**Treatment burden, haemostatic strategies and real world inhibitor screening practice in Non-Severe Haemophilia A**

**Authors:** Paul Batty 1, Steve K. Austin 2,3, Kate Khair 4, Carolyn M. Millar 5, Ben Palmer 6, Savita Rangarajan 3, Jan-Phillip Stümpel 1, Murugaiyan Thanigaikumar 7, Thynn Thynn Yee 8, Daniel P. Hart 1

**Institution Addresses:** 1 The Royal London Hospital Haemophilia Centre, Barts and The London School of Medicine and Dentistry, QMUL, London, UK; 2 St George’s Healthcare NHS Trust Haemophilia Centre, London, UK; 3 The Centre for Haemostasis and Thrombosis, St Thomas’ Hospital, London, UK; 4 Great Ormond Street Haemophilia Centre, London, UK; 5 Hammersmith Hospital Haemophilia Centre, London, UK; 6 The United Kingdom National Haemophilia Database, Manchester, UK; 7 Lewisham Hospital Haemophilia Centre, London, UK; 8 Katharine Dormandy Haemophilia and Thrombosis Centre, Royal Free Hospital, London, UK

**Corresponding Author:** Dr Dan Hart, The Royal London Haemophilia Centre, Haematology Day Unit, The Royal London Hospital, Whitechapel, London, E1 1BB

**Telephone:** 0203 594 1869

**Fax:** 0203 594 1859

**Email:** d.hart@qmul.ac.uk

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**Summary**

Inhibitor formation in non-severe haemophilia A is a life-long risk and associated with morbidity and mortality. There is a paucity of data to understand real-world inhibitor screening practice. We evaluated the treatment burden, haemostatic strategies, *F8* genotyping and inhibitor screening practices in non-severe haemophilia A in seven London haemophilia centres.

In the two-year study period, 44% (377/853) patients received at least one haemostatic treatment. 79% of those treated (296/377) received Factor VIII (FVIII) concentrate. *F8* genotype was known in 88% (331/377) of individuals. Eighteen percent (58/331) had “high-risk” *F8* genotypes.

In patients with “standard-risk” *F8* genotypes treated on-demand with FVIII concentrate, 51.3% episodes (243/474) were screened within one year. However, poor screening compliance was observed after “high-risk” treatment episodes. In patients with “standard-risk” *F8* genotypes, 12.3% (28/227) of treatment episodes were screened in the subsequent 6 weeks after surgery or a bleed requiring ≥5ED. Similarly, in the context of “high-risk” *F8* genotypes after any FVIII exposure, only 13.6% (12/88) of episodes were screened within 6 weeks.

Further study is required to assess optimal practice of inhibitor screening in non-severe haemophilia A to inform subsequent clinical decisions and provide more robust prevalence data to further understand the underlying immunological mechanism.

**Introduction**

Haemophilia A is a bleeding disorder resulting from an inherited defect in the *F8* gene. Severity of haemophilia A is defined by baseline FVIII coagulant activity (FVIII:C) being either severe (FVIII:C <1IU/dL) or non-severe (FVIII:C 1-40 IU/dL) (Blanchette *et al*, 2014). One of the greatest challenges in the management of persons with haemophilia A is the occurrence of FVIII-neutralising antibodies (inhibitors), which occur in 32% of patient with severe haemophilia A (Gouw *et al*, 2013). Although the cumulative incidence of inhibitors in non-severe haemophilia A is lower (5.3%) (Eckhardt *et al*, 2013), these antibodies constitute 22% (120/555) of all previously reported inhibitors in the UK (United Kingdom Haemophilia Centres Doctors' Organisation, 2015). Clinically these antibodies in non-severe haemophilia A are of particular concern due to reported cross-reactivity against endogenous FVIII (Hay *et al*, 1998;Eckhardt *et al*, 2013), change in bleeding phenotype (Hay *et al*, 1998) and increased mortality (Eckhardt *et al*, 2015). In one small study a change in bleeding phenotype and fall in baseline FVIII:C (bFVIII:C) was seen in 22/26 and 24/26 of patients respectively (Hay *et al*, 1998). More recently within the INSIGHT study, decrease in bFVIII:C and a change in bleeding phenotype was reported in 34/54 (58%) and 30/54 (51%) of patients respectively (Eckhardt *et al*, 2013). In both of these reports however, inhibitor screening was performed only in the context of clinical suspicion of a FVIII inhibitor (change in bleeding phenotype, bFVIII:C or impaired treatment efficacy or FVIII recovery) (Hay *et al*, 1998;Eckhardt *et al*, 2013). Only 11 (19%) of inhibitors were diagnosed on routine screening in asymptomatic patients within the INSIGHT study (Eckhardt *et al*, 2013).

