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Factor VIII Prophylaxis Effects Outweigh Other Hemostasis Contributors in Predicting Severe Hemophilia A Joint Outcomes

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Abstract

Introduction: The Joint Outcome Study (JOS) demonstrated that previously untreated children with severe hemophilia A treated with prophylactic factor VIII (FVIII) concentrate had superior joint outcomes at age six years compared to those children treated episodically for bleeding. However, variation in joint outcome within each treatment arm was not well-explained.

Aim: In this study, we sought to better understand variation in joint outcomes at age 6 years in participants of the JOS.

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Authorship

BBW analyzed and interpreted the data and wrote the first draft of the manuscript. LJ performed all of the laboratory assays. GRB, MR, DB, CL, TCA, ADS, and MMJ developed the research plan and performed the research. CK performed the pharmacokinetic analyses. All authors reviewed, edited, and approved the manuscript.

Conflicts of Interest

The remaining authors have no conflicts of interest to declare.

Methods: We evaluated the influence of FVIII half-life, treatment adherence, constitutional coagulant and anticoagulant proteins, and global assays on joint outcomes (number of joint bleeds, total number of bleeds, total MRI score, and joint physical exam score). Logistic regression was used to evaluate the association of variables with joint failure status on MRI, defined as presence of subchondral cyst, surface erosion, or joint-space narrowing. Each parameter was also correlated with each joint outcome using Spearman correlations.

Results: Prophylaxis treatment arm and FVIII trough were each found to reduce risk of joint failure on univariate logistic regression analysis. When controlling for treatment arm, FVIII trough was no longer significant, likely because of the high level of covariation between these variables. We found no consistent correlation between any laboratory assay performed and any joint outcome parameter measured.

Conclusion: In the JOS, the effect of prescribed prophylactic FVIII infusions on joint outcome overshadowed the contribution of treatment adherence, FVIII half-life, global assays of coagulation, and constitutional coagulation proteins. (ClinicalTrials.gov number, NCT00207597)

Keywords

Haemophilia; Prophylaxis; Pediatric; Joints; Blood Coagulation Factors; Global Assays

Introduction

The Joint Outcome Study (JOS) demonstrated that young children with severe hemophilia A (HA) treated prophylactically with FVIII had superior joint health at age six compared to those treated with episodic FVIII [1]. Based on these and other results, prophylactic treatment is the standard of care for children with severe hemophilia [2–4]. However, outcomes of the JOS showed variation on identical treatment regimens. This study aimed to determine the influence of patient constitutional and treatment factors on joint outcomes among the JOS participants.

Previous studies evaluating the influence of prothrombotic proteins on hemophilia outcomes have had varied results. The Factor V Leiden (FVL) and prothrombin G20210A (PT20210) heterozygous mutations concomitantly inherited with severe hemophilia were determined to have no effect on factor consumption [5, 6] or Hemophilia Severity Score [7]. Other studies demonstrated lower bleeding rates in those with severe hemophilia and heterozygous FVL, with inconsistent joint outcomes [8, 9]. Similarly, children with severe HA and FVL, PT20210, or protein C deficiency had a 6 month delay in bleeding onset [10]. A higher frequency of prothrombotic abnormalities was described in “clinically mild” phenotypes (<2 bleeds/5 years) compared to “clinically severe” (>10 bleeds/5 years) in patients with severe HA [11].

Previous studies had limited generalizability, as patients were not treated uniformly. In contrast, JOS participants were treated according to specific prophylaxis or episodic treatment protocols, allowing an analysis of other patient factors impacting outcome.

Characterization of hemostatic and anticoagulant proteins in hemophilia may be particularly important with the development of treatment regimens that utilize alternative hemostatic

pathways. Clinical trials inhibiting tissue factor pathway inhibitor (TFPI) [12] and decreasing antithrombin [13] seek to maximize thrombin generation to prevent bleeding without inducing thrombosis. Awareness of the impact of coagulation proteins on hemophilia phenotype may be important, as the effect may be magnified when proteins are modified non-physiologically.

