Online Supplemental Material

Supplemental Materials and Methods

Western blot analysis for histone H3

Plasma was analyzed by Western blotting for histone H3. Plasma was mixed with Laemmli-Buffer supplemented with 5% beta-mercaptoethanol (Biorad). After 3 min at 95°C, samples were subjected to 15% (w/v) SDS-polyacrylamide gel electrophoresis, followed by immunoblotting procedure with a polyclonal rabbit anti-histone H3 (Abcam, product number ab1791) as primary antibody and goat-anti-rabbit-IgG conjugated to horseradish peroxidase (Biorad) as secondary antibody. Detection was carried out with a Pierce ECL Western Blotting Substrate (Thermo Scientific). Human recombinant histone H3 (New England Biolabs) served as a positive control.

Isolation and visualization of plasma DNA

DNA was isolated from plasma using a DNA isolation kit according to manufacturer's instructions (Omega bio-tek, Norcross, GA), subjected to 2% agarose gel electrophoresis in the presence of ethidium bromide and visualized using a gel documentation system (BioRad, Hercules, CA).

Legends to Supplemental Figures and Tables

Supplemental Table 1. Learning cohort. Summary of demographic and diagnostic data from TMA patients and healthy controls. The learning cohort included plasma from 10 healthy controls (Ctrl), 4 patients with D+HUS, 8 patients with tumor-associated TMA, 11 patients with TMA of unknown etiology and 6 patients with acute acquired TTP before PEX therapy. From 5 of these TTP patients samples were available from the time when they were in remission. The table displays sample number, diagnosis, gender (Male/Female), age at first plasma sampling (years), ADAMTS13 activity (%) measured by FRETS-VWF73 assay, ADAMTS13 inhibitor (Bethesda units/ml), plasma DNA (ng/ml), plasma nucleosomes (fold increase over control), plasma LDH activity (OD/min), plasma myeloperoxidase (fold increase over control), plasma S100A8/A9 (fold increase over control), plasma histone H3 (number of bands detected by Western blot). The data are also displayed in figure 1 and supplemental Fig. 1.

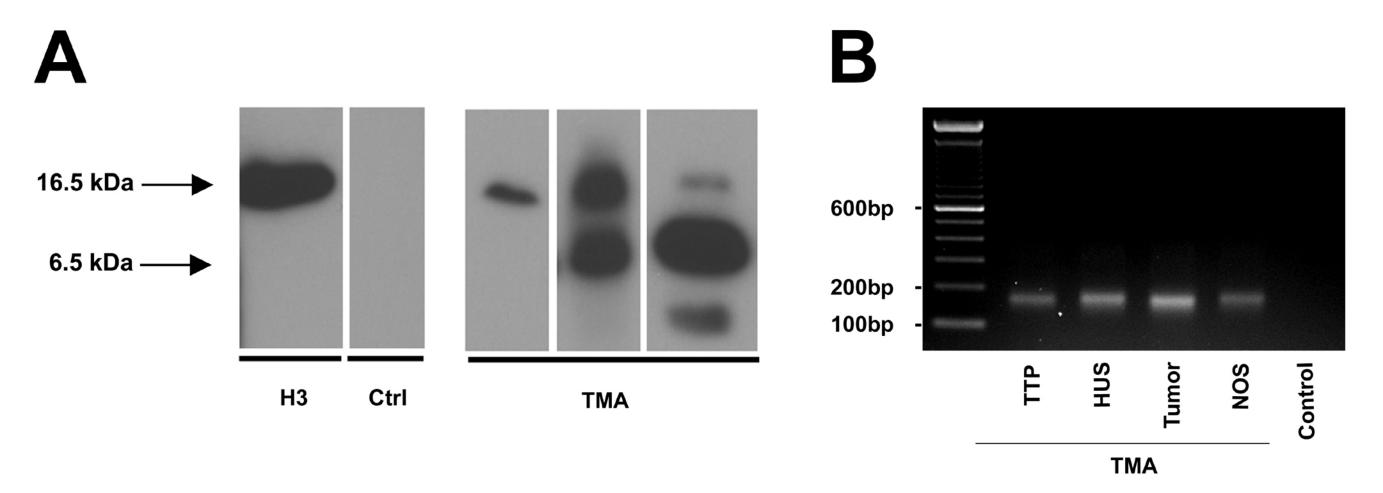
Supplemental Figure 1. Circulating Histones and DNA are fragmented in patients with acute TMA. (A) Immunoblot of histone H3. Recombinant histone H3 was used as a positive control (H3). Histone H3 of approximately 16kDa as well as 2 fragments of histone H3 were detected in 17 of 29 patients. The number of histone H3 bands detected in each patient is listed in Supplemental Table 1. TMA patient samples #31 (left), #35 (middle) and #17 (right) are shown as examples. All controls investigated (n = 10) were negative for Histone H3. (B) Gel electrophoresis of plasma DNA isolated from 4 patients with acute acquired TTP, D+HUS (HUS), tumor associated TMA (Tumor), a TMA of unknown etiology (not otherwise specified [NOS]) and from a healthy control (Ctrl). Patients, but not the control showed a single band of approximately 180 bp.

