

Association of factor expression levels with annual bleeding rate in people with haemophilia B

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ABSTRACT (249 / 250 words)

## Introduction

Gene therapy clinical trials measure steady-state clotting factor expression levels (FELs) to evaluate the modulation of the bleeding phenotype, aiming to offer consistent protection against breakthrough bleeding events. The link between FELs and bleeding risk in people with haemophilia B (PwHB) is not well understood.

## Aim

We evaluated the association between FEL and annual bleeding rate (ABR) in PwHB.

## Methods

This cross-sectional study extended the CHES burden of illness studies in Europe and the United States. Recruitment of additional adult males with haemophilia B supplemented the existing CHES sample size of PwHB and FELs. PwHB receiving prophylaxis were excluded, as fluctuating FELs may have confounded the analysis. Demographic and clinical characteristics were reported descriptively. Any recorded baseline FEL was reported by the haemophilia-treating physicians according to the medical records. Generalized linear models with log link explored the association between changes in FEL and ABR.

## Results

The study included 407 PwHB and no inhibitors receiving on-demand treatment. Mean age was 36.7 years; 56% from the EU, 44% from the US. Mean baseline FEL was 9.95 IU/dL (SD, 10.47); mean ABR was 2.4 bleeds/year (SD, 2.64). After adjusting for covariates, the model showed that for every 1% increase in FEL the average ABR decreased by 0.08 ( $P < 0.001$ ). Predicted number of bleeding events according to FEL showed a significant non-linear relationship between FEL and ABR ( $P < 0.05$ ).

## Conclusion

This analysis showed a significant relationship between FEL and ABR, where increases in FEL were associated with decreases in ABR among men with HB in Europe and the US.

KEYWORDS: haemophilia B; factor expression level; annual bleeding rate; real-world data

## INTRODUCTION

Haemophilia B (HB) is a rare X-linked genetic disorder characterized by the deficiency or dysfunction of clotting factor IX, with a worldwide prevalence of approximately 3.8 per 100,000 males.<sup>1</sup> The severity of HB is classified according to factor IX (FIX) activity levels, where people with <1% of normal (<1 IU/dL) levels are considered severe, 1-5% of normal is considered moderate HB, and 6 to <40% of normal is considered mild HB.<sup>2</sup> This is the same phenotypic classification used for factor VIII (FVIII) deficiency in haemophilia A (HA). The hallmark of haemophilia is bleeding into joints (hemarthrosis). The accumulation of hemarthroses over time is associated with long-term joint damage leading to haemophilic arthropathy and chronic pain. Haemophilia-associated morbidity worsens with severity, as spontaneous bleeding events are more common with severe haemophilia than with mild haemophilia.<sup>3-5</sup> More frequent spontaneous bleeding events and progressive joint damage have been shown to reduce quality of life and work productivity while increasing healthcare resource use.<sup>4-7</sup>

The standard of care for haemophilia aims to prevent bleeding events through prophylactic use of clotting factor replacement therapy, though on-demand treatment is also used to treat bleeding events when they occur.<sup>8</sup> A spectrum of options exists between continuous prophylaxis and on-demand therapy, including 'intermittent' prophylaxis and 'individualised' prophylaxis, meant to be optimised based on the patient's pharmacokinetic profile and lifestyle. Use of factor replacement therapy is widespread, owing to its general safety and effectiveness, but the lifelong requirements of ongoing management is associated with its own treatment burden, and breakthrough bleeding events persist.<sup>9-11</sup>

Gene therapy for haemophilia, designed to enable continuous endogenous production of the missing clotting factor, holds potential for a curative benefit.<sup>12</sup> Gene therapy trials measure steady-state clotting factor expression levels (FELs) to evaluate the modulation of the bleeding phenotype,

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aiming to offer more consistent protection against breakthrough bleeding events compared to that observed with historical standards of care.<sup>13</sup> Den Uijl and colleagues (2010) reported an association between joint bleeding and baseline FEL in a cohort of 433 Dutch patients with mild (73%) or moderate (27%) haemophilia A receiving on-demand treatment; rates of joint bleeding decreased by 18% with every IU/dL increase in baseline FVIII activity level.<sup>14</sup> Nonetheless, our understanding of the link between FELs and bleeding risk in people with HB remains limited, particularly among those with mild HB.

