Thrombosis Research 173 (2019) 1–3

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Letter to the Editors-in-Chief

Presence of thrombophilia and levels of coagulation factors, coagulation inhibitors and TAFI do not affect global haemostasis or bleeding phenotype in patients with haemophilia A

ARTICLE INFO

Keywords:
Bleeding
Haemophilia A
Coagulation inhibitors
TAFI
Thrombophilia

Dear Editors-In-Chief,

Factors such as thrombophilia and different mutations can partially explain the discrepancy between disease severity and the grade of bleeding in some patients with haemophilia A, but the exact mechanisms are unknown [1–3]. The aim of this study is to investigate the associations of coagulation factors and inhibitors, thrombin activatable fibrinolysis inhibitor (TAFI) and global haemostatic tests (overall haemostatic potential, OHP and endogenous thrombin potential, ETP) with the bleeding phenotype, as well as the response to treatment with factor concentrate in a patient cohort with haemophilia A.

1. Patients and methods

The study cohort consisted of 76 patients with haemophilia A followed up at the Haemophilia Centre, Belgrade, Serbia and 30 healthy male controls. According to the presence of a target joint, the time lapsed between birth and the first bleeding episode ($>$ or $\leq$12 months) and the number of spontaneous haemarthroses/year ($>$ 3 or $\leq$3), patients were grouped as having clinically severe or non-severe disease.

Blood samples were collected at least 20 h following the latest dose of factor VIII (FVIII). In 38 patients, blood sampling was additionally performed 30 min after on-demand treatment with 1000–2000 IU of plasma-derived FVIII concentrate [3,4].

The methods for analysing OHP, ETP and FVIII have been described before [3,4]. Assays for coagulation factors (II, V, VII, VIII, IX, X, XI, XII) and inhibitors (proteins C (PC) and S (PS), antithrombin) were performed on an automated coagulation analyser (ACL ElitePro, Beckman Coulter Inc., Brea, CA, USA) using HaemosIL commercial kits from Instrumentation Laboratory (SpA, Milano, Italy). Levels of TAFIa/TAFIai complex were determined using an ELISA assay (Asserachrom TAFIa/TAFIai antigen kit; Diagnostica Stago, Asnieres, France). Factor V (FV) Leiden, prothrombin gene mutation G20210A (PTG20210A) and MTHFR C677T mutations were detected by using a PCR-RFLP method and APC resistance was detected by a modified assay (Hemosyl Factor V Leiden - APC ™ Resistance V).

We used multiple regression analysis to study the effects of coagulation factors and inhibitors on global haemostatic tests, bleeding phenotype and response to treatment. A type I error cannot be excluded since the p-value was not corrected for the multiple testing. We used Fisher's exact test for thrombophilia prevalence and the Mann Whitney *U* test for to compare markers between patients and controls. A p-value $<$ 0,05 was considered statistically significant.

The protocol of the study was approved by the local Ethics Committee in Belgrade, Serbia and all of the participants had given their written consent prior to inclusion in the study.

2. Results

The median age of the patients was 26 (11–65) years and of the controls 36.5 (18–59). Nine patients (12%) had moderate, ten (13%) mild and the rest had severe haemophilia. Fifty-six (73.7%) patients had a clinically severe form and twenty (26.3%) a milder bleeding phenotype.

Of all the markers tested, only FVIII was a significant predictor of the results of OHP, ETP and the bleeding phenotype.

The response to treatment was evaluated by comparing FVIII levels, ETP and OHP before and after administration of factor concentrate. FVIII and factor XI (FXI) had a significant effect on the variance of the response to therapy ($p = 0.045$ for both). PC ($\beta = 0.69$, $t = 4.50$; $p < 0.001$), FX ($\beta = -0.55$, $t = -3.40$, $p < 0.002$) and MTHFR C677T ($\beta = 0.38$, $t = 2.74$; $p < 0.01$) had predictive value for OHP (37% of the total variance). PS ($\beta = 0.49$, $t = 3.55$, $p < 0.001$) and fibrinogen ($\beta = 0.32$; $t = 2.29$; $p < 0.03$) had predictive value for ETP (33% of the total variance). There were no statistically significant partial correlations between ETP, OHP, clinical phenotype, response to therapy and coagulation factors and inhibitors, TAFI and thrombophilia. No statistically significant differences were observed for the coagulation factors, coagulation inhibitors and APC ratios in patients and controls and all levels were within the normal reference range.

The overall prevalence of thrombophilia for patients and controls was 27.6% respectively 23.3% ($p = 0.81$). Three patients (3.9%, 3.3% in controls) were heterozygous for FV Leiden, five had heterozygous prothrombin gene mutation (6.6%, 6.6% in controls) and fifteen had homozygous MTHFR 677TT mutation (19.7%, 13.3% in controls).

There was no statistically significant difference in the prevalence of

https://doi.org/10.1016/j.thromres.2018.11.008
Received 22 May 2018; Received in revised form 22 October 2018; Accepted 8 November 2018
Available online 08 November 2018

Table 1
Levels of inhibitors (antithrombin, PC, PS) and APC ratio in patients with clinically severe and mild haemophilia A.