Supplemental Figure 2. Correlation of plasma markers of patients with acute

TMAs. Values for DNA (ng/ml), nucleosomes (fold increase over healthy controls), LDH activity (OD₄₉₀/min), MPO (fold increase over healthy controls), S100A8/A9 (fold increase over healthy controls), ADAMTS13 activity (%) from patients with acute TMA (n = 29, see Supplemental Table 1) were correlated to each other using Spearman correlation. The table shows Spearman r and Bonferroni-adjusted significance levels. Results were considered significant at p < 0.005. **Supplemental Table 2. Investigational cohort. Summary of demographic and diagnostic data from acquired TTP patients during therapy.** TTP patients were treated as indicated in table 1. Plasma samples were collected at admission, before PEX or as indicated in "Sample Description". The table displays sample number, patient ID, gender (Male/Female), age at first plasma sampling (years), diagnosis, treatment, days after first admission to hospital, ADAMTS13 activity (%) detected by FRETS-VWF73 or immunoblotting (IB), ADAMTS13 inhibitor (Bethesda units/ml), platelet count (1000/µl), DNA (ng/ml), plasma MPO (fold increase over controls). The data are also displayed in figures 2, 3 and 4.

Sample #	Diagnosis	Acute / Remission	Sex	Age	ADAMTS13 activity (% of Ctrl)	ADAMTS13 inhibitor (BU/ml)	DNA (ng/ml)	Nucleosomes (fold increase)	LDH Activity (OD/min)	Myeloperoxidase (fold increase)	S100A8/A9 (fold increase)	# of H3 Bands [WB]
1	Ctrl		М	59	>100%		8	0.5	0.020	2.4	0.7	0
2	Ctrl		F	52	>100%		26	3.7	0.016	1.2	0.5	0
3	Ctrl		F	31	>100%		43	0.5	0.003	0.1	0.5	0
4	Ctrl		M	39	>100%		46	0.5	0.030	1.6	2.2	0
5	Ctrl		M	42	>100%		13	0.2	0.007	0.4	1.7	0
6	Ctrl			43	>100%		29	1.9	0.020	2.2	1.8	0
7			M									
·	Ctrl		M	49	>100%		57	0.7	0.011	0.8	1.0	0
8	Ctrl		M	52	>100%		45	1.4	0.013	1.0	0.8	0
9	Ctrl		F	32	>100%		28	0.1	0.001	0.2	0.4	0
10	Ctrl		F	45	>100%		16	0.4	0.001	0.1	0.4	0
n = 10	Ctrl			Mear	100% >100%		31.0	1.0	0.012	1.0	1.0	0/10
11	D+HUS	Acute	М	2	33%		9316	156.7	12.635	575.8	> 100	3
12	D+HUS	Acute	F	6	85%		2661	133.6	13.113	33.2	> 100	2
13	D+HUS	Acute	F	4	77%		1728	453.6	6.339	34.6	> 100	2
14	D+HUS	Acute	М	5	45%		5789	138.2	7.228	127.4	> 100	3
n = 4	D+HUS	Acute		Mear	n 60%		4873.4	220.5	9.829	192.8	> 100	4/4
15	Tumor-TMA	Acute	F	47	45%		794	39.4	11.097	49.6	> 100	0
16	Tumor-TMA	Acute	М	71	76%		1786	73.3	1.776	15.0	76.3	1