Unlike severe haemophilia A in which FVIII concentrate exposure begins early in life with associated early risk of inhibitor formation (median 14 exposure days (ED)) (Gouw *et al*, 2007b;Gouw *et al*, 2013) and necessity for systematic inhibitor screening (Collins *et al*, 2013),(van den Berg *et al*, 2015); treatment in non-severe haemophilia A often commences later in life and is required less frequently than in severe haemophilia A. There are often substantial periods of time between treatment episodes and many patients will not receive significant FVIII exposure until later in life, if at all (Den Uijl *et al*, 2011). Data from the INSIGHT cohort has suggested a lifelong risk of inhibitor formation in this group of patients (Eckhardt *et al*, 2013). However it is not clear when and how to test for FVIII antibodies in non-severe haemophilia A and there is a lack of data to guide these practices. In the UK, consensus guidance stratifies inhibitor screening according to a combination of genetic (*F8* genotype) and treatment related factors (FVIII exposure intensity) (Hay *et al,* 2006; Collins *et al*, 2013). There are presently no data describing “real-world” inhibitor testing practices in patients with non-severe haemophilia A. We report findings from a retrospective audit and evaluation of treatment burden, inhibitor screening and *F8* genotyping practices from a large cohort of patients with non-severe haemophilia A treated at haemophilia centres in the London region.

**Methods**

*Case and Centre Selection*

A retrospective chart review was performed of all patients with non-severe haemophilia A treated at all seven London haemophilia centres (four comprehensive care centres (CCC) and three haemophilia treatment centres (HTC)). All sequentially treated patients with non-severe haemophilia A receiving treatment between 1/1/11 and 31/12/12 were included.

The primary objective was to audit inhibitor screening activity against United Kingdom Haemophilia Centre Doctors’ Organisation (UKHCDO) inhibitor screening guidelines available at the time of study (Hay *et al*, 2006). These recommended that patients with moderate and mild haemophilia A receive annual inhibitor screening *or* after intensive replacement treatment, especially in “high-risk” *F8* mutations. Subsequent UKHCDO guidance has recapitulated these recommendations (Collins *et al* 2013).

The optimal time period in which testing should be performed following such intensive FVIII replacement (so called “convalescent inhibitor screening”) is not defined. For the purpose of this study, convalescent inhibitor screening was defined as testing within six weeks (≤42 days) of the first treatment day and categorised for *F8* genotype as follows:

“standard-risk” *F8* genotype: Annual inhibitor testing in all patients who have received exposure to FVIII concentrate in that year. Follow-up inhibitor screening after intensive FVIII exposure (≥5EDs) or surgery.

“high-risk” *F8* genotype: Follow up inhibitor testing after *all* episodes of exposure to FVIII concentrate.

Secondary objectives were to assess uptake of *F8* genotype testing, evaluate treatment patterns (including DDAVP use), bleeding patterns, timing of inhibitor screening and the incidence of new inhibitors. The study design and data to be collected were discussed with the Clinical Effectiveness Unit (CEU) at The Royal London Hospital and Research Approvals Office (Joint Research Management Office) and represented service evaluation and clinical audit. The study was registered locally at each centre by the lead treating-physician.

*Diagnosis and Definitions of Non-Severe Haemophilia A*

All patients were diagnosed locally with baseline FVIII:C (bFVIII:C) levels derived from the centres’ patient registration records. Non-severe haemophilia A was defined as a FVIII:C of 1-50 IU/dL (Hay *et al*, 1998). Patients were then further sub-categorised by the investigator as having either, moderate (FVIII:C >1 to ≤5IU/dL) or mild (FVIII:C >5 to ≤50IU/dL) haemophilia A (Hay *et al*, 1998;White *et al*, 2001;Blanchette *et al*, 2014).

*F8 Genotype Testing and Definitions of “High-Risk” F8 Genotypes*

Where available, *F8* genotype was derived from historical personal or family testing, then categorised by centres as “high” or “standard-risk” *F8* genotypes. “High-risk” genotypes were derived from the published HIGS cohort (legacy format with HGVS assignment in parentheses) (Oldenburg & Pavlova, 2006;Astermark *et al*, 2013): Arg593Cys (Arg612Cys); Tyr2105Cys (Ty2124Cys); Arg2150His (Arg2169His); Arg2163His (Arg2182His); Tyr2229Cys (Trp2248Cys); Asn2286Lys (Asn2305Lys) and Pro23000Leu (Pro2319Leu) (Kemball-Cook *et al*, 1998;Oldenburg & Pavlova, 2006;Astermark *et al*, 2013).