We evaluated plasma samples from the JOS to better understand the effects of constitutional variation in coagulation proteins and global assays on joint outcomes in severe HA. We also examined the role of FVIII half-life and treatment adherence.

Materials and Methods

Study Participants

The JOS was approved by the ethics committee at each participating institution and registered with clinicaltrials.gov (NCT00207597). The sixty-five participants were randomized as toddlers to receive either prophylactic FVIII 25 IU/kg every other day (n=32) or enhanced episodic FVIII for treatment of bleeding (3+ doses per joint bleed, n=33) [1]. All received the same recombinant FVIII product (Kogenate®, Bayer HealthCare). Follow up included monthly bleeding and infusion log review; quarterly weight-based dose adjustment; biannual joint physical exam; and study entry and exit MRI and X-ray. Participants who developed and maintained an inhibitor of >10 Bethesda units (BU) for 3 months were removed from the study [1].

Sample Collection and Processing

Blood was collected for all assays except pharmacokinetic studies >72 hours and 48±6 hours after last factor dose in the episodic and prophylaxis arms, respectively. Blood was collected by venipuncture, or from central venous access devices (CVAD) as previously described [14]. Samples were immediately centrifuged twice at 4°C at 2500*g* for 20 minutes. Plasma was stored in 250-μL aliquots at −70°C and thawed at 37°C immediately before testing. Heparin was neutralized from CVAD samples using hepzyme (Dade Behring) [14]. Plasma samples with positive inhibitor titers were not used here. FVIII and FVIII inhibitor assays were run within 6 months of sample collection and processing. Other assays were run within 10 years of sample processing; our laboratory quality assurance determined that factor levels on samples stored at −70° C for 1 month and for 10 years had an acceptable inter-class correlation coefficient (>0.95).

Protein Assays

FVIII levels were determined using one-stage clot-based assay [15]. Von Willebrand Factor (VWF) antigen levels were measured by enzyme-linked immunosorbent assay (ELISA) using the REAADS® kit (Corgenix). TFPI activity was measured chromogenically using the Actichrome® kit (American Diagnostica, Inc). Thrombin activatable fibrinolysis inhibitor (TAFI) antigen levels were evaluated by ELISA using the Imuclone® kit (American Diagnostica, Inc). Activated protein C resistance (APC-R) was measured using the aPTT-based Coatest® APCR-V kit (Chromogenix). Protein C activity and antithrombin heparin cofactor activity were measured chromogenically using StaChrom® kits, and free and total

protein S antigen were measured by latex immunoassay using Sta®Liatest® kits, all using the Stago STA Compact Max®. FVIII inhibitor was measured every three months using the Bethesda assay [16].

Global Assays

Global assays were performed to assess whether decreased thrombin generation, decreased clot formation, or increased clot lysis on trough samples correlated with increased bleeding [17–19]. Computed Automated Thrombography (CAT, Diagnostica Stago, Inc.) was performed without corn trypsin inhibitor using a 1 pM tissue factor trigger. Time to clot initiation (lagtime), estimated thrombin potential (ETP, area under the curve), peak thrombin generation (peak) and time to peak (ttPeak) were calculated from CAT thrombin generation curves. [20]Thrombin-antithrombin complex (TAT) levels were measured by ELISA using the Enzygnost® TAT micro kit (Siemens Healthcare Diagnostics). The Clot Formation And Lysis (CloFAL) assay, which turbidometrically measures clot formation and subsequent lysis of plasma after addition of lipidated tissue factor, calcium, and tissue plasminogen activator, was performed, and maximum amplitude (MA), time to MA (T_1), and coagulation index (CI) were calculated [19, 20]. Euglobulin Lysis Time (ELT) was performed by turbidometrically measuring time to lysis of a a clot formed from a resuspended precipitate using acid buffer enriched for fibrinogen, plasminogen, tPA, PAI-1, FVIII, and TAFI [21].