17	Tumor-TMA	Acute	F	74	63%		3778	85.0	1.256	13.9	9.4	3 (Figure S1A)
18	Tumor-TMA	Acute	F	47	67%		934	31.2	1.672	20.7	10.4	2
19	Tumor-TMA	Acute	M	29	32%		108	9.6	0.411	10.8	54.2	0
20	Tumor-TMA	Acute	M	74	64%		2632	84.5	2.688	39.9	> 100	2
21	Tumor-TMA	Acute		57	51%		101	0.6	0.191	3.5	0.2	0
	Tumor-TMA		F	73	32%		468					2
22 n = 8	Tumor-TMA	Acute Acute	г		<u> </u>		1325.4	124.8 56.1	5.153 3.030	23.4 22.1	> 100	5/8
11 - 0	Turnor-TWA	Acute		wear	1 54 %		1323.4	50.1	3.030	22.1	-	5/6
23	NOS-TMA	Acute	F	72	72%		416	13.3	3.389	9.3	> 100	0
24	NOS-TMA	Acute	F	18	65%		316	25.2	5.251	18.5	> 100	0
25	NOS-TMA	Acute	М	77	67%		1215	50.2	1.672	115.3	> 100	2
26	NOS-TMA	Acute	м	21	63%		1865	53.6	5.153	134.7	> 100	2
27	NOS-TMA	Acute	М	26	71%		266	10.0	0.094	48.9	5.4	0
28	NOS-TMA	Acute	Μ	83	36%		33	3.8	0.058	10.5	0.9	0
29	NOS-TMA	Acute	M	63	65%		128	0.6	0.031	32.6	1.1	0
30	NOS-TMA	Acute	F	34	49%		46	4.5	0.042	11.1	1.1	0
31	NOS-TMA	Acute	M	26	61%		123	28.7	3.802	11.1	> 100	1 (Figure S1A)
32	NOS-TMA	Acute	E	50	>100%		41	2.6	0.010	2.3	0.8	0
	NOS-TMA		Ë	28	72%							-
<u>33</u> n = 11	Tumor-TMA	Acute Acute	г		12% 1 62%		245 426.7	24.1 19.7	3.078 2.053	4.9 36.3	> 100 -	0 3/11
24	TTD #1	Aquita	F	25	< 50/	>>2	2960	12.0	1 202	20.2	> 100	2
34	TTP #1	Acute	F	25	<5%	>>2	2860	12.9	1.282	30.3	> 100	2
35	TTP #2	Acute	F	28	<5%	1	1625	53.5	4.345	32.9	> 100	2 (Figure S1A)
36	TTP #3	Acute	M	57	<5%	1.7	145	5.1	0.554	7.9	2.5	0
37	TTP #4	Acute	F	26	<5%	>2	182	13.7	2.125	17.6	> 100	1
38	TTP #5	Acute	F	57	<5%	1.7	908	57.0	6.459	32.5	> 100	2
39	TTP #6	Acute	М	29	<5%	1.8	348	12.5	2.849	13.8	> 100	1
n = 6	TTP	Acute		Mear	า <5%		1011.3	25.8	2.936	22.5	-	5/6
40	TTP #1	Remission			92%		28	1.0	0.042	1.7	2.1	0
41	TTP #2	Remission			67%		36	2.0	0.062	1.8	1.5	0
42	TTP #3	Remission			73%		34	0.9	0.046	5.2	1.7	0
43	TTP #4	Remission			94%		14	0.9	0.076	6.6	2.2	0
44	TTP #5	Remission			80%		59	1.1	0.11	3.6	2.4	0
n = 5	TTP	Remission		Mear	n 81%		34.0	1.2	0.067	3.8	2.0	0/5
1-5	11F	Remission		wear	10170		54.0	1.2	0.007	5.0	2.0	0/0

Supplemental Table 1



Supplemental Figure 1

DNA	-					
Nucleo- somes	0.84 (<0.0001)	-				
LDH	0.61 (0.0004)	0.79 (<0.0001)	-			
MPO	0.71 (<0.0001)	0.64 (0.0002)	0.55 (0.002)	-		
S100A8/A9	0.58 (0.001)	0.66 (<0.0001)	0.84 (<0.0001)	0.52 (0.004)	-	
ADAMTS13	0.04 (0.85)	0.10 (0.60)	-0.06 (0.76)	-0.01 (0.95)	-0.15 (0.45)	-
Spearman r (p - Value)	DNA	Nucleo- somes	LDH	MPO	S100A8/A9	ADAMTS13