*Data Collection*

A standardised data-collection tool was distributed to each of the haemophilia centres, enabling data retrieval directly from electronic patient records (EPR). Data collection took place between 4/4/13 and 18/6/13.

Baseline demographic data, including age, bFVIII:C, inhibitor history and *F8* genotype result were collected for all patients. Family history of inhibitor was not available for analysis. Details of all treatment episodes included: use of desmopressin (DDAVP); and/or FVIII concentrate; and/or bypassing agents. For episodes treated with FVIII concentrate, information on treatment indication, timing and results of any subsequent inhibitor testing was requested. Treatment indications were categorised as home-treatment or on-demand (hospital based treatment): bleed/trauma; surgery; other; not stated. Surgery was defined as any surgical or dental intervention/procedure requiring haemostatic therapy. An exposure day (ED) was defined as a calendar date in which one or more FVIII infusions was used (Gouw *et al*, 2007a). Where treatment episodes did not include FVIII concentrate, no additional information was retrieved. Only hospital based on-demand episodes and associated inhibitor screens were analysed as these episodes had documented evidence of FVIII being infused. Home-treatment categorised treatment and timing were considered to be too heterogeneous for further analysis.

*Assessment of Timing of Inhibitor Testing*

Participating haemophilia centre laboratories are accredited by Clinical Pathological Accreditation (CPA) and triennial UKHCDO inspections. Centres performed all inhibitor testing locally and provided dates and results of all inhibitor tests performed within the study period.

For evaluation of annual inhibitor screening, treatment episodes were paired to the nearest, subsequent inhibitor test, providing it occurred within one year of the first treatment day. For patients who received multiple treatment episodes within a year period, a single inhibitor test could represent an annual inhibitor screen for multiple treatment episodes.

For evaluation of “paired inhibitor screening”, treatment episodes were paired with the nearest subsequent inhibitor test, providing it occurred within one year of the first day of treatment and no factor FVIII exposure occurred in the intervening time. If inhibitor testing was not performed within that year or if another treatment episode occurred before an inhibitor screen within the same year, the treatment episode was judged as not having a paired inhibitor screen. If more than one inhibitor test was sent, the treatment episode was paired to the first inhibitor screen with subsequent assays being excluded from the analysis. Consequently in this sub-analysis an inhibitor screen could only be paired to a single treatment episode. Screening was assessed as having being sent due to treatment if testing was performed within (±) one day of the subsequent treatment episode. Finally, a true “convalescent inhibitor screen” was defined as a paired inhibitor screen being performed within six weeks after the first day of treatment (≤42 days).

*Statistical Analyses*

Descriptive statistics, including mean, median, standard deviation, inter-quartile range (IQR) and frequency were performed. The annualised bleed rate (ABR) was calculated from the total number of bleeding episodes recorded in the 2-year data collection window, divided by two. Comparative statistics were performed using the Mann-Whitney U and Kruskall-Wallis H tests for continuous and the Chi-squared, Chi-squared goodness of fit or Fisher’s exact test for categorical variables. A post-hoc analysis of factors associated with inhibitor testing was performed by mixed-effects logistic regression for the binary outcome of inhibitor test performed within six weeks. All tests performed were two sided, with a p value of <0.05 taken as being significant. Statistical analyses were performed using either IBM SPSS version 21 (IBM Corp., Armonk, New York, USA), Stata (StataCorp. 2011. Stata Statistical Software: Release 12.1 College Station, TX: StataCorp LP) or GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla California USA).

**Results**

853 persons living with non-severe haemophilia A were registered at the 7 haemophilia centres within the London region. Of these, a treatment episode was recorded in 377 (44%) over the two-year study period (102 (27.1%) moderate and 275 (72.9%) mild haemophilia A patients (Table 1)).

*Uptake of F8 Genotyping*

Personal *F8* genotype was retrievable in 79% (297/377) of patients who received treatment in the study period. Evaluating personal *F8* testing by centre, all except one small HTC (19 patients treated) had performed personal *F8* genotype testing in ≥70% of patients. The causative *F8* familial mutation was known in a further 34 patients (9%) in the absence of personal *F8* genotype testing. Thus, the causative *F8* genotype was known in 88% (331/377) of patients with 18% (58/331) classified as a “high-risk” *F8* genotype.