Normal Ranges

Normal ranges for each assay were determined using plasma samples from 20–50 healthy controls without bleeding or clotting history, and with normal PT, aPTT, and fibrinogen screening. Normal range was defined as [mean]±2*[standard deviation], or as [median] ±1.5*[interquartile range] if values were not normally distributed. Pediatric normal ranges were determined for all assays except antithrombin, TAT, and APC-R, for which adult samples were used due to limited normal pediatric sample availability.

Factor VIII Pharmacokinetics

Pharmacokinetic (PK) studies were performed in participants who agreed by measuring FVIII activity prior to and at least 6 time points ranging from 0.25 to 72 hours following dose administration. Population-based PK parameters were calculated using the WAPPS-Hemo program [22, 23] and confirmed using WinNonlin (version 5.2, Pharsight Corp).

Review of Infusion Logs

Adherence to treatment regimen was determined using paper treatment and bleeding episode logs collected monthly. Adherence was defined as the percentage of total factor doses prescribed that were given; the average of monthly adherence percentages are reported. Study personnel also answered the question “In your professional opinion, what was the family’s level of compliance with the study? 70–100%, 40–69%, or <40%” on quarterly study forms.

Outcome Measurements

The total number of bleeding episodes from study entry to exit (at age 6) was used. MRIs performed on ankles, knees, and elbows were scored on a 10-point scale as previously described [1, 24] by 2 independent radiologists. Joint failure was defined as presence of osteochondral damage, indicated by MRI score ≥7. Scores that discrepantly identified joint failure were adjudicated by a third radiologist, with the two most congruent scores averaged. Because most participants had MRI scores of <1 at study exit, perfect joint outcome (MRI score <1 in all joints) and intermediate joint outcome (maximum MRI score 1 to <7 in any joint) categories were also used. Joint exams were performed as previously described [1, 25]; the summative score of all joints at study exit was used. Joint exam and MRI scoring were performed by investigators blinded to study arm.

Statistics

The odds ratio for MRI joint failure given the value of each experimental parameter was calculated using univariate logistic regression. The variables associated with MRI joint failure with $p<0.2$ were evaluated using multivariable logistic regression. The relationships between each laboratory assay and each joint outcome were also evaluated using Spearman correlations. Statistical software was SAS®9.4.

Results

Descriptive Statistics

Descriptive statistics of each measured parameter and outcome by treatment arm are shown in table 1, along with normal ranges. Among plasma proteins, only FVIII trough differed by treatment arm, with higher mean FVIII trough in the prophylaxis arm. Global assays, measured at times of FVIII trough, showed mild but non-significant increases in ETP, thrombin peak, and time to clot lysis in the prophylaxis vs episodic arms.

Coagulation Proteins and MRI Joint Outcomes

Table 2 shows MRI outcomes at study exit. Odds of joint failure on the episodic arm were 10.5 times that on prophylaxis (95%CI 2.1,51.9, p=0.004, table 3). The odds ratio of a perfect joint on MRI was 3.39 (95% CI 1.18,9.71, p=0.02) on prophylaxis compared to episodic treatment.

Two participants were found to have low VWF, with VWF antigens 18% (joint failure, episodic) and 31% (perfect joints, prophylaxis) (table 2). Two participants, both on the prophylaxis arm, were found to have anticoagulant proteins mildly below the normal range: one with protein C activity 56% and a perfect MRI score, and the other with free protein S antigen 44% and MRI joint failure. Two children had FVL heterozygosity, one on each treatment arm, both with perfect MRI scores (table 2). Eighteen participants had elevated TFPI levels, ranging from 1.7 to 2.4 units/mL. Three of 6 with elevated TFPI in the episodic arm had joint failure compared with zero of 12 on prophylaxis. TAT and ELT values outside of normal were distributed across joint outcomes and treatment groups.

