

Establishing Prognostic Markers Associated with Neutrophil Extracellular Traps and the Activated Contact System in Thrombotic Microangiopathy

Running Title: Prognostic markers in TMA

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Supplementary Table 1 Diagnostic classification of thrombotic microangiopathies in total enrolled patients

Diagnosis	Total (n=138)	Survivors (n=123)	Non-survivors (n=15)
TTP	4 (2.9)	3 (75.0)	1 (25.0)
Congenital	2 (1.4)	1 (50.0)	1 (50.0)
Immune-mediated	2 (1.4)	2 (100.0)	0 (0.0)
Malignancy-related TMA	16 (11.6)	12 (75.0)	4 (25.0)
Transplant-related TMA	35 (25.4)	34 (97.1)	1 (2.9)
Infection-associated HUS	4 (2.9)	4 (100.0)	0 (0.0)
Presumptive atypical HUS	47 (34.1)	43 (91.5)	4 (8.5)
DIC	5 (3.6)	2 (40.0)	3 (60.0)
Others	27 (19.6)	25 (92.6)	2 (7.4)

Values are presented as number of subjects (percentage).

Abbreviations: TMA - thrombotic microangiopathy; TTP - thrombotic thrombocytopenic purpura; HUS - hemolytic-uremic syndrome; DIC - disseminated intravascular coagulation

Supplementary Table 2 Baseline characteristics and the values of markers in the malignancy-related TMA population (n = 16) classified based on the 30-day survival status

Characteristics	Survivors (n=12)	Non-survivors (n=4)	P-value
Age (years)	60.5 (50.5–65.5)	69.5 (65.8–83.8)	0.030
Sex, male/female	8 (66.7) / 4 (33.3)	2 (50.0) / 2 (50.0)	0.604
Platelets ($\times 10^9/L$)	62 (21–87)	21 (9–33)	0.078
Fibrinogen (mg/dL)	224 (199–316)	355 (243–355)*	0.170
D-dimer (ng/mL)	937 (845–2007)	566 (464–566)*	0.073
ADAMTS13 activity (%)	53 (34–86)	44 (29–46)	0.262
vWF:Ag (%)	222.6 (200.5–308.4)	457.0 (325.0–457.0)*	0.089
vWF:Rco (%)	140.3 (106.1–260.7)	270.1 (131.0–370.0)	0.304
Factor XIIIa (U/L)	52.1 (36.2–69.6)	39.0 (28.0–56.4)	0.212
Neutrophil elastase (ng/mL)	49.2 (27.7–89.5)	26.2 (19.7–26.2)*	0.233
Histone-DNA (AU)	59.5 (31.8–242.8)	59.0 (58.0–59.0)*	0.734
Cit H3 (ng/mL)	7.23 (3.70–16.95)	4.75 (1.28–4.75)*	0.365
Cell-free dsDNA (ng/mL)	82.9 (58.9–150.1)	35.0 (24.2–35.0)*	0.233

Values are presented as number of subjects (percentage) or median (interquartile range).

*Due to the small sample size in the non-survivor group, some quartile values are identical to the median.

Abbreviations: AU - arbitrary units; ADAMTS13 - a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; vWF - von Willebrand factor; Ag - antigen; Rco - ristocetin cofactor; Cit H3 - citrullinated histone H3; Cell-free dsDNA - cell-free double-stranded deoxyribonucleic acid

Supplementary Table 3 Baseline characteristics and the values of markers in the transplant-related TMA population (n = 35) classified based on the 30-day survival status

Characteristics	Survivors (n=34)	Non-survivor [†] (n=1)	P-value
Age (years)	52.5 (38.0–65.3)	73.0 (73.0–73.0)*	0.114
Sex, male/female	24 (70.6) / 10 (29.4)	0 (0.0) / 1 (100.0)	0.314
Platelets (× 10 ⁹ /L)	82 (39–121)	32 (32–32)*	0.400
Fibrinogen (mg/dL)	257 (214–319)		
D-dimer (ng/mL)	533 (284–1116)		
ADAMTS13 activity (%)	67 (55–93)	49 (49–49)*	0.353
vWF:Ag (%)	235.0 (192.0–396.8)		
vWF:Rco (%)	148.0 (113.1–295.9)		
Factor XIIa (U/L)	37.8 (28.7–53.1)		
Neutrophil elastase (ng/mL)	59.9 (28.8–173.9)	88.2 (88.2–88.2)*	0.848
Histone-DNA (AU)	65.5 (41.3–126.0)	3.0 (3.0–3.0)*	0.061
Cit H3 (ng/mL)	7.41 (3.53–13.61)	2.52 (2.52–2.52)*	0.364
Cell-free dsDNA (ng/mL)	72.0 (64.1–87.5)	65.3 (65.3–65.3)*	0.545

Values are presented as number of subjects (percentage) or median (interquartile range).