In order to help contextualize emerging results from gene therapy clinical trials, we evaluated the association between FEL and annual bleeding rate (ABR) in patients with HB in Europe and the United States utilizing the multinational CHES research platform.

## MATERIALS AND METHODS

### *The CHES EU and CHES US studies*

This cross-sectional study utilized the data and infrastructure of the 'Cost of Haemophilia in Europe: a Socioeconomic Survey' (CHES EU I-II) and the 'Cost of Severe Haemophilia across the US: a Socioeconomic Survey' (CHES US/US+) bottom-up burden of illness studies. The design, methods and primary findings from CHES EU I-II and CHES US/US+ have been reported previously.<sup>15,16</sup> The CHES EU I and II studies included European males aged  $\geq 18$  years with haemophilia A or B from the five largest European countries (France, Germany, Italy, Spain, and the United Kingdom; EU5), with CHES EU II adding physicians and patients from Denmark, Romania, and The Netherlands. CHES EU I enrolment was limited to people with severe haemophilia A or B; CHES EU II included people with mild or moderate disease. Physicians recruited patients from consecutive routine office visits and completed web-based case report forms (eCRF) based on the patient's medical history. Patients

completed questionnaires on their health status, non-medical costs, and work impairment. Data were collected between 2014–2015 (CHESS EU I) and 2018–2019 (CHESS EU II), and anonymized to ensure protection of personal information. The results of the CHESS EU I survey came from 139 physicians and 1,285 patients across the EU5, with >300 variables for each patient. CHESS EU II continues to recruit participants, with a current sample >700 patients across all haemophilia types, severities, and EU countries.

CHESS US was a retrospective, 12-month cross-sectional study including adult males with severe haemophilia in the United States. A similar design and methodology was employed for the CHESS US+ study to supplement the CHESS US database with patient-reported outcomes using a patient panel approach. Participants completed online forms on costs and productivity outcomes over the past 12 months (conducted in 2019). The CHESS US and CHESS US+ populations were sampled in proportion to the population of people with haemophilia in the US.

The CHESS EU and CHESS US studies were reviewed and approved in accordance with the ethical requirements of the University of Chester's Faculty of Health & Social Care.

#### *Physicians and Patients*

All of the available data from the CHESS EU and CHESS US studies pertaining to men with HB receiving on-demand treatment (who had evaluable FEL and ABR data) were used for this study. In order to gather more FEL and ABR data from men with HB receiving on-demand treatment, CHESS II and CHESS US patient recruitment was extended to capture additional on-demand patients within the moderate and mild severity categories, where data was previously absent. This additional data capture is characterized as complementary recruitment in Figure 1. As such, the CHESS EU and CHESS US data sets were supplemented with additional male adults diagnosed with HB  $\geq 12$  months prior to the date of the enrolment office visit (defined as the index date), recruited by their

haemophilia treating physicians. Physicians recruited via a fieldwork agency using a convenience sampling method had to be in clinical practice in the EU5 or US with a primary specialty of

haematology (with the exception of France where Haemophilia Care Providers could participate).

Physicians also had to have been in practice for  $\geq 3$  years with a minimum caseload of  $\geq 4$  male patients with haemophilia per month. Eligible physicians were invited to retrospectively enrol an average of 4 haemophilia patients seen during clinical consultations and to complete eCRFs for each patient. Physicians retrospectively extracted information from the patients' medical records from the previous 12 months.

All eligible patients had to be male adults ( $\geq 18$  years) with any severity of non-acquired HB, and had to provide voluntary informed consent. Patients receiving prophylactic treatment regimens observe fluctuating FELs (highest following infusion and lowest right before the next infusion) and spend a high proportion of on-treatment time at FELs that are higher than their baseline trough level. Since this may have conflicted with our primary objective to characterize the association between FEL and ABR, patients receiving prophylaxis regimens were excluded. Patients receiving on-demand treatment, however, have more steady-state FELs and were included in this study. Patients with haemophilia A, those diagnosed with an inhibitor at the index date, and those unable to make decisions for themselves or to understand the study materials were also excluded. Eligible patients who agreed to participate in the study completed Patient Public Involvement Engagement (PPIE) questionnaires including socio-demographic characteristics and self-reported clinical characteristics.