Effect of Parameters on MRI Joint Failure

The influence of each laboratory assay on MRI joint failure was evaluated using univariate logistic regression (table 3). Only trough/baseline FVIII and treatment arm significantly influenced odds of MRI joint failure. For every 1% increase in FVIII trough, the odds of joint failure were 0.18 (95%CI 0.05,0.68; p=0.012) times lower. However, when controlling for treatment group, the influence of FVIII trough was not statistically significant (OR 0.28, 95%CI 0.058,1.38; p=0.12, table 4), likely because of the high level of covariance between higher FVIII trough and prophylaxis group (Pearson correlation coefficient 0.64, p<0.0001). The contribution of FVIII trough to MRI joint failure was also analyzed within each treatment arm. Within the narrow range of levels in this study (0.5–7.4%), there was not a significant association between FVIII trough in the prophylactic (OR 0.94, 95%CI 0.004,2.03, p=0.13) or the episodic arm (p=0.81), although the prophylaxis arm, which had a wider range of FVIII troughs, approached significance.

Adherence was high across the study (mean 97%, median 99%, range 83–100%) and did not influence joint outcome, perhaps because of the lack of variability. Study personnel judged the vast majority of participants to have >70% compliance. Two prophylaxis participants, with maximum MRI scores of 2 and 9, were judged to have <40% compliance for more than one quarter.

FVIII half-life was able to be calculated for 19 participants (11 episodic, 8 prophylaxis) using WAPPS-Hemo and confirmed for 15 using WinNonLin. The mean FVIII half-life was 11.5 (range 5.5–14.5) hours using WAPPS-Hemo and 11.7 (range 8.5–18.4) hours using WinNonLin. There was neither an association between half-life and joint failure in logistic regression (table 3), nor a difference in half-life between those with and without joint failure within each treatment arm (p=0.6 prophylaxis, p=0.1 episodic). Additional PK parameters, including area under the curve, clearance, and time to 5% and 1% FVIII were also considered in the logistic regression analysis and were not associated with joint failure.

The laboratory parameters with univariate analysis p<0.2 (FVIII, VWF, Free Protein S) were analyzed with multivariable logistic regression, and FVIII trough continued to have a significant effect on MRI joint outcome (p=0.013) (table 4). The treatment arm effect on outcome was also adjusted for adherence, since poor adherence to prophylaxis could weaken the effect. Adjusted odds of joint failure on episodic treatment were 18.6 times that of prophylaxis (95%CI 2.5,138, p=0.004) (table 4).

Correlation between Parameters and Joint Outcome

The relationship between each parameter and each outcome (total bleeds, total joint bleeds, MRI score, and joint exam score) was evaluated using Spearman correlations. Antithrombin level correlated positively with joint exam score, but not with MRI score or number of bleeds (supplemental table 1). FVIII trough correlated negatively with total and joint bleeds and MRI score but not joint exam score (supplemental table 1) when analyzed in aggregate but not when separated by treatment arms (supplemental table 2), likely because of FVIII/treatment arm covariance. Negative correlations were detected when comparing CAT peak thrombin generation to bleeding episodes in the aggregate and prophylaxis analyses, but this

pattern did not hold for MRI scores, or for any outcome in the episodic arm (supplemental table 2). Scatter plots of joint outcomes with select laboratory parameters are shown in figure 1.

Effect of Inhibitor formation on Outcome

Inhibitory antibodies developed in ten study participants (table 5). Children received a mean of 714 factor exposures (range 338 to 979) on prophylaxis and 207 factor exposures (range 23 to 437) on the episodic arm. Joint failure by MRI developed in 1 of 3 children with a high titer inhibitor, 1 of 2 children with persistent low titer inhibitor and none of 5 children with a low titer (0.5–1.0BU) on one occasion only. One child experienced intracranial hemorrhage following resolution of a low-titer inhibitor.

Discussion

This secondary analysis of the Joint Outcome Study extends the results of the initial JOS analysis, demonstrating that prophylaxis with FVIII was the agent responsible for the prevention of bleeding and preservation of joint structure at age six years. Although there was variability in bleeding and joint outcome within each treatment arm, the explanatory power of prophylaxis outweighed that of all other variables examined.

