Relationship between plasma tissue Factor Pathway Inhibitor (TFPI) levels, thrombin generation and clinical risk of bleeding in patients with severe haemophilia A or B

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Abstract

Introduction: Bleeding severity in severe haemophilic patients, with low thrombin generation (TG) capacity, can vary widely between patients, possibly reflecting differences in tissue factor pathway inhibitor (TFPI) level.

Aim: To compare free TFPI (fTFPI) levels in patients with severe haemophilia A (sHA) and severe haemophilia B (sHB) and to investigate in these patients as a whole the relationships between bleeding and TG potential, between TG potential and fTFPI level and between fTFPI level and bleeding tendency.

Methods: Data on bleeding episodes retrospectively recorded during follow-up visits over 5–10 years were collected and used to calculate the annualised joint bleeding rate (AJBR). fTFPI levels and basal TG parameters were determined in platelet-poor plasma (PPP) and platelet-rich plasma (PRP) using calibrated automated tomography (CAT).

Results: Mean fTFPI levels did not differ significantly between sHA ($n = 34$) and sHB ($n = 19$) patients.

Mean values of endogenous thrombin potential (ETP) and thrombin peak (peak) in PPP and PRP were two-fold higher when fTFPI levels < 9.4 versus > 14.3 ng/mL. In patients treated on demand, ETP and peak in PRP were doubled when AJBR was ≤ 4.9, AJBR being halved in patients with a low fTFPI level (9.4 ng/mL). In patients on factor prophylaxis, no association was found between TG parameters and either fTFPI level or AJBR.

Conclusion: In patients treated on demand, bleeding tendency was influenced by fTFPI levels, which in turn affected basal TG potential. In patients on prophylaxis, bleeding tendency is probably determined primarily by the intensity of this treatment.

KEYWORDS
severe haemophilia A, severe haemophilia B, thrombin generation and bleeding rate, tissue factor pathway inhibitor (TFPI)

1 | INTRODUCTION

The rare X-linked recessive bleeding disorders haemophilia A and B, caused by deficiency of the functional coagulation factors VIII (FVIII) and factor IX (FIX), respectively, may be classified according to endogenous factor activity levels as severe (<1%), moderate (1%–5%) or mild (5%–40%). Individuals with severe haemophilia experience frequent and sometimes life-threatening episodes of bleeding following minor trauma or even spontaneously.

The severity of the bleeding phenotype, characterised by a low thrombin generation (TG) potential, is correlated with the extent of deficiency of the factor concerned.[1,2] However, the clinical expression of haemophilia in terms of bleeding can vary from one patient to another despite their presenting the same degree of coagulation factor deficiency,[1,3] suggesting that other factors may play a role. As regards patients with severe haemophilia, the extent of bleeding seemed to be greater in those with haemophilia A compared to haemophilia B,[4,5] although this has not been unequivocally demonstrated.[6]

Recent data have shown that TG tests could be useful for evaluating bleeding risk in patients with haemophilia.[7–9] Notably, in a population including both haemophilia A and haemophilia B patients, a correlation was found between severe clinical bleeding type and low ETP.[1]

Tissue factor (TF) pathway inhibitor (TFPI) is a potent direct inhibitor of FXa, inhibiting the FVIIa/TF complex in a FXa-dependent manner.[10–12] Free TFPI (fTFPI) is the active form of this molecule[10] (even if LDL bound TFPI could affect TG).[13] The major role of fTFPI as a negative determinant of TG is manifested when the intrinsic coagulation pathway is impaired, as in haemophilic patients and when TG is measured at low TF concentrations; fTFPI is in fact the main determinant of TG in haemophilic patients.[14]

A retrospective study comparing fTFPI levels in haemophilia A and haemophilia B patients indicated lower levels in patients with haemophilia B, irrespective of disease severity,[15] which might possibly contribute to the putatively lower bleeding risk in severe haemophilia B patients.

The primary objective of this study was to confirm the previously reported difference in fTFPI levels between patients with haemophilia A and B[15] in a more homogenous patient population, namely those with severe haemophilia. Secondary objectives were to confirm, in these patients, the association between fTFPI level and TG potential and between TG potential and bleeding phenotype, and finally, to investigate the relationship between fTFPI level and bleeding phenotype expressed as the annualised joint bleeding rate (AJBR).

This study may be qualified at the same time as retrospective, considering the documentation of bleeding events over the preceding 10 years, and as prospective, with regard to plasma collection.