#### *Variables and Outcomes*

Patient demographic and clinical characteristics were reported both by physicians in the eCRF (age, ethnicity, height, weight, body mass index [BMI], smoking status) and patients in the PPIE (employment status, highest level of education, household income). Outcomes were extracted for a 12-month retrospective observation window from the date of extraction. Physicians also reported

the patient's age at diagnosis, disease severity, target joints<sup>2</sup>, problem joints<sup>17</sup>, assay used to determine HB severity, comorbidities including blood-borne viruses, and genetic mutations.

Consultation history with the haematologist and other specialists (frequency of contact and visits), hospital admissions, factor replacement therapy, age at initiation of factor replacement therapy, and concomitant medications were all reported in the eCRF. Patients reported their perceptions of HB severity (mild, moderate, severe), frequency of bleeding events, pain, frequency of infusions, and compliance/adherence to treatment.

Corresponding with the primary objective, any recorded baseline FEL was reported by the physicians in the eCRF according to the patient's medical record. ABR was defined as the physician-reported number of bleeding events in the previous 12 months (prior to data extraction from the medical record).

#### *Statistical analysis*

Descriptive statistics were used to summarize demographic and clinical characteristics of the study cohort. Correlation analyses and scatterplots were used to assess the relationship between baseline FEL and ABR. For ordinal data, Kruskal-Wallis tests were performed to test for differences across groups then, assuming rejection of the first hypothesis, Mann-Whitney U tests were conducted to make pairwise comparisons. Both approaches included Bonferroni correction for multiple comparison where applicable. No imputation of missing responses was conducted; records with missing data were excluded from the regression models.

Poisson regression and generalized linear models (GLM) with log link were used to explore the association between changes in FEL and ABR. The GLM with log link analyzed variation in ABR across FELs, adjusting for age, BMI, and presence of blood-borne viruses. Akaike information criterion (AIC) was used to measure model fit (lower AIC indicates a better fit). Subsequently, a multivariable



restricted cubic spline (RCS) GLM regression was performed to create, model, and test for the potential non-linear relationship between FEL and ABR. Splines are polynomial functions with pre-defined abscissa values, or knots, at the join locations of the individual polynomials, and are intended to yield a more flexible model fit than traditional linear regression modeling.<sup>18,19</sup> In practical terms, for our model, the RCS approach divided the data set at expression levels (knots were used at FEL thresholds for mild, moderate, and severe HB) and modelled each section separately before piecing them together for a smooth continuous function. Thus, our RCS regression employed 3 knots, located at baseline FEL values of 1, 5, and 10 IU/dL (FELs of 1 and 5 represent the thresholds for moderate and mild severity and the FEL of 10 provided a fairly even split of the sample and was very close to the mean baseline FEL), and controlled for age, BMI, and blood-borne viruses. All analyses were performed using STATA 16 (StataCorp LLC, College Station, TX, USA; [www.stata.com](http://www.stata.com)).

## RESULTS

A total of 407 adult males with HB and no inhibitors who were receiving on-demand treatment regimens were included. The mean age of the study cohort was 36.7 years, with approximately half of patients from the EU (56%) and US (44%; Table 1). Mean baseline FEL was 9.95 IU/dL (SD, 10.47) and median FEL was 6.0 (range, 0.2–40.0). Mean ABR was 2.4 bleeds/year (SD, 2.64), and median ABR was 2.0 (range, 0–50.0).

### *Regression analysis: association of FEL with ABR*

The GLM provided the best fit for modeling ABR (results from the Poisson regression are provided in Table A1 in Appendix A). The scatterplot indicated a generally negative relationship between FEL and ABR (Appendix Figure B1). After adjusting for age, BMI, and blood-borne viruses, the model showed

that for every 1% increase in FEL, the average ABR decreased by 0.08, meaning that one bleed would be avoided every 12.5% increase in FEL ( $P < 0.001$ ; Table 2). The predicted number of bleeding events according to FEL using the GLM RCS model showed a significant non-linear relationship between FEL and ABR ( $P < 0.05$ ). The plot of predicted number of bleeds according to ABR in the GLM RCS model for a hypothetical patient with HB (with no blood-borne viruses and mean age and mean BMI of the cohort) showed a sharper rate of decline in ABR at FELs  $< 8\%$ , followed by milder decline in ABR at higher FEL values (Figure 2).

