulting in inclusion of 95% of inhibitors reported for PUPs, and 82% of inhibitors reported in PTPs. Monitoring of source data and consistency checks are performed in the PedNet registry (12), but the United Kingdom Haemophilia Centre Doctors' Organisation (UKHCDO) registry applies consistency checks only (G. Dolan, personal communication).

The risk of misclassification of inhibitor development according to concentrate due to product switching is considered very low as only 4.3% of PUPs reported concentrate switches.

In general, the power of studies in PUPs with severe haemophilia is limited by patient numbers. After four years, EUHASS was powered to detect an absolute increase of 12% in the cumulative incidence of

inhibitors compared to a baseline of 24% in PUPs treated with Advate (post-hoc calculation, one sided alpha 0.05, power 0.80).

Comparison with other studies

In EUHASS, the cumulative incidence of inhibitors in PUPs with severe haemophilia A was 25.9% (CI 21.8-30.4), which is similar to data from large cohorts from Italy (151/565: 27%)(20), the European CANAL study (87/377: 24%)(5), and the RODIN study reporting 32.4% (CI 28.5-36.3%) inhibitors in 574 PUPs with severe A. Although the overlap between EUHASS and the RODIN study could be approximated, it was impossible to exclude overlapping data at patient level for several reasons: first, date of birth was unavailable in EUHASS; second, RODIN followed patients until 75 and EUHASS until 50 EDs; third, the analysis was different: RODIN applied survival analysis and expressed treatment as exposure days (calculating Hazard Ratios), while EUHASS collected data at reaching 50 EDs or inhibitor development only and did not consider time in the analysis (calculating cumulative incidences and relative risks); finally, the EUHASS study did not collect information on other risk factors for inhibitor development and could therefore not perform adjusted analyses. Central testing for inhibitors was not performed in either study. Despite the differences in methods, the proportion of high titer inhibitors was similar: 69% in RODIN and 63% in EUHASS. However, the increased risk of inhibitor development in patients treated with Kogenate Bayer/Helixate NextGen in the RODIN study (unadjusted Hazard Ratio 1.37 (CI 0.93-2.01) adjusted Hazard Ratio 1.60 (CI 1.08-2.37) (12)) was much less pronounced in EUHASS at 30.8% vs 26.2% (relative risk 1.17 (CI 0.81-1.70); odds ratio 1.25 (CI 0.75-2.09), and disappeared when excluding the 120 patients who were likely reported in both studies (25.7% vs 25.9%, relative risk 0.96, CI 0.59-1.56).

So far, the EUHASS data do not provide an explanation for the discrepancy with RODIN findings. As a consequence of the attempt to enrol all eligible patients in all participating centres, it is unlikely that the failure to confirm RODIN findings is explained by patient characteristics. Differences at centre level, especially different distribution of recombinant FVIII concentrates used in the centres

participating in RODIN compared to the other centres and differences in clinical management may play a role, but this can not be studied as details on treatment and other risk factors for inhibitor development were not collected in EUHASS. Unfortunately, the patient numbers were still too low to compare inhibitor development at centre level. In theory, patients with an increased inhibitor risk may have been prescribed Kogenate Bayer/Helixate NextGen following the publication of a very low inhibitor risk of 15% on Kogenate Bayer in 2005 (21). To explain the discrepancy with RODIN however, this selection bias must have occurred in the RODIN centres only.

The results of the present study cannot be directly compared with those of two recent meta-analyses of studies reporting on inhibitor development in PUPs with severe haemophilia A, mainly because all of them only reported indirect comparisons (22,23). Interestingly, these meta-analyses did not observe differences in inhibitor development between concentrates in prospective studies, nor could they identify differences in study design explaining these findings.

Regarding inhibitor development in PTPs with severe haemophilia A, Xi (8) analyzed 33 studies including 4323 patients and observed an overall inhibitor rate of 0.17/100 treatment years (CI 0.13-0.23), which is highly compatible with the rate of 0.16/100 treatment years in EUHASS. Within the limitations of incomplete reporting in some studies, inhibitor rates were similar across FVIII concentrates. Similarly, Kempton et al reported an inhibitor rate of 0.23/100 person years in PTPs with severe haemophilia A (24), with similar inhibitor development across FVIII concentrates. All brands and generations of recombinant factor VIII presented overlapping rates in EUHASS, with the exception of Refacto in PTPs, with four inhibitors in 209 treatment years. Although Refacto has previously been associated with an increased inhibitor rate (13), the present finding in EUHASS needs to be interpreted with caution as it is based on a very limited number of treatment years, and was observed in PTPs only.

In a report on 2528 British patients with severe haemophilia A, Hay reported on inhibitor development according to age, including both PUPs and PTPs. Over a period of 20 years, 51% of inhibitors occurred

after the age of 5 years. In these patients, the inhibitor rate was 160/26447 treatment years (0.60/100 years, CI 0.52-0.71), which is significantly higher than the inhibitor rate reported in PTPs in the current study (14). Moreover, inhibitors developed much earlier in EUHASS: in 4.25 years of follow-up, 98% of inhibitors in PUPs, and 23% of all inhibitors in PTPs were detected before the age of 5 years. Further comparisons are needed to explain this discrepancy.

