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Bleeding phenotype of patients with moderate haemophilia A and B assessed by thromboelastometry and thrombin generation

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Abstract

Introduction: Predicting the bleeding phenotype is crucial for the management of patients with moderate haemophilia. Global coagulation assays evaluate haemostasis more comprehensively than conventional methods.

Aim: To explore global coagulation assays and the bleeding phenotype of patients with moderate haemophilia A (MHA) and B (MHB).

Methods: The MoHem study is a cross-sectional, multicentre study covering Nordic patients with MHA and MHB. Thromboelastometry in whole blood and thrombin generation (TG) in platelet-poor plasma (1, 2.5 and 5 pM tissue factor (TF)) were compared with joint health (Haemophilia Joint Health Score (HJHS)) and treatment modality.

Results: We report on 61 patients from Oslo and Helsinki: 24 MHA and 37 MHB. By TG (2.5 pM TF), patients who had been without replacement therapy during the previous 12 months depicted higher endogenous thrombin potential ($P = .03$). In contrast, those who had low ETP (< median) captured higher HJHS ($P = .02$). Patients who had undergone orthopaedic surgery generated least thrombin ($P = .02$). By thromboelastometry, those without the need of factor consumption had short clotting times, and quick times to maximum velocity (< median values) ($P = .03$). Factor VIII/factor IX activity (FVIII/FIX:C) did not align with the bleeding phenotype, but FIX:C ≤ 3 IU/dL was associated with lower peak thrombin ($P = .03$).

Conclusion: TG differentiated patients with moderate haemophilia according to HJHS, annual factor consumption, and whether orthopaedic surgery had been performed. Thromboelastometry differentiated according to factor consumption only. Global coagulation assays may assist predicting the bleeding phenotype in moderate haemophilia.

KEYWORDS

bleeding phenotype, joint score, moderate haemophilia A, moderate haemophilia B, thrombin generation, thromboelastometry

1 | INTRODUCTION

In haemophilia, patients who have the same degree of factor deficiency may express clinical heterogeneity.[1] A remaining challenge is therefore to predict the individual bleeding phenotype to optimize treatment and follow-up. This is particularly important among patients with moderate haemophilia, as treatment guidelines are lacking for this group. Identifying patients who should receive prophylaxis early in life may translate into clinical benefits.

Thrombin is the lead enzyme in haemostasis, which stimulates and directs the generation of a haemostatic plug. In vitro, the formation of a visible fibrin clot occurs during the initiation phase of coagulation at minor levels of thrombin. Thus, majority of thrombin (96%) is generated after clot formation,[2] which is not captured by the fibrin-clotting endpoints commonly used to evaluate haemostasis. Global coagulation assays, like thrombin generation (TG) and viscoelastic tests, evaluate the haemostatic process comprehensively. In TG, plasma samples are used to determine the rate and extent of thrombin generated after a tissue factor stimulus. Hemker et al. developed the calibrated automated TG method in 2003.[3] Viscoelastic tests, respectively, measure changes in the elastic properties of whole blood during clot formation and fibrinolysis involving all cellular elements. In 2003, Sørensen et al. introduced extrinsic activation at very low concentration of tissue factor (TF).[4] Both thromboelastometry[5,6] and TG[7–10] may reflect the clinical phenotype of patients with haemophilia, but data in moderate haemophilia are scarce. The Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH) have standardized both tests for use in haemophilia.[11,12] The aim of this paper is to explore and compare thromboelastometry and TG to unravel the bleeding phenotype of patients with moderate haemophilia A (MHA) and B (MHB).

2 | MATERIALS AND METHODS

The MoHem study is a cross-sectional, multicentre study covering Nordic patients with MHA and MHB. The study recruitment has been described previously.[13] Evaluation by global coagulation assays was optional, and only patients from Oslo and Helsinki above 5 years of age took part. The MHA and MHB diagnoses were based on lowest historical values of factor VIII/factor IX activity (FVIII/FIX:C) analysed locally by one-stage assay. However, at enrolment we collected washout samples taken minimum 72 h after the last administration of clotting factor concentrates representing baseline FVIII/FIX:C. One-stage and chromogenic FVIII/FIX:C measurements were performed at Skåne University Hospital, Malmö, Sweden. They also analysed FVIII and FIX inhibitory antibodies using the Nijmegen modification of the Bethesda assay. The inhibitory antibodies were considered relevant, if they exceeded 0.6 Bethesda units per mL.[14] Thromboelastometry and TG were all performed at the Research Institute of Internal Medicine, Oslo University Hospital, Norway. Accordingly, only patients from Oslo were evaluated by thromboelastometry. Samples from healthy persons collected as part of a previous study served as controls.[15]