*Treatment Characteristics*

Of those receiving haemostatic treatment, 78%, (296/377) received FVIII concentrate, with 259 patients (69%) solely receiving FVIII concentrate (Figure 1). DDAVP was used as part of treatment for 110 patients (29%), with 76 (20%) only receiving DDAVP, 33 (9%) FVIII & DDAVP and 1 (<1%) DDAVP and bypassing agents. DDAVP was used in the management of 10% (10/102) patients with moderate haemophilia A (DDAVP alone=4, FVIII & DDAVP=6) in comparison to 36% (100/275) patients with mild haemophilia A (DDAVP alone=72, FVIII & DDAVP=27 and DDAVP and bypassing agents=1). Three patients in whom DDAVP was used had an inhibitor history.

An evaluation of the baseline demographics by treatment choices was performed for treatment categories in which there were more than five individuals (FVIII alone, DDAVP alone and FVIII & DDAVP). Patients treated with DDAVP alone were significantly younger (29.2 years, IQR 14.6-44.5) than those treated with FVIII alone (35.8 years, IQR 16.6-57.2, p=0.024). Patients treated with DDAVP alone had significantly higher median bFVIII:C (20.5IU/dL, IQR 11.3-29.0) than those treated with FVIII alone (8.0IU/dL, IQR 4.0-14.0, p<0.0005) or FVIII & DDAVP (10.0IU/dL, IQR 7.0-19.0, p=0.001). No difference in bFVIII:C was seen in those treated with FVIII alone (8.0IU/dL IQR 4.0-14.0) compared to those treated with FVIII & DDAVP (10.0IU/dL, IQR 7.0-19.0, p=0.0760). A “whole-case” analysis of the frequency of “high-risk” *F8* genotypes in patients treated with FVIII alone (40/226, 17.7%), DDAVP alone (9/64, 14.1%), FVIII & DDAVP (4/32, 12.5%) did not differ from that expected by chance (p=0.642).

*On-Demand (Bleeding and Surgery) Treatment with FVIII Concentrate*

Over the study period, 236 patients received FVIII concentrate alone to cover 562 on-demand treatment episodes (surgery=211, bleeding=351). Patients received a median of 4ED (range 1-159) of FVIII in the two-year study period (Table 1). These on-demand episodes resulted in a cumulative treatment burden of 2041ED (bleed=887 and surgery=1154).

In the treatment of bleeding, 157 patients received treatment with a FVIII concentrate at hospital to treat a median of 1 bleeding episode (range 1-15). For surgery, 130 patients received FVIII treatment to cover a median of 1 surgical episode (range 1-11). Ninety six patients (32.4%) were issued FVIII for home treatment, 50 of whom only received home treatment. No further characterisation of home treatment was possible and thus excluded from further analysis.

*Bleeding episodes in Non-Severe Haemophilia A*

An assessment of bleeding events was performed in a subgroup of 148 patients without a history of inhibitor who only received on-demand FVIII treatment (bleeding or surgery) The median annualised bleed rate (ABR) was 0.5 episodes/year (range 0-4.5) with 26% of patients having an ABR ≥1 (39/148) (Figure 2).

*Inhibitor Screening: “Standard-Risk” F8 Genotype*

There were 194 “standard-risk” or unknown *F8* genotype patients treated with FVIII concentrate for one or more on-demand treatment episode (n=474) over a cumulative 1753ED. Of the on-demand treatment episodes, 51.3% (243/474) were followed by an inhibitor screen within one year, at a median of 106 days (range 2-365) (Figure 3 & Supplementary Table 1).

FVIII concentrate was used in 175 surgical episodes (106 patients), treated for a median of 1 day (range 1-157). Inhibitor screening was performed within one year for 40.6% (71/175), but only 9.7% (17/175) of surgical episodes had a true “convalescent inhibitor screen” performed within 6 weeks of treatment (Figure 3 & Supplementary Table 1). Of the episodes screened within 6 weeks, 7/17 tests coincided with a subsequent treatment day. Thus only 10/175 (5.7%) would have a result available to inform the next treatment episode.

There were 299 bleeding episodes (130 patients) treated for a median of 2 days (range 1-75) with FVIII concentrate. Following intensive (≥5 days) FVIII usage for bleeding (n=39), screening within 6 weeks was performed following 21.2% (11/52) of these episodes. Two of these 11 coincided with a subsequent treatment day, leaving 9/52 (17.3%) screened with available results for the next treatment episode.