No consistent correlations were found between any individual coagulant or anticoagulant protein and any outcome. When evaluating laboratory values outside of the normal range, the influence was minimal compared to treatment arm. These data suggest that mild anticoagulant derangement did not prevent joint damage. Current clinical trials in hemophilia attempt to restore functional thrombin generation by decreasing coagulation regulatory proteins. Our data support reports that anticoagulant proteins must be decreased significantly (80–90%) to adequately increase thrombin generation enough to impact bleeding rates [13]. Unfortunately, we were not able to derive TFPI or antithrombin cutoff levels to predict joint failure from our small sample size with low variability (Figure 1).

FVIII trough and treatment arm were the only variables significantly associated with MRI joint failure on univariate analysis. Other reports have highlighted the role of higher FVIII trough in preventing prophylaxis breakthrough bleeding [26]. In multivariable analysis, the influence of prophylaxis was greater than that of the FVIII trough level, although the FVIII trough range was limited. Prophylactic dosing with higher trough levels may have shown a relationship between trough and outcome.

FVIII half-life drives trough FVIII; VWF stabilizes FVIII and extends its half-life [27]. Neither VWF nor FVIII half-life exerted an independent effect on joint outcomes, which was surprising, particularly for prophylaxis participants. This group of young children, treated with standard recombinant FVIII, exhibited a mean FVIII half-life of 11.5 hours, longer than that reported in the literature for children [28–30]. It was not possible to detect PK-driven impacts on outcomes, perhaps because of limited variability.

Adherence is a key driver of treatment outcomes [31, 32]. This population had excellent adherence; therefore the threshold of adherence required to achieve an optimal outcome

could not be determined. Two children on the prophylaxis arm had joint failure. Both children were suspected by their study team to have poor adherence, but this was difficult to discern from treatment logs.

Some authors have reported that global assays of hemostasis predict hemophilia bleeding phenotype. Hugenholtz demonstrated that CAT ETP measured 32 hours after FVIII predicted monthly FVIII usage better than FVIII levels at that time [33]. Trossaert [34] and Dargaud [35] both demonstrated an association between CAT ETP and clinical bleeding phenotype in patients with hemophilia. Goldenberg found that global assays CloFAL and ELT correlated with degree of factor deficiency [19, 36]. In this study, we found no association between joint failure on MRI and any global assay at time of FVIII trough. However, thrombin generation ETP and peak were non-signficantly higher in the prophylaxis group than the episodic group. A larger sample (n=163) in our lab with a wider FVIII range (0–181%) showed a strong Pearson correlation between FVIII and ETP or peak ($r=0.7, p<0.0001$), so a larger sample size or wider FVIII range may have shown an effect. Lagtime in the larger study had a weaker correlation with FVIII ($r=-0.2, p=0.01$), perhaps contributing to the unexpected lower mean lagtime in the episodic group (table 1).

Finally, high-titer inhibitors presented in 10% of children on the prophylaxis arm, similar to the rate of high-titer inhibitor formation in children with severe HA overall [37]. No high-titer inhibitors developed on episodic therapy, suggesting that early institution of prophylaxis may not be sufficient for inhibitor prevention [37]. The children in the episodic arm did have fewer factor exposures, and 2 participants had <50 factor exposures at study exit. Low titer inhibitors were detected in 7 children, probably because of frequent screening, and many did not seem to have clinical impact.

This study had limitations to be noted. Constitutional and treatment variables were used to predict joint outcome at age 6 when evidence of joint damage, where present, was rather mild. This could have limited the value of correlation calculations. However, this analysis was repeated on a subset of 37 JOS participants in the recently completed Joint Outcome Continuation Study (supplementary tables 3–5), with similar results. The study population had a low frequency of abnormal non-FVIII coagulation proteins. The JOS was powered to detect a joint outcome difference between prophylaxis and episodic FVIII treatment; a larger sample size may be required to demonstrate a smaller effect of other parameters on joint outcomes.