Supplemental Figure 2

ample #	Patient ID (Figure)	Sex	Age	Diagnosis & Treatement	Days	Sample Description	ADAMTS13 by FRETS-VWF73	IB	(BU/ml)	Platelets (1000/ul)	DNA (ng/ml)	MPO (fold increase)	Fig. 4
	Patient A (Fig. 2A)	F	38	Acquired TTP PEX, Steroids	0 0 1	Admission before 1. PEX before 2. PEX	<5% <5% <5%	<5% 10%	5.1 2.9 1.6	35 21	457 389 786	23.8 22.1 161.7	x x
					2	before 3. PEX before 5. PEX	<5% <5%	<5%	0.8 0.9	36 75	212 107	29.1 11.1	× ×
					5	before 6. PEX before 7. PEX	<5% <5%	<5%	1.4 1.6	74 80	145 91	8.1 7.1	X
					7	before 8. PEX	<5%	<5%	1.2	110	63	2.2	X
					8 10	before 9. PEX before 10. PEX	<5% 50%	15% 100%	0.6	107 204	13 29	9.6 0.6	X X
					11 12		80% 62%	failed 100%		212 259	53 24	15.7 13.2	X X
					19 26		83% 81%	100% 100%		250 166	41 21	2.9 6.1	X X
	Patient B (Fig. 2B)	М	69	Acquired TTP	33 0	Admission	73% <5%	100%	1.6	198 29	17 729	3.8 20.6	X X
				PEX, Steroids	1 2	before 2. PEx before 3. PEX	13% 24%		0.6	30 78	89 35	13.5 6.2	X X
					8 16		44% 59%			302 375	14 26	1.9 3.9	X X
	Patient C (Fig. 2C)	М	57	Acquired TTP + Catheter Sepsis	0	-	<5% <5%		3.1 1.3	12 21	132 321	20.7 18.6	X X
				PEX, Steroids	2 3	before PEX before PEX	<5% <5%		1.4 1.7	23 27	678 192	19.4 16.8	X X
					4 5	before PEX before PEX	<5% <5%		2.3 5.9	23 32	1080 1027	15.8 15.2	X X
					6 7	before PEX before PEX	<5% <5%	<5%	4.2 9.2	21 13	1116 2520	16.8 23.1	x x
					8 9	before PEX -	<5% <5%	<5%	8.8	18 20	2020 11457	45.7 183.0	x x
					10 10	before PEX Exitus	<5% <5%	<5%	>10	23 32	20846 16404	278.5 221.8	X X
	Patient D (Fig. 2D)	М	29	Acquired TTP PEX, Steroids	0 1	Admission before 2. PEX	<5% 5%		1.8 1	9 21	353 23	19.1 12.5	X X
				+ Rituximab (given 4x, at weekly	2 3	before 3. PEX before 4. PEX	8% 9%		0.8 0.6	94 171	1 7	4.5 4.9	X X
				intervals)	6 7	before 5. PEX before 6. PEX	<5% <5%		7.9 7.2	147 82	49 27	12.5 7.4	X X
					8 12	before 7. PEX before 8. PEX	<5% <5%		4.7 2.1	56 79	91 20	7.9 4.4	X X
					13 14	before 9. PEX before 10. PEX	<5% <5%		3.5 >10	78 65	30 55	5.0 6.4	X X
					15 16	before 11. PEX before 12. PEX	<5% <5%		9.1 5.3	34 47	47 29	3.8 9.4	X X
					17 18	before 13. PEX before 14. PEX	<5% 18%		1.6 0.7	59 82	19 11	3.4 3.4 3.4	X X
					19 21	before 15. PEX (Rituximab) before 16. PEX	50% 16%		0.5	82 87 139	20 26	1.8 4.2	X X
					22 23	before 17. PEX before 18. PEX	13% 7%		1.2 1.1	140 150	11 13	0.2 4.1	X
					24	before 19. PEX before 20. PEX	13% 18%		0.9 0.7	163 126	9	0.6	X
					26 28	before 21. PEX	50%		0.7	122	35 33	2.0 0.0	X
					29 31	before 22. PEX before 23. PEX	74% 77%			146 153	17 10	2.1 2.4	X X
					33 35	before 24. PEX before 25. PEX	81% 81%			146 129	15 12	3.7 1.8	X X
					37 40	before 26. PEX before 27. PEX	88% 82%			173 182	9 12	3.2 0.1	X X
					43 49	2 days after last PEX	76% 71%			184 260	8 28	2.0 3.8	X X
	Patient E (Fig. 2E)	М	18	Acquired TTP	76 0	Admission	81% <5%	<1%	3	250 8	5 1243	2.6 42.3	X X
				PEX, Steroids + Splenectomy	2 3	before 3. PEX before 4. PEX	<5% 6%	<1% <1%	0.8 0.5	35 36	149 62	10.9 11.2	X X