2.1 | Blood sampling and processing

Blood was collected by antecubital venepuncture using 21-gauge needles and minimal stasis. For FVIII/FIX:C measurement, we used Vacutainer® tubes (Becton Dickinson, Plymouth, UK) containing 0.105 M trisodium citrate. Plasma was obtained by centrifugation at 2000 g for 20 min (room temperature). For thromboelastometry and TG, we used S-Monovette® tubes (Sarstedt, Nümbrecht, Germany) containing 0.106 M trisodium citrate, which were manually prefilled with corn trypsin inhibitor (CTI) (Haematologic Technologies, Essex Junction, VT, USA) with final concentration 20 μg/mL. In Helsinki, citrated SCAT tubes containing 50 μg/mL CTI were used for TG. Platelet-poor plasma (PPP) was obtained by double centrifugation (room temperature), first at 2000 g for 10 min, and then at 10,000 g for 10 min. All plasma samples were prepared within 60 min of venepuncture. The supernatant was carefully collected, immediately frozen and stored at -80°C until the samples were transported to Malmö and Oslo on dry ice.

2.2 | Factor VIII and factor IX activities

One-stage FVIII:C was performed with reagent Actin FS and FVIII-deficient plasma (Siemens Healthcare, Marburg, Germany) and chromogenic FVIII:C with reagent Coatest SP (Chromogenix, Mölndal, Sweden). Respectively, one-stage FIX:C was performed with reagent Actin FS (Siemens) and FIX-deficient plasma (Georg King Bio-Medical) and chromogenic FIX:C with Rox FIX reagent (Rossix, Mölndal, Sweden). The automated analyses were performed on Atellica Coag 360 analyser (Siemens).

2.3 | Thromboelastometry

Thromboelastometry according to Sørensen et al.[4] was performed by ROTEM® (Tem Innovations GmbH, Munich, Germany). Whole blood was incubated in water bath at 37°C for minimum 30 min. Prewarmed plastic test cups were prepared with 20 μL buffer 1 (20 mM Hepes, 150 mM NaCl, pH 7.4) and 20 μL buffer 2 (20 mM Hepes, 150 mM NaCl, 200 mM $CaCl_2$, pH 7.4) before adding recombinated relipidated TF, Innovin® (Dade Behring, Liederbach, Germany) diluted in 20 μL buffer 1. Haemostasis was initiated by adding 280 μL whole blood. The final TF dilution was 1:70,000 corresponding to a theoretical concentration of 0.35 pM. The measurements were run in duplicates.

2.4 | Thrombin generation

TG in PPP was performed according to Hemker et al.[3] by calibrated automated TG (Stago, Asnières, France) with Thrombinoscope® software (Thrombinoscope BV, Maastricht, the Netherlands). We used PPP reagents containing 1, 2.5 and 5 pM TF with 4 μM phospholipids to trigger haemostasis. First, 20 μL reagents and 20 μL thrombin calibrator were dispensed in triplicates into the wells of a round-bottom 96

TABLE 1 Patient characteristics and major outcomes of the 61 patients with moderate haemophilia A (MHA) and B (MHB) included in this study

	MHA (n = 24)		MHB (n = 37)	
	Prophylaxis n = 12 (50%)	On-demand n = 12 (50%)	Prophylaxis n = 11 (30%)	On-demand n = 26 (70%)
Age (years)	15 (9-26)	53 (33-63)	19 (8-50)	48 (16-62)
Historical one-stage FVIII/FIX:C (IU/dL)	1 (1-3)	2 (1-3)	2 (1-2)	2 (2-2)
Current one-stage FVIII/FIX:C (IU/dL)	0.7 (0.2-2.0)	2.5 (1.0-4.8)	2.6 (2.2-3.3)	2.2 (1.5-3.1)
Current chromogenic FVIII/FIX:C (IU/dL)	0.7 (0.0-1.5)	1.6 (0.8-2.7)	2.1 (0.6-3.4)	1.8 (0.4-2.7)
History of haemarthrosis	11 (92%)	12 (100%)	11 (100%)	18 (69%)
Age at first joint bleed (years)	2 (1-5)	5 (2-5)	5 (4-7)	7 (4-13)[a]
Age at start of prophylaxis (years)	6 (2-15)	–	9 (3-24)	–
Annual factor consumption (IU/kg/y)	3935 (2332-5046)	190 (0-585)[b]	2109 (1636-2939)	36 (0-89)
Joint bleeds during the last 12 months	1 (0-4)	3 (0-9)	0 (0-1)	0 (0-0)
ISTH-BAT	19 (11-20)	25 (20-29)	14 (11-19)	13 (9-19)
HJHS total (0-120 points)	2 (1-10)	13 (5-27)	5 (2-12)[c]	3 (1-8)
Arthrodesis or arthroplasties	2 (17%)	5 (42%)	1 (9%)	2 (8%)

