Platelet procoagulant activity and thrombin generation in patients with severe hemophilia

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INTRODUCTION

- There is a marked variation in bleeding frequency in patients with severe hemophilia (≤1% of normal activity).
- The biological reasons for these differences are largely unknown, and have hampered the ability to individualize treatment plans for patients.

Proposed Mechanisms for Phenotypic Variability

Gene defects
Factor VIII-linked
Other: i.e. Factor V Leiden mutation
Factor VIII half-life
Other procoagulant/anti-coagulant proteins
Fibrinolytic proteins
Platelet function

BACKGROUND

- Differences in thrombin generation have been indentified as a potential mediator of differences in bleeding patterns in patients with severe hemophilia.
- In strongly-stimulated platelets (e.g. thrombin and collagen stimulation), exposure of platelet phosphatidylserine amplifies prothrombinase and tenase activity.
- The goal of this study was to investigate differences in platelet activation potential, particularly procoagulant platelet potential, that might account for variability in thrombin generation in patients with severe hemophilia, thereby impacting clinical severity.

MATERIALS AND METHODS

Thirty-four patients with severe hemophilia A or B (31 with hemophilia A and 3 with hemophilia B) were recruited from our Hemophilia Treatment Center.

Thrombin Generation

- Thrombin generation in platelet-rich plasma was assessed after initiation by tissue factor (1 pM).
- The thrombin generation test was used to determine lag time, time to peak, endogenous thrombin potential ,and peak.
- The velocity index was calculated using peak, time to peak, and lag time values.

Platelet Procoagulant Potential

- Washed platelets were stimulated with thrombin (0.5 u/mL) and the GPVI agonist convulxin (250 ng/mL) and assessment of procoagulant platelet potential.
  - Evaluation of annexin V+ platelets by flow cytometry after stimulation
- Washed platelets were stimulated with threshold concentrations of agonist (low dose thrombin) and assessment of surface P-selectin and PAC-1.

RESULTS

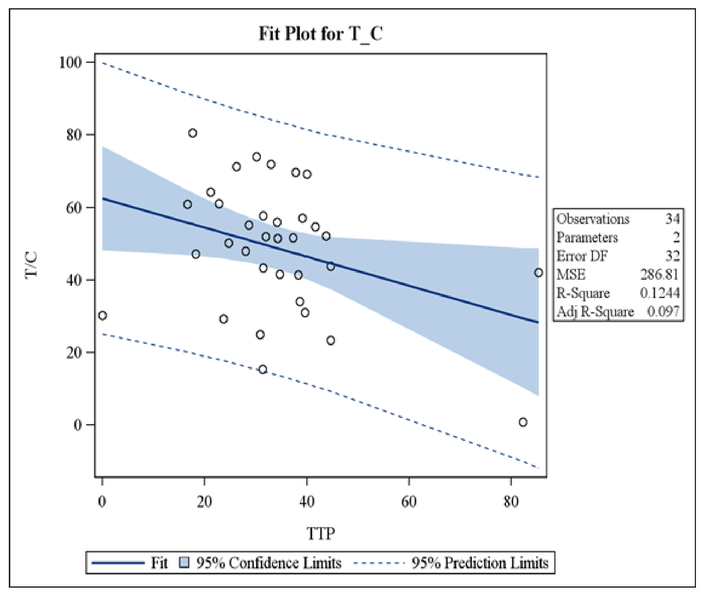
ID	ETP	Time to Peak	Velocity Index	PPP
1	n/a	85.33	-67.79	42
2	1122	41.67	-18.97	54.7
3	1156	40	-13.99	69.2
4	2281.5	31.5	-8.66	43.4
5	1213.5	34.17	-15.72	56
6	2827	38.67	-14.01	34.1
7	795	44.67	-12.93	43.8
8	795	44.67	-18.76	23.4
9	1307	0	-16.33	30.3
11	1566	18.25	0.16	47.1
12	1403	28.67	-9.56	55.2
14	1375	21.17	-3.58	64.2
15	761	22.83	-4.49	61.1
17	429	28	-9.28	48
21	0	31.38	-9.63	15.4
22	944.5	32	-13.83	51.9
23	1426	39.67	-25.52	31
24	1126	30.17	-10.48	74
25	535	39.17	-16.11	57.1
26	658.5	34.67	-10.97	41.6
27	1521	30.83	-11.81	25
28	1386.5	16.67	-0.73	60.9
29	889.5	33	-12.23	71.9
30	1073.5	38.3	-16.32	41.4
31	2078	31.5	-10.61	57.7
32	2167.5	26.2	-5.75	71.3
34	n/a	82.3	-31.05	0.8
35	n/a	43.8	-15.66	52.1
36	413.5	37.3	-16.61	51.6
40	754.5	34.3	-14.1	51.4
43	1201.5	23.67	-9.51	29.2
49	0	37.83	-34.65	69.7
53	662	24.67	-8.61	50.2
56	1052.5	17.67	-3.21	80.5

Following activation with thrombin and convulxin marked variability was noted in PCPP (49% ± 18%) and thrombin generation in PRP (Time to peak (35 ± 16 s); endogenous thrombin potential (1126 ± 627 nm)).

An inverse correlation was noted between PCPP and time to peak in the thrombin generation assay (p = 0.041). No correlation was noted between thrombin generation and activation potential by threshold concentrations of agonists

Correlation and Linear Regression Analysis

- An inverse correlation was noted between procoagulant platelet potential and time to peak in the thrombin generation assay (p=0.041).



CONCLUSIONS

- We have demonstrated for the first time an association between the rapidity of thrombin generation in platelet-rich plasma and platelet procoagulant potential.
- Since thrombin generation was determined without convulxin present, this result suggests that the assay of procoagulant platelet potential to thrombin and convulxin may reflect the platelet's procoagulant potential in multiple settings.
- Studies are ongoing to determine whether these biologic markers are predictive of bleeding severity in patients with severe hemophilia.

References

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