	Phenotype	Median	Range	p
Number of haemarthroses/year				
Antithrombin (IU/mL)	Severe	1.06	0.9–1.33	0.411
	Mild	1.15	0.83–1.38	
Protein C (IU/mL)	Severe	1.01	0.63–1.71	0.110
	Mild	1.05	0.82–1.35	
Protein S (IU/mL)	Severe	1.02	0.61–1.44	0.500
	Mild	1.10	0.73–1.54	
APC ratio	Severe	3.19	1.81–3.54	0.213
	Mild	3.21	2.84–3.46	
Time to first bleeding episode				
Antithrombin	Severe	1.06	0.9–1.33	0.570
(IU/mL)	Mild	1.15	0.83–1.38	
Protein C	Severe	1.01	0.63–1.71	0.875
(IU/mL)	Mild	1.05	0.82–1.35	
Protein S	Severe	1.02	0.61–1.44	0.550
(IU/mL)	Mild	1.10	0.73–1.54	
APC ratio	Severe	3.19	1.81–3.54	0.903
	Mild	3.21	2.84–3.46	
Presence of target joint				
Antithrombin	Severe	1.06	0.9–1.33	0.751
(IU/mL)	Mild	1.11	0.83–1.38	
Protein C	Severe	1.01	0.63–1.71	0.205
(IU/mL)	Mild	1.05	0.67–1.35	
Protein S	Severe	1.02	0.61–1.44	0.523
(IU/mL)	Mild	1.10	0.73–1.54	
APC ratio	Severe	3.20	1.81–3.54	0.341
	Mild	3.17	2.84–3.46	

thrombophilia between the group with clinically mild compared with the group with severe haemophilia, except for the MTHFR 677TT mutation among patients who had their first bleeding episode before respectively after 12 months of age (39.4% and 18.6%, p = 0.04).

The levels of PS, PC, antithrombin and APC ratio according to the grades of clinical severity are shown in the Table 1.

3. Discussion

We found no significant difference in the prevalence of thrombophilia in controls and patients. The PTG20210A was the most common thrombophilia in our cohort, which could reflect differences in geographical prevalence. The thrombophilia rate was higher in our cohort compared to other studies [5], which could be a result of testing for more traits, different prevalence rates of thrombophilias and different cohort sizes. There is additionally no agreement on a potential “protective” role of thrombophilia against bleeding, which was also not shown in our study. Most studies have focused on common thrombophilic agents with varying prevalence, which can explain some of the observed differences.

Global haemostatic tests can help distinguish between patients with different disease severity, by revealing different haemostatic potentials [6]. It would appear that factors other than the coagulation factors and inhibitors analysed in this study are responsible for these discrepancies. It has also been suggested that fibrinolysis can explain variations [7], but, in our study, TAFI had no influence on the test results.

FVIII did not have a recurrent influence on the posttreatment test results, regardless of the response grade. FXI could explain 8.2% of the variation observed in the treatment response. This could be an expression of the intrinsic pathway compensating for the absence of the intrinsic tenase [8] in haemophilia. However, the patients had received exogenous FVIII, which could explain the low contribution of FXI. PC and PS had significant roles in explaining the posttreatment variations in OHP and ETP, respectively. Since PC and PS target FVIII, inhibition of APC has been proposed as an alternative haemophilia treatment [9]. The posttreatment results in our cohort could reflect increased anticoagulant activity in the plasma of patients receiving exogenous factor, secondary to the changed balance in the patient's habitual coagulation (increased procoagulant activity). Exogenous factor concentrate corrects the impaired thrombin generation in the patients and the activation of the APC pathway could thusly be further enhanced by the complex of thrombin and thrombomodulin, which also induces TAFI generation [10]. Even though no significant correlations were found, this finding is of potential importance since no similar studies have been performed and the results could contribute to understanding the function of APC pathway in haemophilia.

One of the strengths of our study is the relatively large cohort, with controls, and the analysis of a number of coagulation factors and inhibitors concurrently, which enabled us to get a more thorough picture of the patients' haemostatic potential and the factors that may affect the bleeding phenotype. The clinical severity of haemophilia was defined by parameters, which have been previously used in other investigations, thus making our results comparable to those in already published studies. Moreover, we used the response to treatment as an additional clinical feature, which has not been attempted before. However, our study did not uncover any strongly significant results or recurrent associations. This could be a result of low power for some of the questions in the study (such as the effect on treatment response), especially in subgroup analysis (disease severity).

Overall, our study showed that thrombophilia and coagulation factors and inhibitors in patients with haemophilia A cannot explain variations in the phenotype, but the activity of the APC pathway may influence the treatment response. Larger studies, designed specifically to evaluate variations in the posttreatment response, could help further clarify our findings.

Declaration of interests

Jovan P. Antovic has received speaker fees from Werfen, Stago, Siemens, Roche, Sysmex, Shire, research grants from Shire (previously Baxter) and is on the advisory board of NovoNordisk, Sobi and CLS Behring. Danijela Mikovic, Iva Pruner and Roza Chaireti have no conflicts of interest to declare.

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