2 | PATIENTS, MATERIALS AND METHODS

2.1 | Patients

Consecutive patients with severe haemophilia A or B receiving prophylactic or on-demand treatment, and attending regular follow-up visits in one of the participating haemophilia centres, were eligible for inclusion in this prospective, multicentre study. The inclusion criteria comprised age above 18 years, no current or previous presence

of an anti-FVIII or anti-FIX inhibitor, availability of clinical data for the 5–10 years preceding inclusion, regular follow-up (at least one visit every 12-15 months) and last treatment with standard half-life FVIII or FIX concentrate more than 72 and 96 h, respectively, before withdrawal of the blood sample required for the study (to avoid any impact of the residual factor administered on the results of TG assays). The annualised bleeding rate (ABR) and the annual joint bleeding rate (AJBR) were calculated from the number of bleeds necessitating infusion of the deficient coagulation factor. The study was approved by the ethics committee of Saint–Etienne Sud Est and registered with ClinicalTrials.gov (identifier: NCT02540187). Patients were enrolled in eight French university hospitals during a routine follow-up consultation, after having received full information on the study and having signed an informed consent form.

2.2 | Blood draws and plasma

Blood was drawn into S-Monovette© tubes (Sarstedt, France) containing 0.106 mol/L trisodium citrate and 1.45 µmol/L Corn Trypsin Inhibitor (CTI, Cryopep, Montpellier, France) for TG analyses in platelet-poor plasma (PPP) and platelet-rich plasma (PRP), as well as into citrated tubes containing 0.105 M tri-sodium citrate for fTFPI assay. Blood samples for TG analysis were centrifuged first at 150 × *g* for 10 min at room temperature (RT) to obtain PRP. TG was analysed in PRP within 2 h after blood draw in each centre. The remaining plasma was centrifuged twice at 2500 × *g* for 15 min at RT to obtain PPP, which was stored frozen at −80°C until centralised TG assay. Citrated blood samples intended for fTFPI assay and FVIII and FIX measurement were centrifuged twice at 2500 × *g* for 15 min at RT and the PPP was stored frozen at −80°C until analysis.

2.3 | Thrombin generation measurements

TG was evaluated in fresh PRP and in (frozen-defrozen) PPP according to the calibrated automated thrombinography (CAT) method.[16] Thrombin generation was triggered in PRP by adding 1 pM tissue factor (TF) (PRP reagent, Diagnostica Stago) and in PPP by adding 1 pM TF and 4 µM phospholipids (PL) (PPP Low reagent, Diagnostica Stago).

TG was measured at 37°C in a Fluoroscan Ascent Fluorometer (Thermolab Systems, Helsinki, Finland). PRP reagent or PPP reagent (20 µL) was added to 80 µL of PRP or PPP respectively. TG was then initiated by adding 20 µL of FluCa reagent containing a thrombin-specific fluorogenic substrate and $CaCl_2$ (100 mmol/L). All samples were analysed in triplicate.

TG was recorded using Thrombinoscope software, version 5.0 (Biodis; Signes, France). Endogenous thrombin potential (ETP), corresponding to the area under the thrombin generation curve, and peak values were derived from each TG curve.

TG tests in fresh PRP, necessitating analyses within 2 h after blood sampling, were performed locally according to a detailed experimental protocol common to the eight investigating centres, using the same reagent batches and controls and the same Thrombinoscope software version. A frozen PRP control (prepared by the central laboratory) was provided to every centre and run in each TG assay performed locally. The mean CVs for ETP and peak (calculated taking into account all the control CV values determined in the 8 centres) were 9.1% and 7.2%, respectively. TG analyses in PPP were performed centrally blind to the patients' biological and clinical data.

2.4 | fTFPI and plasma factor assay

fTFPI levels were determined in PPP by ELISA (Asserochrom free TFPI kit; Diagnostica Stago, Asnières-sur-Seine, France) according to the manufacturer's protocol. fTFPI levels were determined for all severe haemophilia patients, mean and median values, range (min–max) and Q1–Q3 values (first and third quartiles) being calculated for the patient population as a whole and according to the type of haemophilia.

Plasma levels of FVIII and FIX were measured by one-stage activated partial thromboplastin time (aPTT)-based clotting assays (Actin FS; Siemens, Marburg, Germany) on a BCS automated blood coagulation analyser (Siemens, Marburg, Germany).