## DISCUSSION

This analysis of adult males with HB in Europe and the US showed a significant relationship between FEL and ABR, where every 1% increase in FEL was associated with an average decrease in ABR of 0.08. Our population included patients with all severities of HB, most commonly mild disease (41%) with an overall mean FEL of 9.95% and median FEL of 6%, consistent with our particular interest in patients with mild disease ( $> 5$  to 25% factor IX levels). The overall mean ABR was 2.4 bleeds/year (median 2 bleeds/year) in the context of chronic pain and  $\geq 1$  problem joint reported in approximately half of the overall cohort (57% and 45%, respectively). Understanding the relationship between FEL and ABR across levels of HB severity should inform interpretation of clinical trial findings, including those emerging from gene therapy trials where FELs are used to estimate the potential for prevention of bleeding events. The results of this study, interpreted in the context of HB clinical trial results, suggest that FIX expressions within the upper mild haemophilia range (i.e.,  $> 25\%$  FIX levels) following gene therapy administration could be sufficient to eradicate all bleeding events and eliminate the need for prophylactic FIX replacement therapy, based on typical ABR reported in severe and moderately severe HB patients within studies examining outcomes of PwHB receiving prophylaxis.<sup>12,20,21</sup>

Our work aims to address a lack of information regarding FEL and ABR in patients with HB, particularly for those with non-severe disease. Den Uijl and colleagues (2010) showed a non-linear relationship of the association of the increase in FEL and decrease in annual frequency of joint bleeds using negative binomial regression.<sup>14</sup> They used a sample of patients receiving on-demand treatment regimens only, as was used in our study, due to the nature of FEL control and variability among patients receiving prophylaxis regimens. The GLM log link regression provided the best fit for our data, which showed a relationship between FEL and ABR. Our RCS model provided further insight to the relationship between FEL and ABR in patients with mild to moderate HB, reporting a non-linear relationship, as the sharp decline in ABR with increasing FELs appeared to flatten out at approximately the 8% FEL threshold. Den Uijl (2011) also performed an analysis of FELs in patients with HA that showed a clear distinction between those with severe and non-severe disease, but those with mild or moderate HA were not well differentiated.<sup>22</sup> The relationship between predicted joint bleeds and FELs reported by Den Uijl also showed a steeper reduction in bleeding events with FELs <10%, which became more gradual with increasing FELs. Soucie and colleagues (2018) have also reported decreasing joint bleed rates with increasing FELs.<sup>23</sup> This large US study predicted a 'typical' patient with HB (white, commercially insured, normal BMI, HIV negative, 25 to 44 years old) and FEL of 20% to have one joint bleed every 2 years. Similar to the present study, Soucie and colleagues reported a non-linear relationship between FELs and bleeding frequency, where bleeding rate decreased by 10% for every 1% increase in FELs.<sup>23</sup> Soucie et al presented curves for predicted joint bleeds by FEL by age group, which showed a similarly steep decline in bleeding events with FELs <10% for the 25- to 44-year-old group in particular, but not for children aged 2 to 9 years (mean age for our study was 37 years).

To our knowledge, this is one of the few robust, recent analyses of FEL and ABR among patients with mild or moderate HB. The GLM log link analysis provided the best fitting model, and RCS analysis was

consistent with the primary model findings and provided additional insight. The use of physician-reported joint and non-joint bleeding data offered valuable footing in real-world reported outcomes, with a potential trade-off in precision versus using joint-only bleeding data. Den Uijl (2010) used joint bleed data to minimize potential misclassification of bleeding events and recall bias, though our interest in mild or moderate HB patients (where there is a notable absence of data related to FELs) was likely best served with a more conventional, clear outcome definition that was easily extractable from the patients' medical records (bleeding events).

The results of this analysis should be considered in the context of certain limitations. Firstly, data from patients with available information on FELs were used, which did not include any patients from The Netherlands or Denmark. Secondly, differences in healthcare systems and medical management practices across countries should also be noted, and may have influenced reporting and care provision related to bleeding events. Additionally, it should be noted that participation in the CHES studies by physicians and patients is entirely voluntary and patient selection is done so by physicians, based on the next occurring patient consultations and therefore a degree of selection bias cannot be excluded. Patients who participated in this study may also have been fundamentally different than those who did not participate in ways that could have been related to their haemophilia severity, care, and management; however, since the data for nonparticipants is not available, this cannot be evaluated. The nature of each bleed recorded (spontaneous / trauma-related) was not captured in adequate detail to allow for analysis across datasets, with both trauma-related and spontaneous bleeding events captured within the ABR outcome. Finally, given the nature of our sample (patients of all severities treated on-demand), the on-demand treatment strategy for severe and moderate patients may be associated with a milder bleeding phenotype or a lower likelihood to bleed, rendering the results a potential underestimation of the reduction in ABR associated with increasing

FELs. Considering these conservative results, one might expect the decrease in ABR to be larger in the overall haemophilia population.