*Quartile values in the non-survivor group are identical to the median because only one observation was available.

[†]Data for fibrinogen, D-dimer, vWF:Ag, vWF:Rco, and Factor XIIa were missing in the non-survivor group and thus excluded from the analysis.

Abbreviations: AU - arbitrary units; ADAMTS13 - a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; vWF - von Willebrand factor; Ag - antigen; Rco - ristocetin cofactor; Cit H3 - citrullinated histone H3; Cell-free dsDNA - cell-free double-stranded deoxyribonucleic acid

Supplementary Table 4 Cox regression analyses of circulating markers to predict mortality in the malignancy-related TMA population

Variables	Univariable			Multivariable [†]		
	HR	95% CI	P-value	HR	95% CI	P-value
ADAMTS13 activity (> 49 vs. ≤ 49%)	62.62	0.0–2.0×10 ⁵	0.314			
vWF:Ag (≤ 218.4 vs. > 218.4%)	36.92	0.0–1.0×10 ⁷	0.572			
vWF:Rco (≤ 175.7 vs. > 175.7%)	3.56	0.4–34.4	0.272			
Platelets (> 49 vs. ≤ 49 × 10 ⁹ /L)	62.62	0.0–2.0×10 ⁵	0.314			
Factor XIIa (≤ 26.06 vs. > 26.06 U/L)*						
Neutrophil elastase (> 110.9 vs. ≤ 110.9 ng/mL)	22.50	0.0–5.9×10 ⁹	0.753			
Histone-DNA complexes (≤ 56 vs. > 56 AU)	51.07	0.0–6.4×10 ⁵	0.414			
Cit H3 (> 9.12 vs. ≤ 9.12 ng/mL)	41.20	0.0–7.7×10 ⁵	0.459			
Cell-free dsDNA (> 65.25 vs. ≤ 65.25 ng/mL)	3.67	0.3–40.7	0.290			

*Factor XIIa was not analyzed because all patients were in the same category.

[†]Multivariable analysis did not yield a model because the model did not converge due to an insufficient number of events relative to the number of variables, combined with complete separation in several predictors.

Abbreviations: AU - arbitrary units; ADAMTS13 - a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; vWF - von Willebrand factor; Ag - antigen; Rco - ristocetin cofactor; Cit H3 - citrullinated histone H3; Cell-free dsDNA - cell-free double-stranded deoxyribonucleic acid; HR - hazard ratio; CI - confidence interval

Supplementary Table 5 Cox regression analyses of circulating markers to predict mortality in the transplant-related TMA population

Variables	Univariable			Multivariable [†]		
	HR	95% CI	P-value	HR	95% CI	P-value
ADAMTS13 activity (> 49 vs. ≤ 49%)	2.18×10 ³	0.0–6.2×10 ²¹	0.723			
vWF:Ag (≤ 218.4 vs. > 218.4%) [*]						
vWF:Rco (≤ 175.7 vs. > 175.7%) [*]						
Platelets (> 49 vs. ≤ 49 × 10 ⁹ /L)	2.01×10 ²	0.0–3.6×10 ¹⁰	0.585			
Factor XIIa (≤ 26.06 vs. > 26.06 U/L) [*]						
Neutrophil elastase (> 110.9 vs. ≤ 110.9 ng/mL)	45.40	0.0–7.5×10 ⁹	0.653			
Histone-DNA complexes (≤ 56 vs. > 56 AU)	0.01	0.0–1.4×10 ⁵	0.596			
Cit H3 (> 9.12 vs. ≤ 9.12 ng/mL)	45.40	0.0–7.5×10 ⁹	0.653			
Cell-free dsDNA (> 65.25 vs. ≤ 65.25 ng/mL)	0.03	0.0–9.4×10 ⁶	0.730			

^{*}vWF:Ag, vWF:Rco, and Factor XIIa were not analyzed because all patients were in the same category.

[†]Multivariable analysis did not yield a model because the model did not converge due to an insufficient number of events relative to the number of variables, combined with complete separation in several predictors.

Abbreviations: AU - arbitrary units; ADAMTS13 - a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; vWF - von Willebrand factor; Ag - antigen; Rco - ristocetin cofactor; Cit H3 - citrullinated histone H3; Cell-free dsDNA - cell-free double-stranded deoxyribonucleic acid; HR - hazard ratio; CI - confidence interval