Plasma concentrations of fTFPI, FVIII and FIX were determined centrally.

2.5 | Data collection

Data were recorded in case report forms during the numerous routine patient follow-up visits conducted over 5−10 years. Data on bleeding events included all spontaneous and trauma induced bleeds requiring factor substitution, including joint bleeding, hematoma, epistaxis, gum bleeding, haematuria and intracranial haemorrhage, during the period evaluated. Initially, we planned to use these data to calculate both the AJBR and the annualised bleeding rate (ABR). However, as only bleeds prompting antihemophilic treatment were taken into account in calculating these rates, estimation of ABR was challenging. In contrast to joint bleeding, causing acute pain, other haemorrhagic events, depending on patient perception, did not systematically lead to antihemophilic treatment. The calculation of ABR was therefore potentially based on less reliable data than that of AJBR and for this reason, bleeding tendency was evaluated solely on the basis of AJBR.

2.6 | Statistical analyses

Numerical data are presented as means ± standard deviation (SD), median, range and first to third quartiles (Q1–Q3).

The Mann–Whitney test was used to compare fTFPI levels between patients with severe haemophilia A and severe haemophilia B.

A *p*-value below .05 was considered as statistically significant.

TABLE 1 Patient demographic data.

	Severe haemophilia ($n = 53$)
Age (years)	
Mean (SD)	38.6 (14.9)
Median	35.1
Min–Max	18.3–73.2
Q1–Q3	27.1–48.8
Type of haemophilia	
Haemophilia A	34
Haemophilia B	19
Regimen	
Prophylaxis	22
On demand therapy	31

Dunn's multiple tests were used to compare basal ETP and peak (in PRP and in PPP) according to fTFPI levels in all severe haemophilia patients with a residual factor level <2%, to compare ETP and peak (in PRP and in PPP) according to AJBR in patients treated on demand and in those receiving prophylactic treatment with a residual factor level <2%, and to compare AJBR according to fTFPI levels in patients treated on demand and in those receiving prophylactic treatment. A *p*-value below .05 was considered as statistically significant. All analyses were performed using GraphPad Prism 7 (GraphPad software).

3 | RESULTS

3.1 | Patient characteristics

A total of 53 patients with severe haemophilia aged from 18.3 to 73.2 years (median 35.1 years) were included in this study, 34 presenting haemophilia A (aged from 18 to 72 years; median: 33 years) and 19 with haemophilia B (aged from 25 to 73 years; median: 36 years). In total, 22 patients were receiving regular prophylactic therapy (all with standard half-life products) and 31 patients were being treated on demand (Table 1). The median level of the deficient coagulation factor in the total population was 0.40% (range 0.2%–6.8%), seven patients on prophylaxis showing baseline levels of the deficient factor ≥2%, corresponding to the residual factor level after factor VIII or IX infusions. As these residual factor levels could influence the basal TG results, these patients were excluded from the analyses of TG parameters.

3.2 | fTFPI levels

In patients with severe haemophilia A or B as a whole ($n = 53$), mean fTFPI level was 12.5 ng/mL, median fTFPI level was 11.3 ng/mL (range: 6.9–26.5 ng/mL) and the Q1-Q3 interval was 9.4–14.3 ng/mL. fTFPI levels did not differ significantly between patients with severe haemophilia A (median: 10.5 ng/mL; range: 7.8–26.5 ng/mL; Q1-Q3 interval: 9.4–13.6 ng/mL) and those with severe haemophilia B (median: 13.3 ng/mL; range: 6.9–23.7 ng/mL; Q1-Q3 interval: 9.4–16.5 ng/mL) (Table 2).

TABLE 2 fTFPI levels in patients with severe haemophilia A, B and A or B.

	Haemophilia A and B $n = 53$	Haemophilia A $n = 34$	Haemophilia B $n = 19$
TFPI ng/mL		$p = .133$	
Mean ± SD	12.5 ± 4.1	11.9 ± 3.8	13.5 ± 4.4
95% CI of mean	[11.3–13.6]	[10.6–13.2]	[11.4–15.6]
Median (range)	11.3 (6.9–26.5)	10.5 (7.8–26.5)	13.3 (6.9–23.7)
Q1–Q3	9.4 –14.3	9.4–13.6	9.4–16.5

3.3 | Values of thrombin generation parameters in patients with severe haemophilia

Only patients with severe haemophilia showing residual levels of the deficient coagulation factor below 2% were included in the analyses of TG parameters. This population comprised 46 patients (86% of the total study population) for determination of TG parameters in PPP and 43 patients (81% of the total population included) for their determination in PRP.