A striking observation in EUHASS is that 11/26 inhibitors in PTPs with haemophilia A developed between 50 and 150 EDs, including five of these eleven 'early' inhibitors developing before the age of five years. Unfortunately, denominator data are for patients reaching 150 EDs are unavailable and the exact inhibitor rate up to 150 EDs cannot be estimated. Overall, 81% of all FVIII inhibitors developed before 50 EDs, and only 8% between 51 and 150 EDs. These data are insufficient to change the current observation period for inhibitor development in PUPs, but they suggest that regular inhibitor testing should be continued after reaching 50 EDs.

Data on inhibitor development in patients with haemophilia B are very scant. From the American Universal Data Collection project, Peutz reported a prevalence of 67/1367 (4.9%) in severe haemophilia B patients, without considering differences across concentrates (25).

Clinical relevance

The issue of potentially increased inhibitor development in PUPs on Kogenate Bayer/Helixate NextGen remains unresolved. The data generated from this study suggest that all products available can be used in PUPs and PTPs with severe haemophilia A, since no differences large enough to be of clinical and statistical relevance were seen. If a concentrate is associated with increased inhibitor development, this should be observed independent of other risk factors. The present study was only powered to detect a 50% increase in inhibitor risk: from 24% to 36%. To detect an increase from 24% to 31, 500 PUPs on Kogenate Bayer/Helixate NextGen and 1000 PUPs on other concentrates are needed (alpha error 0.05 and power 80%). Multivariate analyses adjusting for other risk factors of

inhibitor development would have more power to detect small differences and would further increase our confidence in the absence of a difference.

Currently, the UKHCDO and the French Coag registries (www.francecoag.org) are also analysing the effects of different recombinant FVIII concentrates on inhibitor development in PUPs. Combining data from different studies may be used to achieve sufficient data to detect smaller differences, but great care should be taken to avoid double reporting of single patients due to overlaps between databases. Combining data with additional studies may soon be possible: since 2012 Canada is running the CHESS registry based on the EUHASS design (http://www.ahcdc.ca/index.php/national-studies/chess), and a similar system open to other countries is currently being developed. The American Universal Data Collection system has been in place since 1998 and is expected to generate information too (26). Until then, we are not in the position to provide treatment recommendations but clinicians need to make decision based on all the available published evidence. Our data suggest that the issue is not definitively settled and there is uncertainty.

Conclusion:

The cumulative incidence of inhibitors in PUPs with severe haemophilia A was 25.9%, and 6.9% in PUPs with severe haemophilia B. Inhibitor rates in PTPs with severe haemophilia A were 0.15/100 treatment years, and 0.04/ 100 treatment years for severe haemophilia B. Non-adjusted inhibitor rates were similar across currently available FVIII and FIX concentrates for both PUPs and PTPs.

Authorship:

K Fischer, M Makris, R Lassila, F Peyvandi, G Calizzani, A Gatt, T Lambert and J Windyga conceived and designed the study. M Makris and E Gilman secured funding and managed study procedures (governance and data management). M Makris, K Fischer, and E Gilman coordinated and performed datachecking. K Fischer planned and undertook the statistical analysis, which was independently repeated by A Iorio. All authors performed data interpretation. K Fischer and M Makris drafted the manuscript, which was completed with input from all authors. All the authors approved the final manuscript.

Datacollection was performed by K Fischer, R Lassila, F Peyvandi, A Gatt, T Lambert, J Windyga ,A Iorio, M Makris and all collaborators/centers collaborating in the EUHASS project (separate list)

A list of collaborators to the EUHASS project is shown in the supplementary material.

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Competing interests:.

KF has acted as a consultant and participated in expert groups for Bayer, Baxter, Biogen, CSL Behring, NovoNordisk and Pfizer, has received research grants from Baxter, NovoNordisk, Pfizer, and has given invited educational lectures for Bayer, Baxter, NovoNordisk, and Pfizer, and has received travel support from Baxter and Bayer.

FP has given invited educational lectures for Novo Nordisk, CSL Behring, Bayer and Baxter, and has recerved a research grant from Novo Nordisk

Al had received research grants from Bayer, Baxter and Pfizer and honoraria for advisory board participation and invited educational lectures from Bayer, Baxter and Pfizer.

TL received honoraria for consultancy, advisory board participation and /or invited educational lectures from Baxter, Bayer, CSL Behring, and Pfizer.

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 has received travel support from Baxter and Bayer.

AG, GC and EG reported no competing interests.

RL received honoraria for advisory board participation for Novo Nordisk, Pfizer and Sanquin. FP has received honoraria for invited educational lectures from Novo Nordis, CSL Behring, Bayer and Baxter, in addition she has received research support from Novo Nordisk.

JW has acted as a consultant and participated in expert groups for Bayer, Baxter and Novo Nordisk, and has received speakers fees from Bayer, Baxter, CSL Behring, Novo Nordisk, Octapharma, and Pfizer.