Numbers (%) or medians (interquartile ranges). Abbreviations: FVIII/FIX:C, factor VIII/factor IX activity; HJHS, Haemophilia Joint Health Score; ISTH-BAT, International Society on Thrombosis and Haemostasis bleeding assessment tool. The number of the patients (n) is noted if it deviates from the total number:
[a]n = 16/18.
[b]n = 11/12.
[c]n = 10/11.

well microtiter plate. Then 80 μL PPP was added to each well. The plate was preheated at 37°C for 10 min in the fluorometer before the starting reagent containing a fluorogenic substrate and $CaCl_2$ was automatically dispensed (20 μL per well). All reagents were obtained from Thrombinoscope BV.

2.5 | Bleeding phenotype

The bleeding phenotype was characterised by joint health according to the Haemophilia Joint Health Score (HJHS) 2.1 (0-120 points)[16] and treatment modality. We defined a mild bleeding phenotype as low HJHS (0-3 points) or no use of replacement therapy during the previous 12 months. However, those who had undergone arthrodesis or arthroplasties were considered to have a severe bleeding phenotype. In addition, the ISTH/SSC bleeding assessment tool (ISTH-BAT) included lifelong bleeding history.[17]

2.6 | Statistical analyses

Continuous data were summarized as medians and interquartile ranges (IQR). Categorical data were presented as numbers and percentages. Spearman's correlation (*r*) was used to compare one-stage and chromogenic FVIII/FIX:C. The medians of thromboelastometry and TG were used as thresholds to define groups with low and high values. Mann-Whitney *U* test was used to compare continuous variables with skewed distribution, and Student's *t* test was used for normally distributed data. Chi-square test was used to compare categorical data. A *P*-value < .05 was considered statistically significant. The analyses were performed using SPSS Statistics 27.

3 | RESULTS

The main clinical results from the MoHem study have been reported previously.[13] Thromboelastometry was performed on 52 patients from Oslo, and TG was performed on 66 patients from Oslo and Helsinki. A female carrier was excluded due to outlier values and 4 patients (MHA) were removed due to one-stage and chromogenic FVIII:C > 5 IU/dL at the current samples. Thus, the results comprise 61 patients (Table 1). FVIII or FIX inhibitory antibodies were not detected. All participants had platelet counts and von Willebrand factor antigen and activity within reference values. No one had mutations in Factor V Leiden or prothrombin genes. The real washout time for patients on prophylaxis was 96 h (IQR 81–100) for MHA and 120 h (120-198) for MHB. Two patients with MHB were treated by extended half-life products, and their washout time was 228 h. The correlation between one-stage and chromogenic FVIII/FIX:C was strong overall (r = .86, P < .001); for MHA (r = .91) and MHB (r = .84) (Figure 1). FVIII/FIX:C was generally higher by one-stage than chromogenic assay, capturing one-stage/chromogenic ratio 1.4 (1.0-2.4). Seventeen patients (28%) demonstrated classic assay discrepancy (one-stage/chromogenic ratio > 2.0) (Table 2). FVIII/FIX:C (both assays) did not align with the bleeding phenotype. However, of the total MoHem population (107/145 patients), chromogenic FVIII/FIX:C ≤ 3 IU/dL was associated

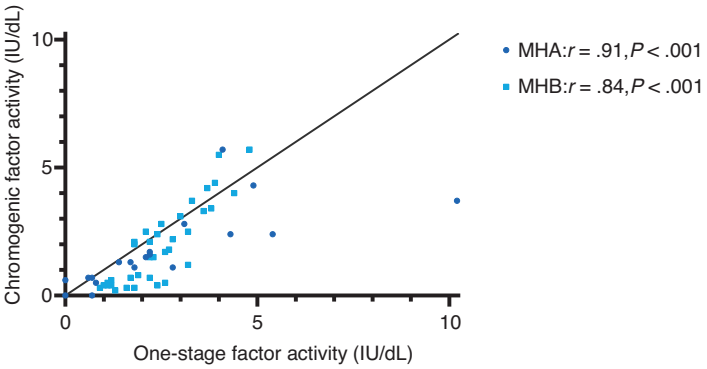

FIGURE 1 Spearman's correlation (*r*) between one-stage and chromogenic baseline factor activity (IU/dL) for patients with moderate haemophilia A (MHA) (n = 24) and B (MHB) (n = 37)