Patients with haemophilia A and those with haemophilia B did not differ to a statistically significant extent with respect to TG measured in PRP (median ETP 390 nmol*min and 470 nmol*min, respectively; median peak 13.1 and 15.7 nmol, respectively), but TG determined in PPP differed to a statistically significant extent (median ETP 215 and 134 nmol*min, respectively; median peak 8.1 and 4.5 nmol, respectively) (Table 3)

3.4 | Relationship between thrombin generation parameters and fTFPI

The mean values of ETP and thrombin peak determined in PPP and PRP as a function of fTFPI level (<9.4, 9.4–14.3 and > 14.3 ng/mL) are shown in Figure 1, the middle range of fTFPI values (9.4–14.3 ng/mL) corresponding to the Q1–Q3 interval. Mean values of both ETP and peak determined in PPP were statistically significantly doubled in patients with a fTFPI level below 9.4 ng/mL ($n = 10$) compared to the mean values determined in patients with a fTFPI level above 14.3 ng/mL ($n = 11$) (287.4 ± 111.1 vs. 142.6 ± 69.1 nmol; $p = .002$ and 12.4 ± 5.6 vs. 5.1 ± 2.6 nmol; $p = .001$, respectively). The mean values of both ETP and peak determined in PRP were also doubled in patients with a fTFPI level below 9.4 ng/mL ($n = 9$) compared to those determined in patients with a fTFPI level above 14.3 ng/mL ($n = 10$) (ETP: 587.6 ± 306.2 vs. 282.2 ± 198.4 nmol; $p = .069$; peak: 22.3 ± 13.8 vs. 10.2 ± 8.4 nmol; $p = .061$). These differences nevertheless failed to reach statistical significance, possibly owing to the large standard deviations of the mean ETP and peak values.

TABLE 3 TG values (ETP and peak) in PRP and in PPP according to the type of haemophilia (A and B).

	Haemophilia A	Haemophilia B	Haemophilia A	Haemophilia B
	n = 30	*n* = 13	*n* = 32	*n* = 14
ETP, nmol*min	**PRP**		**PPP**	
	p = .705		*p* = .0016	
Mean ± SD	462 ± 306	488 ± 305	244 ± 95	157 ± 111
95% CI of mean	[348–577]	[304–672]	[209.5–277.8]	[92.6–220.4]
Median (range)	390 (63–1101)	470 (55–1124)	215 (122–560)	134 (0–473)
Q1–Q3	199–711	288–510	184–303	92–205
Peak, nmol	**PRP**		**PPP**	
	p = .391		*p* = .0003	
Mean ± SD	15.9 ± 12.6	18.9 ± 13.0	9.6 ± 4.0	5.9 ± 5.5
95% CI of mean	[11.2–20.6]	[11.0–26.7]	[8.2–11.0]	[2.8–9.1]
Median (range)	13.1 (1.1–57.1)	15.7 (3.0–44.9)	8.1 (3.5–21.7)	4.5 (1.3–23.5)
Q1–Q3	6.1–21.7	11.1–19.1	7.1–12.6	2.8–6.6

Abbreviations: TG, thrombin generation; ETP, endogenous thrombin potential.