*Inhibitor Screening: “High-Risk” F8 Genotype*

Fifty-eight patients with “high-risk” *F8* genotypes were treated in the study period. Of these, 4 received home treatment and were excluded and 42 (72%) received one or more on-demand FVIII treatments undergoing 36 surgical episodes and 52 bleeding episodes. Surgical episodes received a median of 1 day (range 1-20) and bleeding episodes a median of 2 days (range 1-19) FVIII treatment.

A paired inhibitor screen was performed following 33.0% (29/88) of episodes at a median of 93.5 days (range 2-365) after the first treatment day. Of these episodes, a true “convalescent inhibitor screen” was performed in only 13.6% (12/88) within six weeks of treatment (Figure 3 & Supplementary Table 1). Of the episodes that received inhibitor screening within 6 weeks, 2/12 tests coincided with a subsequent treatment day. Thus, 10/88 (11.3%) episodes had an inhibitor screen for which the result would be known for the subsequent treatment episode.

*Factors Influencing Convalescent Inhibitor Screening Practises*

Multi-variate analysis of factors influencing convalescent screening uptake identified imminent treatment (i.e. screened on day of subsequent treatment) as having a strong association, OR 12.13 (95%CI 5.13-28.67, p<0.001) and treatment episode exposure days a weak association, OR 1.12 (95%CI 1.06-1.19, p<0.001). Non-significant variables included: bFVIII:C; reason for treatment; *F8* genotype (standard/unknown or high risk) and age (Supplementary Table 2)

*FVIII Inhibitor Formation*

Thirteen patients had an inhibitor history at the start of the study, with a mean age of 53.6 years (7.5-80.5) and bFVIII:C of 7.6IU/dL (1.9-16.0). These patients were treated with FVIII alone (n=5, 38.5%), DDAVP alone (n=2, 15.4%), bypassing agents alone (n=4, 30.8%), FVIII and bypassing agents (n=1, 7.7%) and DDAVP and bypassing agents (n=1, 7.7%). Home-treatment was used for 3/5 patients treated with FVIII alone.

For those without a prior inhibitor history, still at risk of inhibitor formation (n=290), three developed a new FVIII inhibitor within the study period, detected during treatment for bleeding (n=2) or upon inhibitor screening (n=1). A change in the bleeding phenotype was seen in all three patients and fall in bFVIII:C to <1% in two patients. One of these inhibitors was initially only detectable by the inhibitor assay following pre-analytical heat treatment. Two patients had “high-risk” and one “standard-risk” *F8* genotype. Although all three new inhibitors were detected in a single centre, the total inhibitor frequency for each centre did not differ significantly than that expected by chance (p=0.654).

**Discussion**

Within the London region, nearly half of those living with non-severe haemophilia A (44%, 377/853) received haemostatic treatment within the two-year study period with a large proportion (79%, n=296/377) receiving exposure to a FVIII concentrate. For those treated with FVIII concentrate alone (n=236), this resulted in a cumulative treatment burden for the haemophilia centres of 2,041 EDs over 562 treatment episodes and for the patients, an individualised cumulative median treatment burden of 4EDs over the 2 years. Our cumulative data identifies a substantial and likely increasing treatment responsibility for haemophilia centres given the ageing haemophilia population and additional co-morbidity risks. This is important to highlight to justify appropriate haemophilia centre staffing levels, ensure clear access to specialist care and knowledge of the emergency pathway for this patient group who are likely dependent on the hospital team to administer haemostatic treatment, in contrast to most of those living with severe haemophilia.

The compliance with inhibitor screening in accordance with the available national guidance was generally found to be poor whether judged by the then current 2006 or subsequent 2013 UKHCDO guidance (Hay *et al,* 2006; Collins *et al*, 2013). Half of treatment episodes (n=243, 51.3%) in patients with “standard-risk” *F8* mutations were followed by an annual inhibitor screen. However, of these patients, only 12.3% (28/227) were screened within six weeks of treatment when indicated by higher treatment intensity or surgery. Similarly in patients with “high-risk” *F8* genotypes, only 13.6% (12/88) of episodes were screened within six weeks of treatment. Screening was predominantly passive and reactive to subsequent treatment demands (imminent treatment prompting inhibitor screen, OR 12.13, p<0.001) rather than an episode being risk stratified and pro-actively followed up post-exposure.