TABLE 2 Genotype and bleeding phenotype among patients presenting one-stage/chromogenic factor VIII/factor IX:C (FVIII/FIX:C) assay discrepancy (n = 17/61)

Patient (age)	Diagnosis	*F8/F9* mutation	One-stage FVIII/FIX:C (IU/dL)	Chromogenic FVIII/FIX:C (IU/dL)	Treatment	Bleeding phenotype
1 (60 y)	MHA	c.5374G>T, p.Val1792Leu	2.8	1.1	Od	-/HJHS>3
2 (32 y)	MHA	c.871G>A, p.Glu291Lys	5.4	2.4	Od	Zero/HJHS>3
3 (54 y)	MHA	c.1648C>T, p.Arg550Cys	10.2	3.7	Od	Non-zero/ HJHS>3/surgery
4 (61 y)	MHB	c.1217G>A, p.Ser406Asn	1.2	0.4	Od	Non-zero/HJHS 0–3
5 (62 y)	MHB	c.1193G>C, p.Gly398Ala	1.7	0.7	Od	Non-zero/HJHS>3
6 (68 y)	MHB	c.1046G>T, p.Gly349Val	1.1	0.4	Od	Non-zero/HJHS>3
7 (16 y)	MHB	c.1193G>C, p.Gly398Ala	1.1	0.5	Od	Non-zero/HJHS 0–3
8 (6 y)	MHB	c.1193G>C, p.Gly398Ala	1.9	0.8	Od	Non-zero/HJHS 0–3
9 (9 y)	MHB	c.1193G>C, p.Gly398Ala	1.6	0.3	Od	Non-zero/HJHS 0–3
10 (5 y)	MHB	c.1193G>C, p.Gly398Ala	1.3	0.2	Od	Non-zero/HJHS 0–3
11 (10 y)	MHB	c.1046G>T, p.Gly349Val	0.9	0.3	Od	Zero/HJHS>3
12 (8 y)	MHB	c.1046G>T, p.Gly349Val	1.0	0.4	Od	Non-zero/HJHS 0–3
13 (13 y)	MHB	c.1136G>A, p.Arg379Gln	2.6	0.5	Prophylaxis	Non-zero/HJHS 0–3
14 (16 y)	MHB	c.1136G>A, p.Arg379Gln	2.4	0.4	Prophylaxis	Non-zero/HJHS>3
15 (28 y)	MHB	c.1193G>C, p.Gln398Ala	1.8	0.3	Od	Non-zero/HJHS 0–3
16 (8 y)	MHB	c.421T>G, p.Cys95Gly	2.2	0.7	Prophylaxis	Non-zero/HJHS 0–3
17 (5 y)	MHB	c.421T>G, p.Cys95Gly	3.2	1.2	Prophylaxis	Non-zero/HJHS 0–3

Abbreviations: *F8/F9* mutation, factor VIII/factor IX gene mutation; MHA, moderate haemophilia A; MHB, moderate haemophilia B; Od, on-demand; zero/non-zero, factor consumption during the previous 12 months; HJHS, Haemophilia Joint Health Score (0-3 vs > 3 points); surgery, arthrodesis or arthroplasties.

with higher HJHS (6 points (1-12) versus 2 points (0-6)) ($P = .05$). In MHA, the difference in HJHS was larger (9 points (2-22) versus 2 points (0-7)) ($P = .01$).

depicted lower peak thrombin than those with levels > 3 IU/dL (5 nM (4-7) versus 7 nM (6-8)) ($P = .03$). Otherwise, TG and thromboelastometry did not align with FVIII/FIX:C.

3.1 | Global coagulation assays and factor activity levels

Thromboelastometry and TG discriminated patients with moderate haemophilia from healthy controls (Table 3 and 4). By TG in response to 1 pM TF, patients with MHB having chromogenic FIX:C ≤ 3 IU/dL

3.2 | Global coagulation assays and bleeding phenotype

TG was able to discriminate patients with moderate haemophilia according to HJHS, annual factor consumption, and whether they had undergone arthrodesis or arthroplasties. The results were best

TABLE 3 Thromboelastometry in patients with moderate haemophilia (n = 49) and healthy controls (n = 40)

	Moderate haemophilia (n = 49)				
	MHA (n = 19)	MHB (n = 30)	*P*-value	Healthy controls (n = 40)	*P*-value
Clotting time (s)	961 (828-1165)	1186 (1069-1583)	.02	730 (550-813)	< .001
Clot formation time (s)	313 (258-343)	297 (270-363)	.92	191 (157-220)	< .001
Maximal clot firmness (mm)	55 (49-61)	53 (49-59)	.64	56 (52-60)	.24
Time to max V (s)	1276 (1071-1524)	1434 (1347-1800)	.11	877 (715-987)	< .001
Max V (mm/s)	6 (5-7)	6 (5-7)	.86	8 (7-9)	< .001
Area under curve (mm x s)	5544 (4950-6302)	5360 (4944-5913)	.52	5657 (5280-5989)	.32

Medians and interquartile ranges (IQR). Abbreviations: MHA, moderate haemophilia A; MHB, moderate haemophilia B; Max V, maximum velocity.