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Table 1. Data submitted according to treatment status and diagnosis

	PUPs		PTPs	
Centres included	57/60 (95%)		68/74 (92%)	
Inhibitors included	113/119 (95%)		27/33 (82%)	
	Inhibitors < 50 ED	Reaching 50 ED without inhibitors	inhibitors	Treatment years
Severe haemophilia A	108	309	26	17667
Severe haemophilia B	5	67	1	2836

PUPs: Previously Untreated Patients; PTPs: Previously Treated Patients; ED: exposure day

Table 2. Characteristics of inhibitor patients in PUPs

	All PUPs with inhibitors reported to EUHASS		excluding overlap with RODIN study
	haemophilia A median (P25-P75)	haemophilia B median (P25-P75)	haemophilia A median (P25-P75)
Number	108	5	69
Age (yrs)	1.3 (1.0-2.0)	2.1 (1.6-3.5)	1.3 (1.0-2.0)
Nr of ED before inhibitor development	13 (9-20)	11 (8-20)	12 (9-20)
Median 1st titer	3.9 (1.8-14.8)	1.0 (0.7-217)	3.3 (1.2-14.5)
% high titer within first two measurements	63%	40%	64%

PUPs: Previously Untreated Patients; ED: exposure day

Table 3. Inhibitor development in PUPs with severe haemophilia according to concentrate type

	All PUPs reported to EUHASS						**excluding overlap with RODIN study**				
	inhibitors	**Total**	**Cumulative incidence**	**Lower CI**	**Upper CI**		**Inhibitors**	**Total**	**Cumulative incidence**	**Lower CI**	**Upper CI**
Haemophilia A											
FVIII pd	11	51	21.6%	11.3%	35.3%		8	38	21.1%	9.6%	37.3%
FVIII rec	97	366	26.5%	22.1%	31.3%		62	259	23.9%	19.1%	29.5%
recFVIII BHK	44	143	30.8%	23.3%	39.0%		27	105	25.7%	17.7%	35.2%
recFVIII CHO	53	223	23.8%	18.3%	29.9%		35	154	22.7%	16.4%	30.2%
Advate	37	141	26.2%	19.2%	34.3%		22	85	25.9%	17.0%	36.5%
Helixate NexGen	12	37	32.4%	18.0%	49.8%		11	33	33.3%	18.0%	51.8%
Kogenate Bayer	32	106	30.2%	21.7%	39.9%		16	72	22.2%	13.3%	33.6%
Recombinate	1	24	4.2%	0.1%	21.1%		1	24	4.2%	0.1%	21.1%
Refacto	0	6	0.0%	0.0%	45.9%		0	1	0.0%	0.0%	97.5%
Refacto AF	15	52	28.8%	17.1%	43.1%		12	44	27.3%	15.0%	42.8%
Haemophilia B											
FIX pd	3	30	10.0%	2.1%	26.5%						
FIX rec (Benefix)	2	42	4.8%	0.6%	16.2%						

Pd:plasma derived; rec: recombinant; FVIII BHK: FVIII produced by Baby Hamster Kidney cells (Kogenate Bayer and Helixate Next Gen); FVIII CHO: FVIII produced by Chinese Hamster Ovary cells.

Inhibitor development on Kogenate Bayer/Helixate NextGen compared to Advate: All study (44/143)/(37/141)=1.17; RODIN excluded: (27/105) /(22/85)=0.99; RODIN only (17/38)/(15/56) =1.67

Table 4. characteristics of inhibitor patients in PTPs according to diagnosis

	Severe haemophilia A median (P25-P75)	Severe haemophilia B median (P25-P75)
Number	26	1
Age (yrs)	25.0 (7.6-44.8)	1.9
Nr of EDs before inhibitor development	294 (95- >999)	60
Median 1st titer	4.6 (1.3-15.3)	4.0
% high titer within first two measurements	54%	0%

PTPs: Previously Treated Patients; ED: exposure day

Table 5. Inhibitor development according to concentrate type for PTPs with severe haemophilia.

Severe PTP			
	Inhibitors (N)	Treatment years (N)	Inhibitors (N/100 yrs) (95% CI)
FVIII rec	19	12959	0.15 (0.09-0.23)
FVIII pd	7	4708	0.15 (0.06-0.31)
FVIII BHK	7	5506	0.13 (0.05-0.26)
FVIII CHO	12	7454	0.16 (0.08-0.28)
Advate	5	4656	0.11 (0.03-0.25)
Helixate NexGen	1	1987	0.05 (0.00-0.28)
Kogenate Bayer	6	3519	0.17 (0.06-0.37)
Recombinate	0	251	0.00 (0.00-1.46)
Refacto	4	209	1.91 (0.52-4.82)
Refacto AF	3	2338	0.13 (0.03-0.37)
FIX pd	0	1150	0.00 (0.00-0.32)
FIX rec (Benefix)	1	1686	0.06 (0.00-0.33)

Pd:plasma derived; rec: recombinant; FVIII BHK: FVIII produced by Baby Hamster Kidney cells (Kogenate Bayer and Helixate Next Gen); FVIII CHO: FVIII produced by Chinese Hamster Ovary cells.

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