Conclusion

In a thorough analysis of the effect of patient constitutional coagulation protein levels, global assays, and treatment variables on joint outcome in severe HA patients in the JOS, prophylaxis was found to be the only predictor of joint preservation. It is likely that the benefit of prophylaxis outweighs any contribution of other coagulation proteins on global hemostasis. Unfortunately, this analysis did not reveal a reason for variation in joint outcome within each treatment arm, which could include individual susceptibility to bone and cartilage damage or a variable frequency of subclinical bleeding. Prophylaxis remains the most important treatment regimen for maintaining joint health in patients with severe HA.

However, more studies are needed to understand why some severe HA patients with excellent adherence to prophylaxis develop arthropathy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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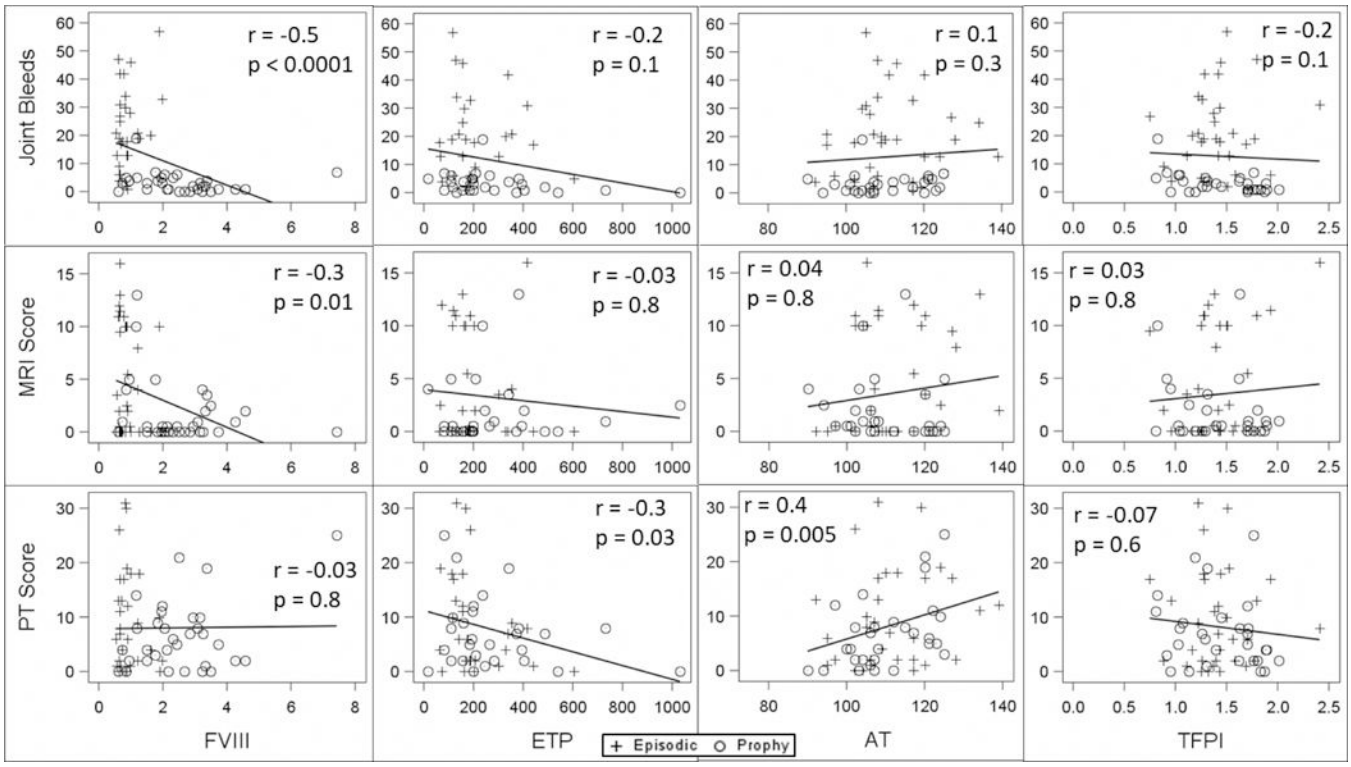

Figure 1:

Scatter plots and regression lines of joint outcomes vs FVIII, thrombin generation ETP, antithrombin (AT) levels and TFPI levels.