TABLE 4 Thrombin generation in patients with moderate haemophilia (n = 61) and healthy controls (n = 40) according to tissue factor concentration of 1, 2.5, and 5 pM

	Moderate haemophilia (n = 61)			Healthy controls (n = 40)			
	1 pM[a]	2.5 pM[b]	5 pM[c]	1 pM	2.5 pM	5 pM	*P*-value
Lag time (min)	11* (8-19)	5 (4-6)	3 (3-4)	7 (6-8)	5 (4-6)	3 (3-4)	< .001
ETP (nM x min)	160* (116-192)	314* (231-398)	721* (518-826)	1199 (837-1372)	1201 (1072-1394)	1294 (1176-1481)	< .001
Peak (nM)	6* (5-8)	17* (11-24)	56* (34-74)	163 (68-224)	166 (103-222)	226 (193-247)	< .001
Time to peak (min)	27* (22-37)	14* (11-19)	9* (8-11)	11 (10-13)	10 (9-10)	7 (6-8)	< .001
Velocity index (nM/min)	0.4* (0.2-0.5)	2* (1-3)	10* (4-15)	39 (12-70)	38 (19-65)	67 (52-83)	< .001

Medians and interquartile ranges (IQR). Abbreviations: ETP, Endogenous thrombin potential. The number of patients (n) is noted if it deviates from the total number:
[a]n = 50/61 (ETP only).
[b]n = 60/61 (ETP only).
[c]n = 60/61 (all parameters).
*$P < .001$ vs healthy controls.

demonstrated at 2.5 pM TF exposure, and endogenous thrombin potential (ETP) and peak thrombin were the best predictive variables. Patients who captured HJHS 0–3 points showed a trend towards higher ETP and peak than those with > 3 points (Table 5). Correspondingly, patients with ETP below median value captured higher HJHS than those with ETP in the upper half (7 points (2-19) versus 3 points (1-7)) ($P = .02$). Long time to peak ($P < .01$) and low velocity index ($P = .03$) also aligned with higher HJHS. According to the 75 percentile of ETP, the difference in HJHS was more pronounced (6 points (2-16) versus 2 points (0-4)) ($P < .01$).

Moreover, at 2.5 pM TF, TG discriminated the patients according to factor consumption during the previous 12 months (Figure 2a, Table 5). In line with this, those who had been without replacement therapy more commonly had ETP in the upper quartile (40% (6/15) versus 11% (5/44)) ($P = .01$). TG using 1 pM TF also to some extent dissociated according to annual factor consumption (Table S1).

Furthermore, TG using 2.5 and 5 pM TF distinguished patients who had undergone arthrodesis or arthroplasties from those without surgery (Figure 2b, Table 5 and S2). At 2.5 pM TF, surgeries were more commonly performed among those with ETP below median (27% (8/30) versus 3% (1/30)) ($P = .01$). Similarly, all but one who had undergone orthopaedic surgery had low and delayed peak thrombin, and low velocity index ($P < .01$).

By thromboelastometry those who had been without replacement therapy during the previous 12 months had shorter clotting time and quicker time to maximum velocity (tMaxVel) (33% (8/24) versus 8% (2/24) in the upper half) ($P = .03$). In MHB, all but one without the need of replacement therapy had short clotting times and quick tMaxVel (88% (7/8) versus 13% (1/8) ($P = .01$). For both diagnoses, those with shorter clot formation time captured lower scores by ISTH-BAT (13 points (8-20) versus 19 points (13-24)) ($P = .02$).

4 | DISCUSSION

We explored global coagulation assays and the bleeding phenotype of patients with MHA and MHB. TG was highly sensitive, since patients without factor consumption during the previous 12 months had highest ETP. Moreover, those who had low ETP captured higher HJHS, implying impaired joint health. Furthermore, patients who had undergone orthopaedic surgery generated least thrombin. Thromboelastometry differentiated according to factor consumption only.