## CONCLUSION

Understanding bleeding outcomes in the context of FELs is important to the evaluation and interpretation of increasingly effective treatment options for patients with haemophilia. As the risk of breakthrough bleeding events decreases with ever more effective prevention strategies, refining our use and confidence in clinical measures such as FELs becomes essential. This study should help to interpret and contextualize emerging findings from clinical trials of preventative therapeutic options such as gene therapy, where the potential to offer a curative benefit will make measurement of effectiveness by the incidence of bleeding events challenging. Further understanding the association between FELs and non-clinical outcomes, such as on the subsequent humanistic and economic burden of haemophilia morbidity, will offer meaningful holistic insights to clinicians, policymakers and patients with HB worldwide.

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## CONFLICT OF INTEREST STATEMENT

TB, AS, and JOH are employees of HCD Economics which received research funding for this study from uniQure Inc. TMA and NL are employees of uniQure Inc. BAK, DN, BOM and SP have no relevant relationships or financial interests related to this work.

## DATA SHARING AND ACCESSIBILITY

The data that support the findings of this study may be available from the corresponding author upon reasonable request.

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TABLES

Table 1. Demographic and clinical characteristics of the study cohort

Characteristic, n (%) unless noted	Patients with HB (N=407)
Age	
Mean (SD)	36.7 (15.7)
Median (range)	33 (18–89)
Race/ethnicity	
White	321 (79)
Other	86 (21)
Country <sup>a</sup>	
United States	181 (44)
Italy	69 (17)
France	55 (14)
Germany	42 (10)
Spain	30 (7)
United Kingdom	29 (7)
Romania	1 (<1)
BMI	
Mean (SD)	27.6 (14.0)
Haemophilia severity	
Mild (>25%)	40 (10)
Mild (>5–25%)	166 (41)
Moderate (1–5%)	98 (24)
Severe (<1%)	103 (25)
Baseline FEL	
Mean (SD)	9.95 (10.5)

Characteristic, n (%) unless noted	Patients with HB (N=407)
Median (range)	6.0 (0.2–40.0)
ABR	
Mean (SD)	2.4 (3.6)
Median (range)	2.0 (0–50)
Chronic pain	
None	174 (43)
Mild	172 (42)
Moderate	55 (14)
Severe	6 (1)
Problem joints	
None	222 (55)
1	118 (29)
≥2	67 (16)
Comorbidities	
Anxiety	51 (13)
Depression	34 (8)
Type 2 diabetes mellitus	14 (3)
Osteoarthritis	20 (5)
Osteoporosis	3 (1)
HIV	1 (<1)
Hepatitis B	4 (1)
Hepatitis C	8 (2)

BMI, body mass index; FEL, factor expression level; HB, haemophilia B; HIV, human immunodeficiency virus; SD, standard deviation.

<sup>a</sup>No data on FELs was available for patients from Denmark and the Netherlands

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Table 2. GLM log link results: Association between FEL and ABR

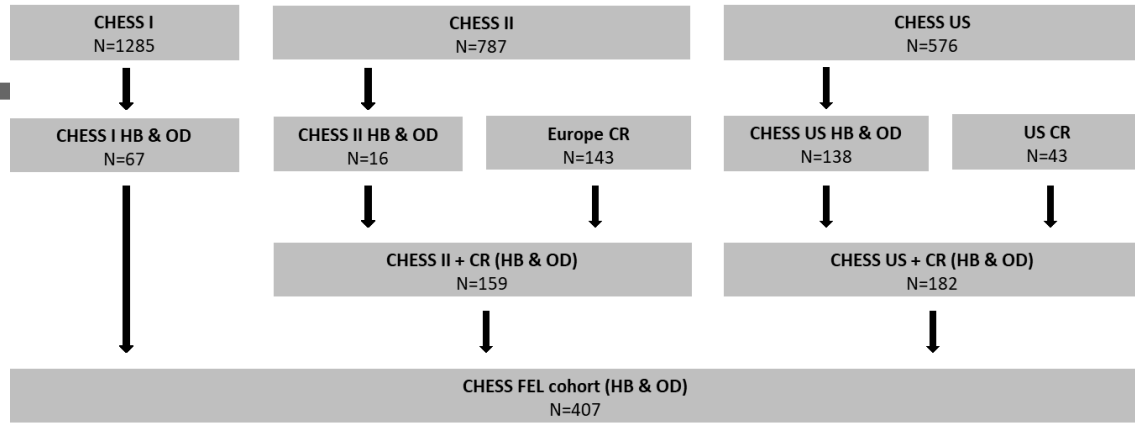
Covariate	Coefficient <sup>a</sup>	Standard error	P value
FEL	-0.079	0.015	<0.001
Age	-0.004	0.009	0.664
BMI	-0.001	0.011	0.918
HIV (present)	-0.033	3.10	0.992
HBV (present)	1.434	1.58	0.364
HCV (present)	-0.998	1.12	0.373