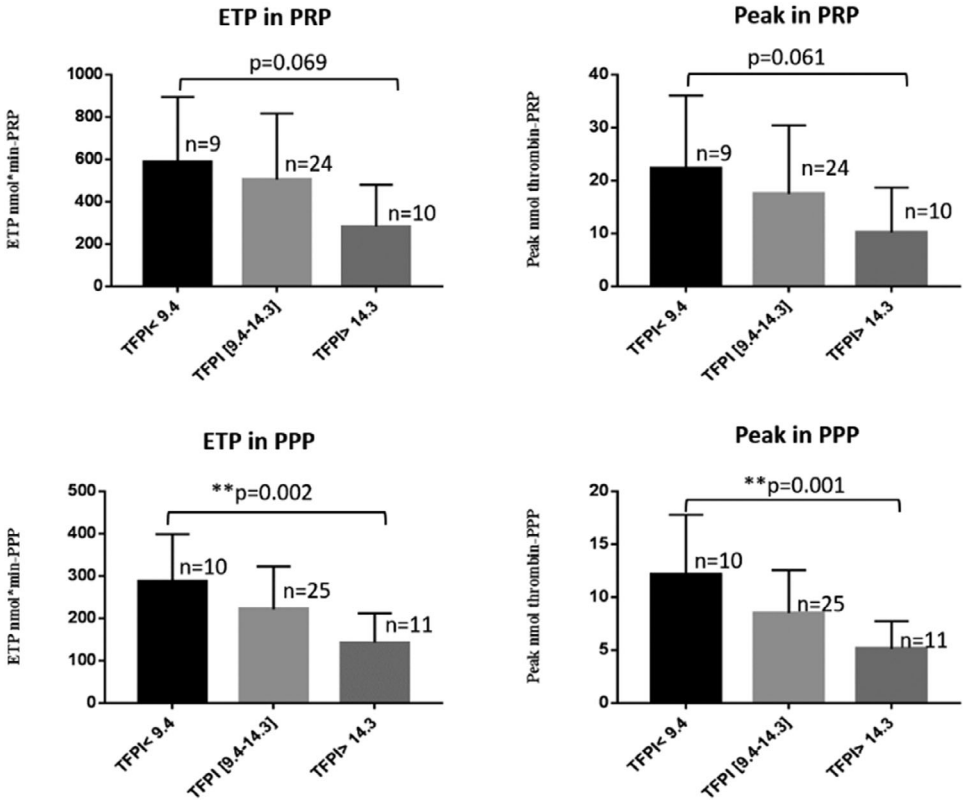

FIGURE 1 ETP and peak in PPP or PRP according to fTFPI level classes (<9.4 ; [9.4–14.3] ; >14.3 ng/mL) in severe haemophilia patients. Mean values of both ETP and peak determined in PPP were statistically significantly doubled in patients with a fTFPI level below 9.4 ng/mL (*n* = 10) compared to the mean values determined in patients with a fTFPI level above 14.3 ng/mL (*n* = 11) (287.4 ± 111.1 vs. 142.6 ± 69.1 nmol; *p* = .002 and 12.4 ± 5.6 vs. 5.1 ± 2.6 nmol; *p* = .001, respectively). Even though, the mean values of both ETP and peak determined in PRP were doubled in patients with a fTFPI level below 9.4 ng/mL (*n* = 9) compared to the mean values determined for those with a fTFPI level above 14.3 ng/mL (*n* = 10) (ETP: 587.6 ± 306.2 vs. 282.2 ± 198.4 nmol; *p* = .069; peak: 22.3 ± 13.8 vs. 10.2 ± 8.4 nmol; *p* = .061). ETP, endogenous thrombin potential; peak, peak thrombin generation; PPP, platelet-poor plasma; PRP, platelet-rich plasma; TFPI, tissue factor pathway inhibitor.