*Testing of F8 Genotype and “High-Risk” F8 mutations in Non-Severe Haemophilia A*

For unbiased assessment of genetic risk of inhibitor formation in non-severe haemophilia A there is a need for high levels of uptake of *F8* genotype testing. Within the London haemophilia centres, good uptake of *F8* genotype testing was observed (88% known *F8* genotype). Marked variation in *F8* genotype testing practice has been previously described in an international survey of 13 centres (Gomez & Chitlur, 2013). Outside of the USA, genetic testing was performed in >75% of cases in 7/8 centres and in the USA, 4/5 centres had performed genetic testing in <50% cases. Similarly, in the recent INSIGHT cohort, the assessment of inhibitor risk associated with *F8* genotype could only be performed using data from centres that had genotyped ≥70% of patients, resulting in the exclusion of 59% (20/34) of centres and 1599 patients from the final analysis (Eckhardt *et al*, 2013). Risk evaluation of inhibitor formation in such large observational studies is also dependent on adequate laboratory detection of the primary endpoint. Poor compliance with convalescent inhibitor testing, as seen in this London cohort, may significantly impact on data quality within observational studies. This makes interpreting data on inhibitor risk associated with *F8* mutations (Eckhardt *et al*, 2013) and whether patients with inhibitors respond to immunosuppression or subsequent FVIII exposure (van Velzen *et al*, 2015) difficult due to probable under reporting. Although clinically evident inhibitors (i.e. change in bleeding phenotype) are likely to be detected, those with weaker inhibitory or sub-clinical effect may be missed. However, the totality of immune response to FVIII is vital when trying to understand mechanisms of inhibitor formation and for the development of risk stratification tools.

The selected “high-risk” *F8* genotypes in our evaluation were based on information available within a recently published international study (Astermark *et al*, 2013) to represent mutations that clinicians may be aware of and could influence inhibitor screening practices. Of these seven “high-risk” *F8* mutations included in the London cohort, 4/7 (Arg593Cys, Tyr2105Cys, Arg2150His and Trp2229Cys) have subsequently been described as being associated with increased inhibitor incidence in the INSIGHT study (Eckhardt *et al*, 2013). These four *F8* mutations accounted for 16% (179/1112) of patients within the INSIGHT study and 57% (29/51) of inhibitor patients had one of these *F8* mutations. No inhibitors were seen in the remaining three “high-risk” *F8* mutations in the INSIGHT study although the number of patients with these mutations was low, accounting for only 9 patients of the whole cohort (Eckhardt *et al*, 2013). All three of these mutations (Arg2163His (Hay *et al*, 1998;Waseem *et al*, 1999), Asn2286Lys(Sharathkumar *et al*, 2003) and Pro2300Leu(Liu *et al*, 2000)) have been described to have been associated with inhibitor formation within the HADB (Kemball-Cook *et al*, 1998). The data within the London cohort appears to further support the concept of “high-risk” *F8* genotypes with these mutations being over-represented (50%, 8/16) within the group of patients with an inhibitor. Further study into *F8* genotype inhibitor risk is required but potentially limited by difficulty in case-control matching, since some controls will develop inhibitors later in life. Given the number of patients and length of follow-up required, construction of such prospective studies is challenging. *In-silico* inhibitor risk prediction offers an interesting approach to address some of these difficulties. Work performed by investigators at INSERM (Pashov *et al*, 2014) and within our group (Shepherd *et al*, 2015) have attempted to identify prediction models based around peptide presentation for *F8* genotypes at the MHC Class II / T-cell receptor interface. These data suggest that risk of inhibitor formation in non-severe haemophilia A is more complex than simple knowledge of the *F8* genotype and addition of MHC Class II into clinical prediction algorithms may improve risk prediction.