					4 5	before 5. PEX before 6. PEX	<5% <5%	<1% <1%	0.5 1	37 27	20 4	7.3 8.9	X X
					6 7	before 7. PEX before 8. PEX	<5% <5%	<1% <1%	4 >10	20 16	45 178	9.2 16.9	X X
					8 9	before 9. PEX before 10. PEX	<5% <5%	<1% <1%	>10 >10	13 14	247 194	16.2 11.9	X X
					12 13	before 13. PEX before 14. PEX	<5% <5%	<1% <1%	>10 5.5	43 49	250 100	6.5 10.4	X X
					14 15	before 15. PEX before 16. PEX	<5% <5%	<1% <1%	2.4 1.7	29 10	809 311	9.2 9.1	X X
					16 17	before 17. PEX before 18. PEX	<5% 6%	5% 30%	1.3 0.6	9 9	399 125	15.9 9.7	X X
					18 19	before 19. PEX before 20. PEX	15% 40%	50% 100%	none	20 39	76 15	8.0 4.8	X X
					20 21	before 21. PEX before 22. PEX	59% 39%	100% 50%	none	62 96	25 110	4.3 4.1	X
					22 23	before 23. PEX before 24. PEX	7% <5%	25% <3%	0.8 1.5	130 179	70 30	7.6 7.3	X X
					24 25	before 25. PEX	<5% <5%	<3% <3%	3.2 4.1	192 64	52 288	8.0 6.4	X X
					27 28	before 27. PEX before Splenectomy	5% 10%	10% 25%	none 0.5	33 44	1 17	9.0 0.3	X X
					28 29 30	before 28. PEX Stop PEX	30% 69%	25% 50% 100%	none	44 135 173	17 7 8	0.3 0.7 3.7	X X X
					31		81%	100%	none none		8 12 4	0.9	
					32 74 478	in Remission	64% 39% 53%	100% 100% 100%	none none	326 355	+ 1 7	1.3 0.0	X X
					478 1255	in Remission in Remission	53% <5%	100% <5%	5.2	435	7 6 5	0.0 1.6	X
					1316 1394	in Remission in Remission in Remission	<5% <5% <5%	<5% <3% <3%	>10 >10 >10	365	5 14 1	5.9 0.0 1.5	Х
	Patient F	М	37	Recurrent, acquired TTP	1449 0	in Remission Admission, 1st TTP episode	<5% <5%	<3% <5%	>10 > 2	9	1 222 26	1.5	
				PEX, Steroids + Rituximab	620 704	in Remission in Remission	50% 42%	100% 40%			26 25		
					781 844	in Remission in Remission	31% <5%	30% 25%	none		18 32		
					907 977	in Remission Admission, 2nd TTP episode	<5% <5%	<5%	0.5 none		71 173		
					1104 1363	in Remission in Remission	>100% >100%				22 12		
					1482 1649	in Remission in Remission	84% 54%				71 43		
					1712	Admission 3rd TTP episode,	<5%		0.6		30		
					1742 1931	after 7-10 days PEX in Remission	8% >100%				47 29		
	Patient G	Μ	36	Recurrent, acquired TTP PEX, Steroids	0 669	in Remission, after 2nd TTP episod Admission, 3rd TTP episode		<3% <5%	>>1 >>1	365 53	33 226	3.1 8.2	X X
					711 754	in Remission Admission, 4th TTP episode	<5% <5%	<3% <3%	>2 >2	302 35	22 812	1.3 16.5	X X
	Patient H	М	59	Recurrent, acquired TTP	876 0	Admission, 5th TTP episode in Remission after 3rd TTP episode	<5%	<3% <3% <5%	traces	40 155	630 42	8.8 1.9	× ×
		141	53	PEX, Steroids	0 15 22	4th TTP episode in Remission	<5% <5% <5%	<5% <3% <3%	1-2 1-2	63 249	42 90 50	1.9 3.0 1.7	X X X
	Patient I	М	51	Hereditary TTP	0	Admission, 2nd TTP episode in Remission	<5%	<3%	none	249 20	11	9.8	Λ
		R 4	04	PEX, FFP infusion	54 1108	in Remission, FFP 1x/week	15%	<5%	0000		31 30	14.0 18.0	
	testing of 1	Μ	61	Hereditary TTP	0	Admission, 1st TTP episode	<5%		none		482	20.9	
	Patient J			PEX, no FFP prophylaxis	52 206	in Remission in Remission	<5% <5%	5%	none none		33 58	7.6 20.6	

Supplemental Table 2