Table 1:

Descriptive statistics for each laboratory assay and joint outcome by treatment arm. MRI and joint exam scores are the sum of scores for all joints (elbows, knees, and ankles) on exams performed at study exit (age 6 years). Normal ranges were defined as [mean ± 2*standard deviation] of patients without bleeding disorders in our lab unless otherwise indicated.

	Prophylaxis					**Episodic**					**p**	**Normal Range**
Plasma Protein Levels	Mean	Median	Std Dev	Min	Max	Mean	Median	Std Dev	Min	Max		
FVIII Trough (%)	2.5	2.4	1.4	0.6	7.4	0.90	0.80	0.4	0.5	1.9	<0.0001	53–200
FVIII Half-Life (hrs)	10.8	11.8	2.7	5.5	14.3	12.10	11.80	1.4	10.8	14.5	0.2	
VWF Antigen (%)	90.4	84.9	27.1	31.25	148.2	90.9	91.4	27.6	18.6	138.6	0.9	40–127[a]
Protein C Activity (%)	82.8	81	12.3	56	114	84.8	86	11.8	65	112	0.5	60–113
Free Protein S Antigen (%)	94.1	86	24.2	44	141	93.4	93	19.5	59	138	0.9	47–140
Total Protein S Antigen (%)	92.9	92	14.7	65	130	96.3	93	15.2	70	135	0.4	63–114
Antithrombin Activity (%)	110.2	107	10.0	90	125	111.2	109	11.0	92	139	0.7	92–118
TAFI antigen (%)	79.3	81.5	14.2	40.1	102.6	79.6	77.9	17.5	48.3	124.5	0.9	52–135
TFPI (units/mL)	1.4	1.5	0.4	0.8	2.01	1.4	1.4	0.3	0.7	2.4	0.7	0.7–1.7
APC-R (ratio)	1.0	1.0	0.1	0.7	1.3	1.0	1.0	0.1	0.7	1.2	0.8	0.9–1.1
Global Assays												
CAT Lagtime (min)	11.0	8.7	5.9	3.73	26.7	7.3	6.6	4.0	1.9	21.2	0.01	1.6–4.2
CAT ETP (nM*min)	280.1	203.4	216.0	17.5	1031	208.2	168.5	126.9	61.4	605.2	0.1	603–2036
CAT Peak (nM)	18.2	11.8	14.8	0.73	62.7	11.9	9.5	10.6	2.4	55.7	0.08	81–421
CAT ttPeak (min)	22.7	21.1	5.9	12.11	39.7	21.6	21.1	6.2	8.3	39.0	0.5	8–5.9
CloFAL CI (%)	13.9	9.1	14.2	0.7	58.1	19.5	8	23.8	0.7	82.4	0.3	0–145
CloFAL T1 (min)	56.7	48.4	22.7	29	110.7	55.3	48.3	33.3	21.5	158	0.9	0.2–0.6
CloFAL MA (units)	0.2	0.171	0.1	0.046	0.5	0.2	0.2	0.1	0.0	0.413	0.9	16–50
TAT (ug/L)	2.3	1.7	1.5	1.1	8	3.8	2.0	5.1	1.1	23.4	0.1	1.1–4.3
ELT (min)	305.1	307.5	107.2	145	512	266.8	257.5	80.6	120	506.5	0.1	49–273[a]
Joint Outcomes												
Total Joint Bleeding Episodes	3.2	2.5	3.6	0	19	21.0	19	14.6	1	57	<0.0001	
Total Bleeding Episodes	14.6	12	14.4	1	70	80.1	78	43.1	6	168	<0.0001	
MRI Score	1.8	0.5	3.0	0	13	5.0	2.5	5.2	0	16	0.004	
Joint Exam Score	6.5	5	6.4	0	25	9.4	7	8.9	0	31	0.14	

[a]= Normal range defined as [median ± 1.5*interquartile range], as values were not normally distributed.