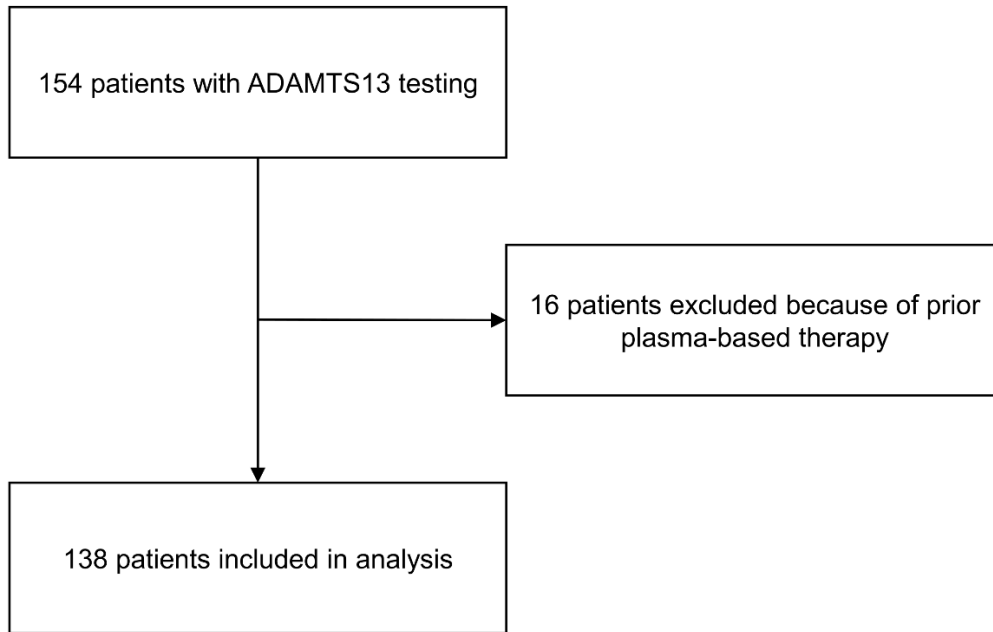


Fig. S1 Flow diagram of patient selection for the final analysis

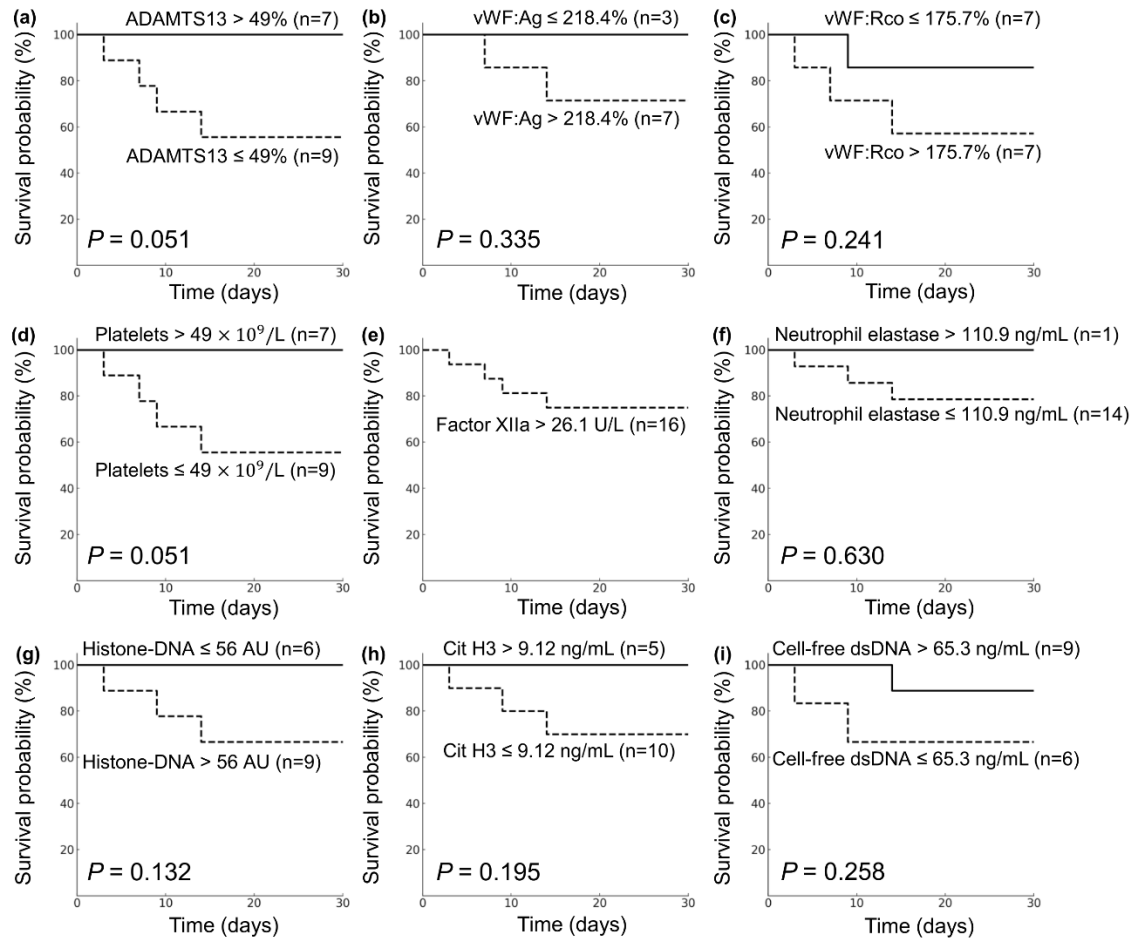


Fig. S2 Kaplan-Meier survival curves in the malignancy-related TMA population. Patients were divided into survivors and non-survivors according to the indicated cutoff values of (a) ADAMTS13 activity, (b) vWF:Ag, (c) vWF:Rco, (d) Platelets, (e) Factor XIIa, (f) Neutrophil elastase, (g) Histone-DNA complexes, (h) Cit H3, and (i) Cell-free dsDNA