TABLE 5 TG with 2.5 pM tissue factor according to Haemophilia Joint Health Score (HJHS), factor consumption during the previous 12 months (zero and non-zero), and orthopaedic surgery (arthrodesis or arthroplasties)

	HJHS 0–3 p (n = 26)	HJHS > 3 p (n = 34)[a]	*P*-value	Zero factor (n = 11)	Non-zero factor (n = 49)[b]	*P*-value	Surgery (n = 10)[c]	No surgery (n = 51)	*P*-value
Lag time (min)	5 (4-6)	5 (4-8)	.30	5 (4-6)	5 (4-7)	.63	6 (4-12)	5 (4-6)	.11
ETP (nM x min)	341 (288-462)	290 (204-378)	.06	409 (293-473)	304 (218-384)	.03	232 (166-298)	331 (257-419)	.02
Peak (nM)	19 (14-29)	15 (9-22)	.06	19 (17-33)	15 (9-23)	.05	9 (8-14)	19 (13-25)	<.01
Time to peak (min)	13 (11-18)	15 (12-21)	.13	13 (10-16)	14 (11-21)	.23	20 (15-31)	14 (11-18)	<.01
Velocity index (nM/min)	2 (1-4)	1 (1-3)	.10	2 (2-6)	2 (1-3)	.05	0.7 (0.5-1.3)	2 (1-4)	<.01

Medians and interquartile ranges (IQR). Abbreviation: ETP, Endogenous thrombin potential. The number of patients (n) is noted if it deviates from the total number: ETP:
[a]n = 33/34.
[b]n = 48/49.
[c]n = 9/10.