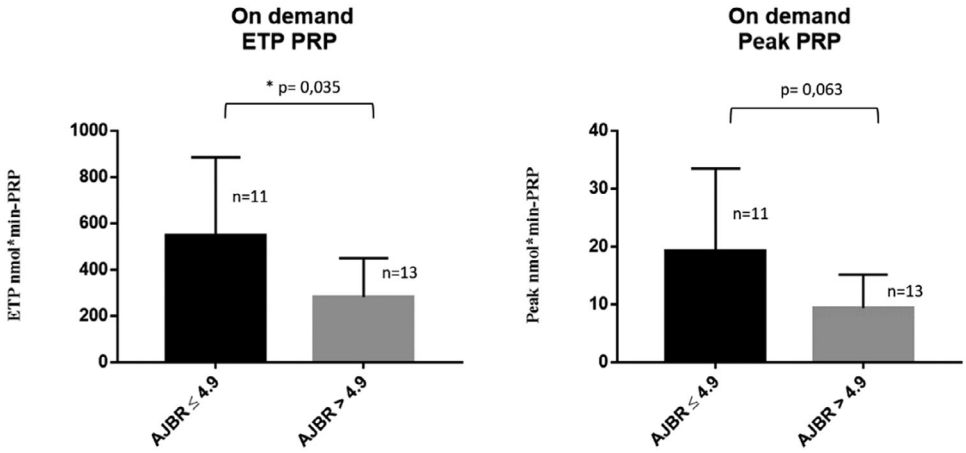

FIGURE 2 ETP and peak in PRP according to AJBR category (≤4.9 and >4.9) in severe haemophilia patients receiving treatment on demand. Mean values of ETP determined in PRP ($n = 24$) differed significantly ($p = .035$) as a function of AJBR (≤4.9; $n = 11$ vs. >4.9; $n = 13$). Mean values of ETP were 547; SD 339 for AJBR ≤4.9 and 282; SD 168 for AJBR > 4.9. The difference in mean values of peak in PRP between patients with AJBR ≤4.9 (mean value 19.2; SD 14.3) and those with AJBR > 4.9 (mean value 9.3; SD 5.8) did not reach statistical significance($p = .063$). AJBR, Annualised joint bleeding rate; ETP, endogenous thrombin potential; peak, peak thrombin generation; PPP, platelet-poor plasma; PRP, platelet-rich plasma.

3.5 | Relationship between TG parameters and AJBR

As expected, median values of AJBR were higher ($p = .001$) in patients receiving treatment on demand (median: 4.9; range: 0–15; 95% CI of median [2–8]) than in patients on prophylaxis (median: 0.95; range: 0–7.2; 95% CI of median [0.4–2.1]). Moreover, AJBR in haemophilia A patients (median: 2.6; range: 0.2–15; 95% CI of median: [1.3–5]) did not differ ($p = .435$) from that in haemophilia B patients (median: 2.45; range: 0–13; 95% CI of median: [0.1–8]).

In patients receiving treatment on demand, mean values of ETP determined in PRP ($n = 24$) differed significantly as a function of AJBR (≤4.9; $n = 11$ vs. > 4.9; $n = 13$), higher values of ETP corresponding to lower values of AJBR ($p = .035$). A similar trend was observed with respect to peak ($p = .063$; Figure 2). In contrast, analyses performed in PPP ($n = 25$) showed no statistically significant differences in mean values of either ETP or peak according to AJBR (Table 4).

In patients receiving prophylaxis, neither basal ETP nor peak values differed significantly according to AJBR (≤0.95 vs. >0.95) irrespective of whether the analyses were performed in PRP ($n = 18$) or PPP ($n = 18$; Table 4).

3.6 | Relationship between AJBR and fTFPI

AJBR as a function of fTFPI level (<9.4 ng/mL, 9.4–14.3 ng/mL and >14.3 ng/mL) is shown in Figure 3. In patients receiving treatment on demand ($n = 28$), mean AJBR was nearly two-fold high in patients with fTFPI levels >14.3 ng/mL (mean AJBR = 6.64) than in patients with fTFPI levels <9.4 ng/mL (mean AJBR = 3.48; Table 5).

TABLE 4 Comparison of ETP and peak (in PRP and in PPP) according to AJBR category for patients with severe haemophilia A or B with a residual factor level <2% receiving treatment on demand and for those receiving prophylactic treatment.

	TREATMENT on demand		Prophylaxis	
	AJBR ≤ 4.9	AJBR > 4.9	AJBR ≤ 0.95	AJBR > 0.95
ETP in PRP, nmol, mean (SD)	547 (339)*	282 (168)*	525 (303)	625 (307)
	$n = 24$	$n = 24$	$n = 18$	$n = 18$
PEAK in PRP, nmol, mean (SD)	19.2 (14.3)	9.3 (5.8)	22.3 (16.1)	20.3 (10.5)
	$n = 24$	$n = 24$	$n = 18$	$n = 18$
ETP in PPP, nmol, mean (SD)	181 (128)	167 (45)	277 (130)	278 (86)
	$n = 25$	$n = 25$	$n = 18$	$n = 18$
PEAK in PPP, nmol, mean (SD)	7.2 (6.0)	6.2 (2.3)	11.4 (5.4)	10.7 (3.5)
	$n = 25$	$n = 25$	$n = 18$	$n = 18$

Abbreviations: AJBR, annualised joint bleeding rate; ETP, endogenous thrombin potential; peak, peak thrombin generation; PPP, platelet-poor plasma; PRP, platelet-rich plasma.

*$p = .035$ (Dunn's multiple test).