BMI, body mass index; FEL, factor expression level; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus.

<sup>a</sup>Coefficient represents the average marginal effect at the mean.

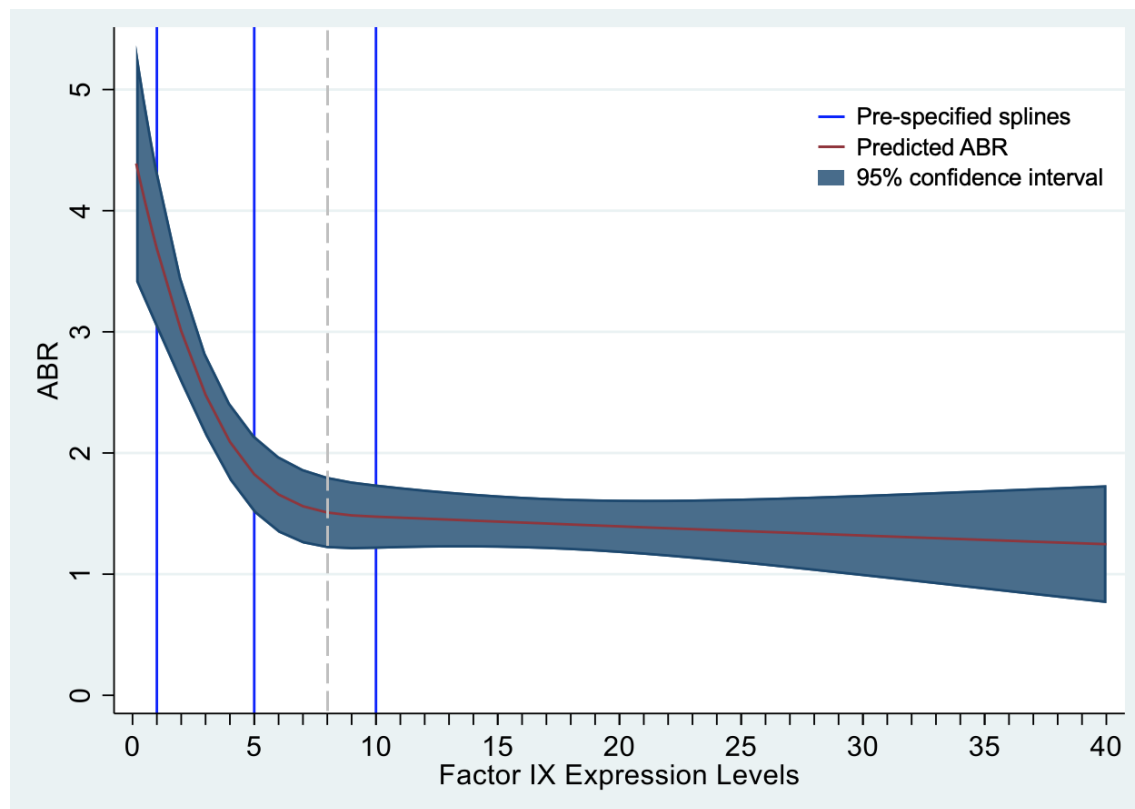
FIGURE

Figure 1. CHES studies recruitment flowchart



Abbreviations: HB, Haemophilia B; OD, On-demand; CR, Complementary Recruitment; FEL, Factor Expression Level

Figure 2. Scatterplot of predicted bleeds in the GLM RCS model



Scatterplot represents the predicted number of bleeds by ABR for a hypothetical patient with HB with the mean age (36.7 years) and mean BMI (27.6 kg/m<sup>2</sup>) of the cohort and no blood-borne viruses.