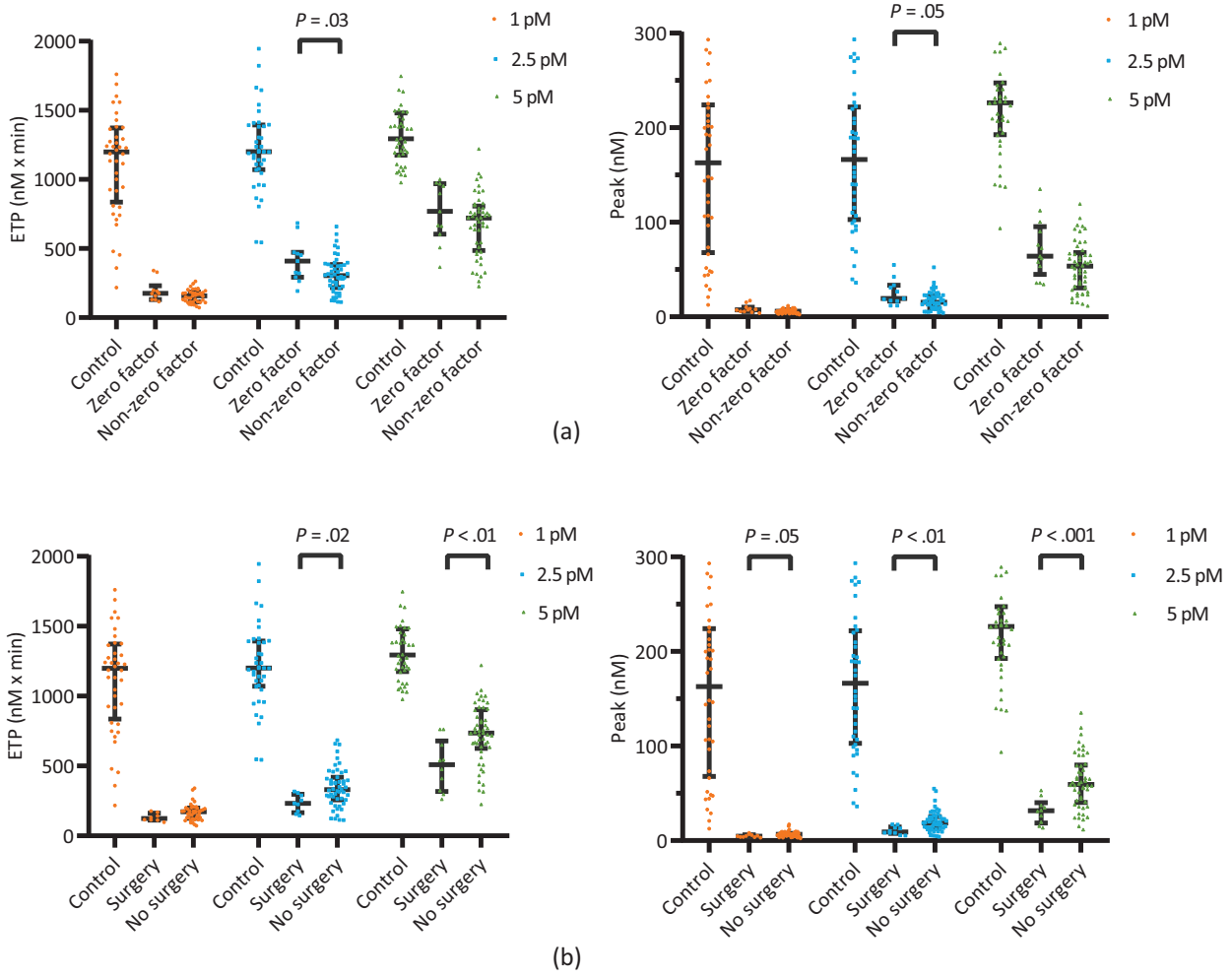

FIGURE 2 a. Endogenous thrombin potential (ETP) and peak thrombin according to factor consumption during the last 12 months (zero and non-zero) at tissue factor concentration 1, 2.5, and 5 pM. b. ETP and peak thrombin according to orthopaedic surgery (arthrodesis or arthroplasties) at tissue factor concentration 1, 2.5, and 5 pM. The lines represent median values and interquartile ranges

4.1 | Thrombin generation

In TG, clinical findings representing a mild bleeding phenotype were associated with relatively higher ETP, peak, velocity index, and shorter time to peak. At 1 and 2.5 pM TF exposure, patients who had been without replacement therapy during the previous 12 months differed from those who consumed factor concentrates. Moreover, at 2.5 pM TF, low ETP and delayed peak thrombin (< median) was associated with higher HJHS. Furthermore, at 2.5 and 5 pM TF those who had undergone orthopaedic surgery generated thrombin more slowly and attained lower peak and ETP. Hence, all TG parameters, except lag time, were able to discriminate patients with moderate haemophilia according to mild or severe bleeding phenotype. A normal lag time is usually seen among patients with haemophilia.[18]

We report higher ETP in PPP among patients with moderate haemophilia displaying a mild bleeding phenotype. In severe haemophilia, Santagostino et al. reported higher ETP among mild bleeders, but only in platelet-rich plasma (PRP).[8] PPP has better utility in clinical practice than PRP because the preparation is less time-consuming, and the samples can be stored (-80°C) before testing. Respectively, in PPP Dargaud et al. detected ETP < 50% of normal among patients with haemophilia A and B of all severities who presented with a severe bleeding phenotype.[7] In mild haemophilia A, Trossaert et al. reported ETP < 50% and peak thrombin < 30% of normal among those having ISTH-BAT ≥ 4 points.[9] In the MoHem study, peak and velocity index also to some extent reflected the patients' bleeding phenotype. Beltrán-Miranda et al. detected differences in peak according to frequency of haemarthrosis and FVIII:C among families with haemophilia A of all severities.[19] However, the peak differences did not correlate with the individual bleeding phenotypes. Recently, Aghighi et al. reported peak as applicable to discriminate between severe and MHA, but this study lacks comparison with clinical endpoints.[20] In the present study, TG did not align with FVIII/FIX:C, except lower peak (1 pM TF) among those having chromogenic FIX:C ≤ 3 IU/dL. Twenty-eight per cent demonstrated one-stage/chromogenic assay discrepancy, which is similar as reported in mild haemophilia.[9,21–23] In accordance with Trossaert et al., the genotype p.Arg550Cys (MHA) was associated with a more severe bleeding phenotype reflecting the lower chromogenic FVIII:C.[9] Nevertheless, both one-stage and chromogenic assays are recommended to detect all forms of haemophilia and avoid misclassification.[9,21,22,24–26]

4.2 | Thromboelastometry

By thromboelastometry, most patients who had been without replacement therapy during the previous 12 months had short clotting times and quick tMaxVel (< median values), particularly in MHB. Moreover, those with short clot formation time captured lower ISTH-BAT scores. Respectively, Chitlur et al. reported more target joints among children with severe and moderate haemophilia who had low (< mean) maximum rate of clot/fibrin generation.[6] According to Sørensen and Ingerslev, patients with severe and MHA presented huge variance in their haemostatic characteristics.[5] Some MHA had velocity profiles similar to normal, while others resembled severe haemophilia. However, they did not compare thromboelastometry with clinical endpoints reflecting the bleeding phenotype.

We did not find any correlation between thromboelastometry and baseline FVIII/FIX:C among patients with moderate haemophilia. However, previous publications reported correlations between FVIII:C and clotting time, tMaxVel, and MaxVel in haemophilia A when all severity groups were evaluated together.[20,27] Naturally, this relationship is less prominent in a narrow group of FVIII/FIX:C 1–5 IU/dL compared with groups containing more widespread levels. In none of these studies was thromboelastometry compared with the bleeding phenotype.