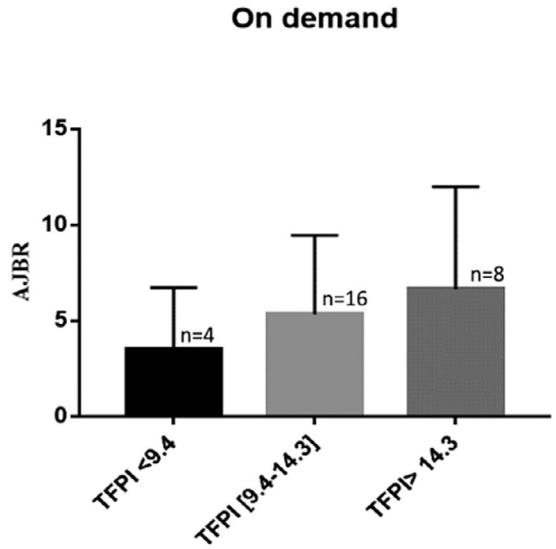

FIGURE 3 AJBR according to TFPI level classes (<9.4; [9.4–14.3]; >14.3 ng/mL) in severe haemophilia patients receiving treatment on demand. Mean AJBR was nearly two-fold higher in patients with fTFPI levels >14.3 ng/mL (mean AJBR = 6.64; SD: 5.36) than in patients with fTFPI levels <9.4 ng/mL (mean AJBR = 3.48; SD: 3.24). This difference did not reach the level of statistical significance ($p = .998$). AJBR, annual joint bleeding rate; TFPI, tissue factor pathway inhibitor.

However, this difference did not reach the level of statistical significance ($p = .998$). AJBR did not differ significantly according to the level of fTFPI in patients on prophylaxis ($n = 22$, Table 5).

4 | DISCUSSION

This study, focused exclusively on patients with severe haemophilia, did not confirm the results of a previous retrospective study performed in patients with haemophilia A or B patients irrespective of severity that showed a difference in fTFPI levels between patients with haemophilia A and those with haemophilia B.[15] We have no valid explanation for these discordant results. In addition, considering the already demonstrated link between fTFPI levels and TG in haemophilia patients,[14] the absence of any difference in fTFPI levels between patients with severe haemophilia A and B, respectively, was consistent with the absence of statistically significant differences in ETP and peak measured in PRP between these two patient populations in our study. These results are in accordance with those of other studies[17,18] which did not find any difference in bleeding tendency between patients with severe haemophilia A and those with severe haemophilia B. Although TG measured in PPP differed to a statistically significant extent between severe haemophilia A and B patients, we cannot be sure that this difference is clinically relevant, given the absence of any difference in AJBR between these two patient populations.

As already demonstrated in a previous study,[14] we confirmed the effect of fTFPI level on both ETP and peak in patients with severe haemophilia: the higher the fTFPI level, the lower the values of ETP and peak and conversely the lower the fTFPI level, the higher the values of ETP and peak. The influence of fTFPI level on ETP and peak values was particularly evident in the case of fTFPI values outside the Q1–Q3 interval.

The differences in ETP and peak values between the lowest (<9.4 ng/mL) and highest (>14.3 ng/mL) values of fTFPI were highly significant when TG parameters were measured in PPP with a trend toward a similar result when these parameters were evaluated in PRP. Keeping in mind that platelets represent a major source of TFPI,[19] the absence of a statistically significant difference in ETP and peak values in PRP according to fTFPI level measured in PPP may have reflected the fact that TFPI present within the platelets was not taken into account. Previous in vitro studies indirectly demonstrated the impact of fTFPI on TG in severe haemophilia patients by determining the correction of TG after fTFPI neutralisation, the level of TG correction depending on the anti-TFPI antibody concentration. This concentration had to be higher in PRP (owing to the presence of platelet TFPI) than in PPP in order to obtain TG normalisation. Moreover, the anti-TFPI antibody concentration needed to obtain complete TG normalisation varied between patients, according to their basal fTFPI levels.[20] The results of in vitro studies are in accordance with those of the clinical Explorer 8 study evaluating an anti-TFPI product (concizumab, Novonordisk). In this study, there was a large variation in treated bleeding events both among the 80 haemophilia A patients and among the 64 haemophilia B patients (0–4.5 and 0–6.4, respectively), the concizumab dose being adapted according to the plasma concentration of this product.[21]

TABLE 5 Comparison of AJBR according to TFPI level categories (<9.4; [9.4–14.3]; > 14.3 ng/mL) for severe haemophilia patients receiving treatment on demand and for those receiving prophylactic treatment.