*Timing and Methodology of Inhibitor Testing in Non-Severe Haemophilia A*

There is currently no data to guide the optimal timing and methodology for performing inhibitor screening in non-severe haemophilia A. Within our cohort, inhibitor testing was performed using a functional inhibitor assay, which has limitations in the detection of inhibitors in non-severe haemophilia A due to likely presence of endogenous FVIII:C in samples. An inhibitor screening test with high specificity (i.e. correctly identifies negative results) insensitive to residual FVIII:C, such as FVIII ELISA (Martin *et al*, 1999;Sahud *et al*, 2007;Batty *et al*, 2015) or modifications to a functional inhibitor assay such as pre-analytical heat treatment (Miller *et al*, 2012;Batty *et al*, 2014;de Lima Montalvao *et al*, 2015;Batty *et al*, 2016), may facilitate inhibitor detection in this patient group. A cut-off of ≤6 weeks between treatment event and screening was selected to reflect the likelihood of detecting a primary or anamnestic immunological response and to provide some certainty that screening was intentionally performed following treatment. The FVIII subcommittee of the Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH) has proposed guidance suggesting that all patients with mild haemophilia A have “convalescent inhibitor screening” using the Bethesda assay six weeks after FVIII exposure (Makris, 2015). This provides a pragmatic approach for inhibitor screening in an area where there are still many unanswered questions as to what constitutes a “high-risk” exposure or indeed a “high-risk” *F8* genotype. Inhibitor screening based on a subset of *F8* genotypes pre-selected as “high-risk” may skew toward detection of inhibitors in these groups, particularly if a founder effect is possible. For other *F8* genotypes where there is less data, this may lead to false reassurance that other groups of individuals are not at risk of antibody formation and result in lack of detection if these are not screened following treatment. Recently presented data from the INSIGHT case-control study has suggested increased risk of inhibitor development with treatment intensity at first exposure (≥10ED), any surgical history and higher (>45IU/dL) peaks of FVIII treatment (van Velzen *et al*, 2016). More systematic, pro-active inhibitor screening that does not discriminate by perceived risk of *F8* genotype will facilitate study of both genetic and environmental risks of FVIII antibody formation and allow further characterisation of the immune response to FVIII. Whether these approaches will increase the detection rate of low-titre or transient inhibitors, as recently described in patients with severe haemophilia A (van den Berg *et al*, 2015) is not clear. Finally, the health economic assessment of increased inhibitor screening should be considered given the substantial associated costs.

*DDAVP*

The number of patients managed with DDAVP within this cohort was possibly lower than expected. Twenty nine percent of patients (110/377) were DDAVP exposed in the study period, with 1 in 5 (76/377) receiving DDAVP alone for their haemostatic treatment. DDAVP offers a cheap and safe (no inhibitor risk) method of treating responsive patients (without medical contraindication) for minor bleeding or surgery (Srivastava *et al*, 2013). There have been varying reports in the literature on factors affecting response to DDAVP which include age, bFVIII:C and *F8* genotype (Castaman & Fijnvandraat, 2014). As expected, patients within our cohort who were treated with DDAVP had higher bFVIII:C levels than those patients treated with FVIII alone. It is possible that the use of DDAVP could further minimise exposure to treatment with FVIII concentrate in responsive patients without medical contraindications (Castaman & Fijnvandraat, 2014).

**Conclusions**

Despite nearly half of individuals in this large cohort receiving haemostatic treatment in the observation period and the causative *F8* genotype being known in the majority, we have shown that only a minority undergo targeted inhibitor screening following treatment with FVIII concentrate. This may limit the availability of information valuable to guide future treatment decisions and to inform patients about their future inhibitor risk, whilst also undermining efforts to unpick the underlying immunological mechanisms. Further service development and awareness is required to implement optimal screening practice, timing and methodology of inhibitor screening in patients with non-severe haemophilia A.

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**Authorship Contribution**

PB & DPH designed the research study

PB performed the research

PB, JPS, SKA, KK, CMM, SR, MT, TTY and DPH collected the data

PB, DPH and BP analysed the data

PB & DPH wrote the first draft of the paper

All authors reviewed and critically edited the manuscript

**Conflicts of Interest**

None declared

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|  |  |  |  |
| --- | --- | --- | --- |
|  | Bleeding | Surgery | Total |
| Patients (episodes) | 157 (351) | 130 (211) | 236 (562) |
| Age (years)  Mean±SD  Median (range) | 34.9±22.8  32.7 (0.2-89.0) | 46.6±23.0  48.5 (2.1-89.0) | 39.1±24.1  39.9 (0.2-89.0) |
| bFVIII:C  Mean±SD  Median (range) | 10.0±7.6  8.0 (1.0-42.0) | 13.0±9.5  10.0 (1.5-43.8) | 12.2±9.1  10.0 (1.0-43.8) |
| Severity  Moderate  Mild | 56  101 | 28  102 | 67  169 |
| *F8* genotype  “High-risk”  “Standard-risk”  Not known | 27 (17.2%)  109 (69.4%)  21 (13.4%) | 24 (18.5%)  88 (67.7%)  18 (13.8%) | 42 (17.8%)  162 (69.1%)  31 (13.1%) |
| Treatment Episode  Mean±SD  Median (range) | 2.2±2.2  1 (1-15) | 1.6±1.4  1 (1-11) | 2.38±2.2  1 (1-15) |
| Total ED / Patient  Mean±SD  Median (range) | 7.4±10.2  4 (1-82) | 6.8±15.5  2 (1-159) | 8.7±14.6  4 (1-159) |