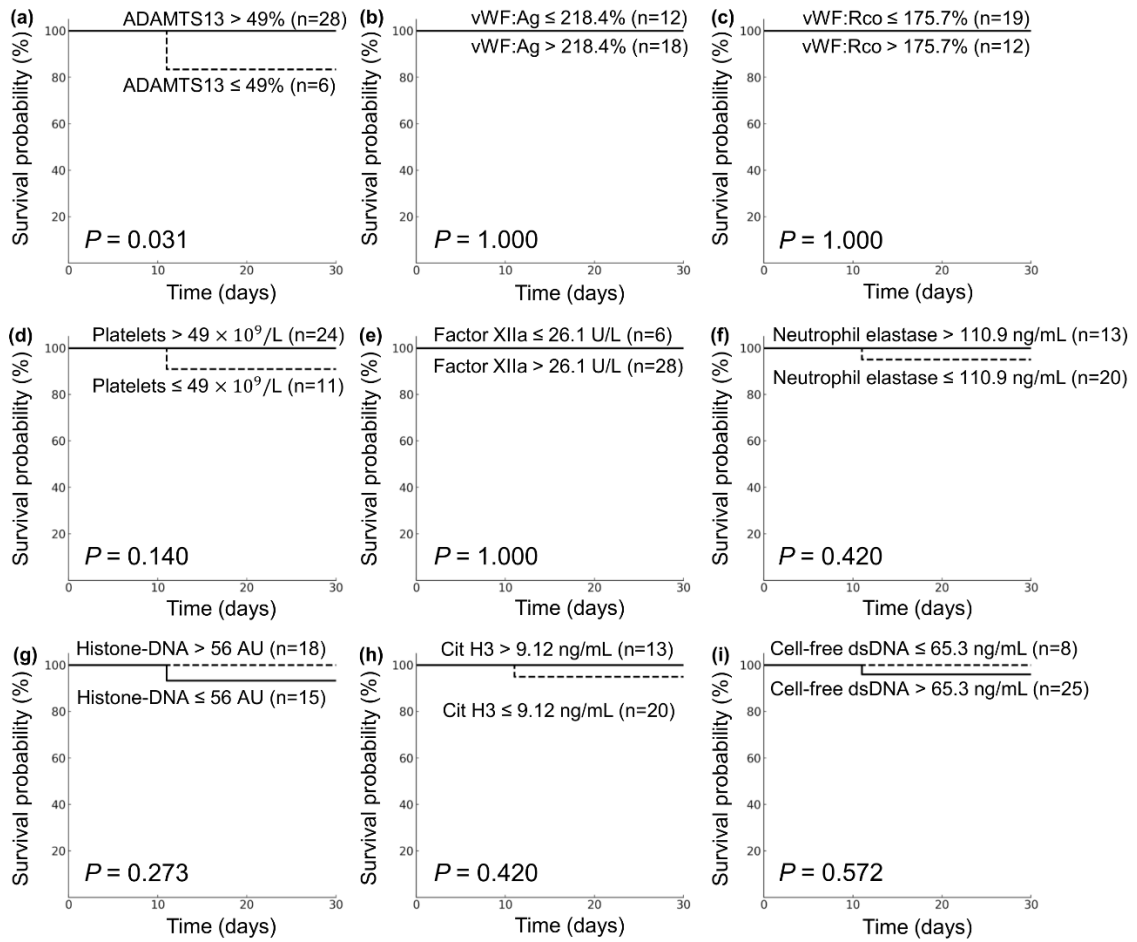


Fig. S3 Kaplan-Meier survival curves in the transplant-related TMA population. Patients were divided into survivors and non-survivors according to the indicated cutoff values of (a) ADAMTS13 activity, (b) vWF:Ag, (c) vWF:Rco, (d) Platelets, (e) Factor XIIa, (f) Neutrophil elastase, (g) Histone-DNA complexes, (h) Cit H3, and (i) Cell-free dsDNA