4.3 | Strengths and limitations

By thromboelastometry in whole blood and TG in PPP, we explored the coagulation process in patients with MHA and MHB having variant clinical presentation. The bleeding phenotype was characterised by HJHS, which is a sensitive, reliable, and validated tool to detect early arthropathy in haemophilia.[16] FVIII/FIX:C was analysed by one-stage and chromogenic assays and centralised to Malmö to avoid inter-laboratory variation. Similarly, all thromboelastometry and TG analyses were performed in Oslo. In accordance with ISTH/SSC recommendations, the sample tubes contained CTI to minimize contact activation.[11,12] CTI reduces assay variability at low TF concentrations.[28,29] The sample tubes in Helsinki contained higher CTI concentration than in Oslo. However, increasing the CTI concentration above 20 μg/mL does not suppress TG.[28,30] Furthermore, PPP was prepared by double centrifugation. By TG, we compared three TF concentrations. Using 1 pM TF, however, some curves were incomplete due to the very low levels of thrombin generated, which excluded estimation of ETP. Similar findings have been reported previously.[29] Thus, in line with others, 2.5 and 5 pM TF turned out most useful.[27,31]

We collected at least 72 h washout samples, which might be an insufficient time off treatment to represent baseline FVIII/FIX:C, particularly in MHB. However, near all patients used replacement therapy without extended half-life. Moreover, the real washout time exceeded 72 h, reaching a median of 120 h in MHB. Nevertheless, four patients having one-stage and chromogenic FVIII:C > 5 IU/dL at the current samples were excluded from the results, even if they all were diagnosed as moderate haemophilia based on historical values. Inter-laboratory differences in FVIII/FIX:C assays are well known, but the precision of these analyses has improved over time. Thus, there might be some misclassification of FVIII/FIX:C.

Furthermore, FVIII/FIX:C did not align with the bleeding phenotype among patients who were evaluated by global coagulation assays, but for the overall MoHem population. The lack of association in the global assay group is probably due to less power. Previously, we reported a younger age at first joint bleed and higher HJHS among those with FVIII/FIX:C ≤ 3 IU/dL based on historical values.[13] A correlation between FVIII:C and clinical phenotype was also detected by den Uijl et al.[32] However, according to our results TG and

thromboelastometry were more sensitive than FVIII/FIX:C to depict the bleeding phenotype, supporting a more widespread use of global coagulation assays. Unfortunately, not all haemophilia treatment centres have an easy access to these assays. We did not adjust for biological variation, but more than one measurement may be necessary to establish the individual TG capacity.[27]

In our study, TG corresponded better with the bleeding phenotype in moderate haemophilia than thromboelastometry did. Previous publications have reported a preference of TG to reflect the severity classification in haemophilia.[20,21,27] As thromboelastometry depends upon fibrin generation, it does not cover the whole TG process because fibrin is exhausted before TG is over.[33] This might explain a higher sensitivity by TG. Moreover, the low TF concentration used by thromboelastometry may increase the variability of this assay.

5 | CONCLUSION

In the MoHem study, we explored global coagulation assays and the bleeding phenotype of Nordic patients with MHA and MHB. By TG, patients presenting a mild bleeding phenotype, characterised by no need of replacement therapy during the previous 12 months, depicted higher ETP. In addition, those who had low ETP more commonly captured higher HJHS, indicating impaired joint health. Furthermore, patients who had undergone orthopaedic surgery generated least thrombin. The ability to distinguish between these groups was best at 2.5 pM TF exposure. Thromboelastometry differentiated according to annual factor consumption only. Global coagulation assays may therefore assist predicting the bleeding phenotype in moderate haemophilia and complements FVIII/FIX:C assays. In turn, this may improve the management of these patients.

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CONFLICT OF INTEREST

Erik Berntorp has acted as paid consultant to Bayer, CSL Behring, Octapharma, Takeda and received funding for research from Bayer, CSL Behring, Shire, and Sobi/Bioverativ. Pål A. Holme has received grants from Bayer, Octapharma, Sobi, and Pfizer. Ragnhild J. Måseide, Vuokko Nummi, Riitta Lassila, and Geir E. Tjønnfjord stated that they had no interests which might be perceived as posing a conflict or bias.

AUTHOR CONTRIBUTIONS

Erik Berntorp, Pål A. Holme and Ragnhild J. Måseide designed the study. Ragnhild J. Måseide and Vuokko Nummi collected the clinical data. Ragnhild J. Måseide planned and took part in the thromboelastometry and TG analyses. Ragnhild J. Måseide analysed the data and drafted the manuscript. Ragnhild J. Måseide, Pål A. Holme, Erik Berntorp and Geir E. Tjønnfjord interpreted the data. All authors contributed to critically revision of the manuscript and gave approval of the final version.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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