Table 2:

Summary of MRI Joint Outcomes and Laboratory Value Outliers. The joint with the maximum MRI score is reported for each participant. Values listed are the laboratory values outside of the normal range.

Joint Outcome (Max MRI Score)		Prophylaxis	Episodic
Perfect (<1)	# (%) Participants	22 (69%)	13 (39%)
	Laboratory Outliers	1 FVL 1 VWF = 31.25% 1 PC 56%	1 FVL
Intermediate (1–6)	# Participants	7 (22%)	5 (15%)
	Laboratory Outliers	None	None
Failure (≥7)	# Participants	2 (6%)	13 (39%)
	Laboratory Outliers	1 PS Free = 44%	1 VWF =18.6%
MRI not done or Inadequate	# Participants	1 (3%)	2 (6%)
	Laboratory Outliers	None	1 TAFI = 48.3
Total	# Participants	32	33

FVL=factor V Leiden, PC = protein C, VWF=Von Willebrand Factor, PS Free = free protein S, TAFI = thrombin activatable fibrinolysis inhibitor.

Table 3:

Results of Univariate Logistic Regression for the influence of each parameter on MRI joint failure.

Parameter	Odds Ratio Estimate (95% CI)	P-value
Plasma Protein Levels		
VWF Antigen (%)	0.98 (0.96,1.01)	0.14
Protein C Activity (%)	0.99 (0.95, 1.05)	0.90
Free Protein S Antigen (%)	0.97 (0.94, 1.01)	0.10
Total Protein S Antigen (%)	0.99 (0.95, 1.03)	0.58
Antithrombin Activity (%)	1.03 (0.97, 1.09)	0.33
TAFI antigen (%)	0.98 (0.94,1.02)	0.33
TFPI (units/mL)	1.17 (0.21,6.65)	0.86
APC-R (ratio)	1.10 (0.003, 388.8)	0.98
Global Assays		
CAT Lagtime (min)	0.84 (0.70, 1.02)	0.072
CAT ETP (nM*min)	0.99 (0.99, 1.00)	0.37
CAT Peak (nM)	0.95 (0.88, 1.03)	0.26
CAT ttPeak (min)	0.96 (0.85,1.07)	0.44
CloFAL CI (%)	1.00 (0.97, 1.03)	0.90
CloFAL T1 (min)	1.00 (0.98, 1.02)	0.92
CloFAL MA (units)	0.04 (<0.001, 46.10)	0.37
TAT (ug/L)	1.07 (0.94,1.22)	0.33
ELT (min)	1.00 (0.99,1.01)	0.41
Treatment Parameters		
Treatment Group (Episodic vs Prophy)	10.47 (2.11, 51.9)	0.004
FVIII Half-life	0.9 (0.57, 1.43)	0.66
FVIII Trough	0.18 (0.05, 0.68)	0.012
Adherence (% Prescribed Doses Given)	<0.001 (<0.001, 395.8)	0.27

Table 4:

Multivariable Logistic Regression for the influence of each parameter on MRI joint failure.

Parameter	Adjusted For	Adjusted Odds Ratio (95% CI)	P-value
FVIII Trough	Treatment Group	0.28 (0.058, 1.38)	0.12
FVIII Trough	VWF, Free Protein S	0.17 (0.04, 0.69)	0.013
Treatment Group (Episodic vs Prophy)	Adherence	18.59 (2.50, 138.1)	0.0043

Table 5:

Inhibitor development in study participants

	Prophylaxis	**Episodic**
# Randomized	32	33
High Titer Inhibitor	3 (9%)	0
Low titer persistent or recurrent	0	2 (6%)
Transient, single detection	2 (6%)	3 (9%)
Total Inhibitors	5 (15%)	5 (15%)