APPENDIX A: Poisson regression results

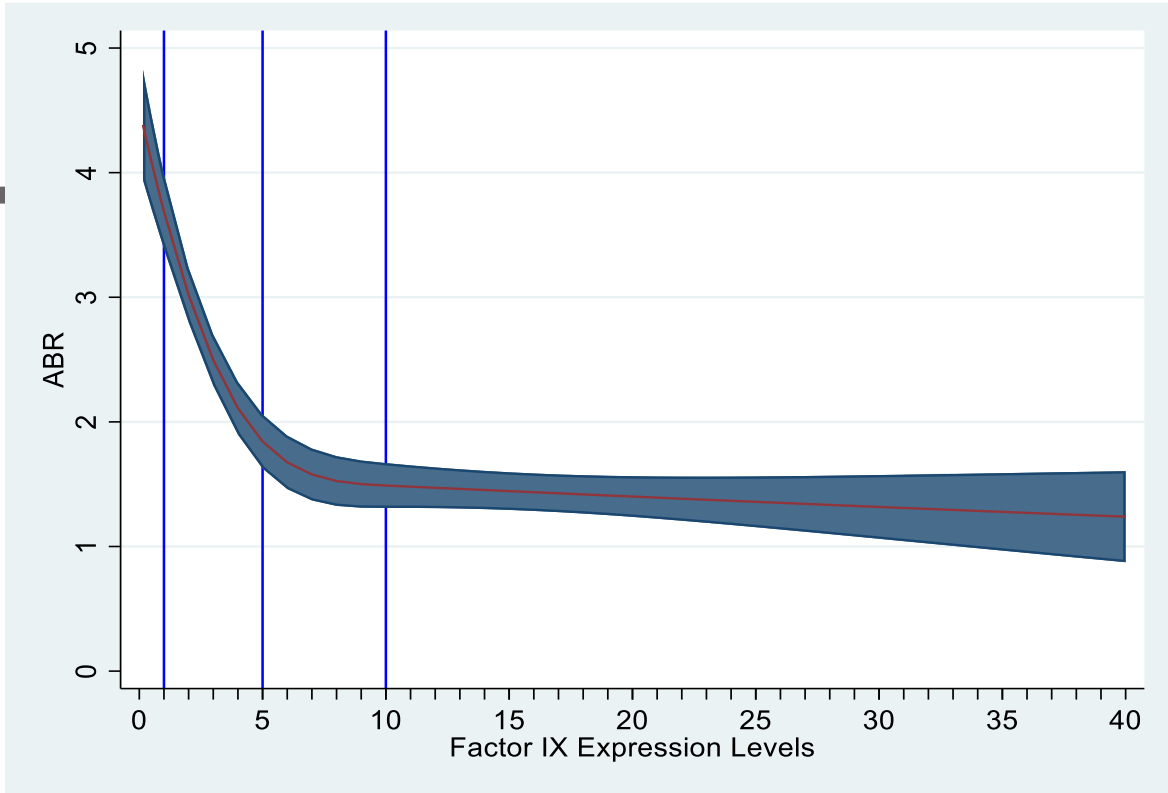
Table A1. Poisson regression results (AIC, 1971.564)

ABR	IRR	SE	z	P> z	95% CI
FEL	0.958	0.004	-10.53	0.000	0.950, 0.965
Age	0.996	0.002	-1.70	0.089	0.992, 1.000
BMI	0.999	0.002	-0.56	0.574	0.994, 1.00
HIV	0.929	0.542	-0.13	0.900	0.296, 2.915
HBV	1.854	0.444	2.58	0.010	1.160, 2.964
HCV	0.737	0.193	-1.16	0.244	0.441, 1.232
Intercept	3.905	0.433	12.29	0.000	3.143, 4.854

ABR, annual bleeding rate; AIC, Akaike Information Criterion; BMI, body mass index; CI, confidence interval; FEL, factor IX expression level; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IRR, incidence rate ratio; SE, standard error.



Figure A1. Poisson spline output



P-value to test for non-linear relationship between FEL and ABR:  $P < 0.0001$

APPENDIX B: GLM results

Figure B1. Scatterplot of FEL vs. ABR (N=407)