**Table 1: Baseline demographics of patients with non-severe haemophilia A receiving on-demand treatment with a FVIII concentrate. bFVIII:C= baseline FVIII activity. ED=exposure days; SD=standard deviation.**

**Figure 1: Treatment modalities used in the management of non-severe haemophilia A, representing the percentage of patients (total: 377) treated within the observation period.**

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**Figure 2: Frequency of bleeding in patients with non-severe haemophilia A. A right (positive) skew in distribution of bleeding episodes (annualised bleed rate) was seen for patients treated with on-demand FVIII (median 0.5 bleeds/year)**



**Figure 3: Inhibitor screening in non-severe haemophilia A. Standard = “standard-risk” *F8* genotype or *F8* genotype not recorded. High Risk = “high-risk” *F8* genotype. Convalescent screened = inhibitor screen performed within 6 weeks of first exposure day of a given treatment.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | Tested | | Not Tested | | |
|  | | Episodes | Tested (%) | Test within 24 hours of treatment (%) | | Not Tested (%) | Treatment episode ≤42 days (%) |
| “High-Risk” *F8* Genotype | Annual | 88 | 42 (47.7%) | 9 (21.4%) | | 46 (52.3%) | - |
| Paired | 88 | 29 (33.0%) | 9 (31.0%) | | 59 (67.0%) | - |
| ≤42 days | 88 | 12 (13.6%) | 2 (16.7%) | | 76 (86.4%) | 15 (19.7%) |
| “Standard-Risk” *F8* Genotype | All Annual | 474 | 243 (51.3%) | 42 (17.3%) | | 231 (48.7%) | - |
| All Paired | 474 | 158 (33.3%) | 42 (26.6%) | | 316 (66.7%) | - |
| All ≤42 days | 474 | 57 (12.0%) | 21 (36.8%) | | 417 (88.0%) | 75 (18.0%) |
| Surgery Annual | 175 | 71 (40.6%) | 14 (19.7%) | | 104 (59.4%) | - |
| Surgery Paired | 175 | 48 (27.4%) | 14 (29.2%) | | 127 (72.6%) | - |
| Surgery ≤42 days | 175 | 17 (9.7%) | 7 (41.2%) | | 158 (90.3%) | 22 (13.9%) |
| ≥5ED Bleed Annual | 52 | 28 (53.8%) | 2 (7.1%) | | 24 (46.2%) | - |
| ≥5ED Bleed Paired | 52 | 21 (40.4%) | 2 (9.5%) | | 31 (59.6%) | - |
| ≥5ED Bleed ≤42 days | 52 | 11 (21.2%) | 2 (18.2%) | | 41 (78.8%) | 13 (31.7%) |
| All Episodes | Annual | 562 | 285 (50.7%) | 51 (17.9%) | | 277 (49.3%) | - |
| Paired | 562 | 187 (33.3%) | 51 (27.3%) | | 375 (66.7%) | - |
| ≤42 days | 562 | 69 (12.3%) | 23 (33.3%) | | 493 (87.7%) | 90 (18.3%) |

**Supplementary Table 1: Frequency of inhibitor screening in non-severe haemophilia A. Treatment Episode ≤42 days=Frequency of episodes in which there was another treatment with a FVIII concentrate in ≤42 days and where a paired inhibitor test was not performed within ≤42 days.**

|  |  |  |  |
| --- | --- | --- | --- |
| Covariate | N | Odds Ratio (95% CI) | p |
| Test due to imminent treatment  No\*  Yes | 477  51 | 1  12.13 (5.13-28.67) | <0.001 |
| Baseline FVIII:C  Per IU/dL | 528 | 0.96 (0.91-1.01) | 0.156 |
| ED within treatment episode  Per Day | 528 | 1.12 (1.06-1.19) | <0.001 |
| Treatment indication  Bleed/trauma\*  Surgery | 325  203 | 1  0.67 (0.32-1.40) | 0.282 |
| F8 mutation  Standard/unknown risk\*  High risk | 445  83 | 1  1.16 (0.46-2.95) | 0.752 |
| Age at treatment  Per Year | 528 | 1.01 (0.99-1.02) | 0.432 |

Supplementary Table 2: Multivariate analysis of factors influencing convalescent (≤42 days) inhibitor screening. Analysis performed on 528 episodes in 224 patients at the 7 centres. \*corresponds to baseline category. ED=exposure day