	AJBR		
	TFPI < 9.4 ng/mL	TFPI [9.4–14.3] ng/mL	TFPI > 14.3 ng/mL
	Mean (SD)	Mean (SD)	Mean (SD)
Treatment on demand	3.48 (3.24)	5.33 (4.11)	6.64 (5.36)
	$n = 4$	$n = 16$	$n = 8$
Prophylactic treatment	1.2 (.88)	2.17 (2.03)	0.1 (0.1)
	$n = 6$	$n = 13$	$n = 3$

Abbreviations: AJBR, annualised joint bleeding rate; TFPI, tissue factor inhibitor.

The parameter used to evaluate bleeding tendency, namely AJBR, is imprecise as the evaluation of joint bleeding is based on the patient's perception, which is recognised to potentially result in both false-negative and false positive reporting of this event.[22] The retrospective nature of our study is a further source of imprecision, as the patients' reporting of joint bleeding episodes at their clinical follow-up visits was not necessarily exhaustive. Taking all these factors into account, we chose to evaluate the relationship between TG parameters and bleeding phenotype (expressed as AJBR) according to high and low AJBR categories (comprising values above and below the median AJBR respectively), rather than according to individual AJBR values).

Our results indicated that in the "on demand treatment" group, patients who bled more (with an AJBR above the median value of 4.9) showed lower ETP and peak values (as measured in PRP) than patients who bled less (AJBR $\leq$ 4.9). Other studies reported the same relationship between TG potential and bleeding phenotype in patients with severe haemophilia A or B.[9,23] In contrast, this relationship was not evident in analyses performed in PPP, suggesting that PRP approaches in vivo conditions more closely than PPP. In patients receiving prophylaxis, TG parameters did not significantly differ according to AJBR. In these patients, it is logical that AJBR depends more on the intensity of prophylaxis than on baseline TG values, the effect of these baseline TG values on AJBR being completely hidden by the greater impact of the treatment.

Analysis of bleeding rates according to fTFPI levels suggested that in patients receiving treatment on demand, the higher the fTFPI level, the higher the AJBR, even though the difference in AJBR according to the level of fTFPI was not statistically significant. This absence of significance could be related to the small number of patients in our study. It may also have reflected the difficulties encountered in determining bleeding rates, as noted above and emphasised in a recent review.[22] The impact of TFPI on bleeding tendency in haemophilia patients is reflected by the clinical efficacy of anti-TFPI therapies in controlling bleeding in haemophilia animal models[24,25] and in haemophilic patients.[20,26–28] As the results of TG assay in haemophilia patients are affected by fTFPI levels, this assay could be used to monitor anti-TFPI therapy, especially if performed in PRP, in which case both plasma and platelet TFPI are taken into account.

The main limitations of our study were related to the retrospective documentation of bleeding episodes (even though plasma was collected prospectively at the time of patient inclusion). The fact that TG assays in PRP were not performed centrally cannot be regarded as a limitation considering the low interlaboratory variability of TG assays in our study. As the patient population included in our study was relatively small, our results warrant confirmation in a larger number of patients.

5 | CONCLUSION

This study indicated that in severe haemophilic patients receiving treatment on demand, AJBR (expressing bleeding tendency) is related to basal TG, which is itself related to fTFPI level. Increasing levels of fTFPI were indeed associated with increasing values of AJBR although this relationship was not statistically significant, possibly owing to the small patient population. In severe haemophilia patients receiving prophylactic treatment, AJBR is related neither to basal TG nor to the level of fTFPI. In these patients, AJBR is probably more closely related to the intensity of prophylactic treatment.

AUTHOR CONTRIBUTIONS

Brigitte Tardy contributed to conceptualisation, funding acquisition and project administration. Brigitte Tardy, Aurélie Montmartin, Fanny Collange and Bernard Tardy contributed to methodology. Aurélie Montmartin performed all the assays. Brigitte Tardy, Hervé Chambost, Anne Lienhart, Birgit Frotscher, Pierre Morange, Céline Falaise, Yesim Dargaud, Marie Toussaint-Hacquard, Laurent Ardillon, Benedicte Wibaut, Emanuelle Jeanpierre, Philippe Nguyen, Fabienne Volot took part to the inclusions. Aurélie Montmartin performed to statistical analysis. Brigitte Tardy and Aurélie Montmartin contributed to writing the manuscript. All authors had access to relevant data of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors state that they have no interests which might be perceived as posing a conflict or bias.

DATA AVAILABLE STATEMENT

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

ETHICS STATEMENT

The study was approved by the ethics committee of Saint-Etienne Sud Est and registered with ClinicalTrials.gov (identifier: NCT02540187).

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