SUPPLEMENTARY MATERIALS

Procoagulant platelets mediate cerebral arterial thrombosis in cancer

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Normal value for routine laboratory data of Istituti Ospedalieri of Cremona

Laboratory test	Reference values
Red Blood Cells (10^6/µL)	4.6 - 10
Hemoglobin (g/dL)	13.70-17.50 (male) 11.20-15.70 (female)
White Blood cells (10^9/L)	3.9-10.6
Lymphocytes (absolute number) (10^9/L)	0.7-5
Lymphocytes (%)	20-45
Neutrophils (absolute number) (10^9/L)	1.50-8
Neutrophils (%)	43-75
Platelets (10^9/L)	150-400
INR	0.8-1.2/<1.2
aPTT	<1.2
D-dimer (µg/L)	<0.5
Fibrinogen (mg/dL)	>200
ESV	
CRP (mg/L),	
Procalcitonin (ng/mL)	<0.05
LDH (U/L)	<248
Glucose levels (mg/dL)	60-100
Total cholesterol (mg/dL)	
LDL cholesterol (mg/dL)	<115
HDL cholesterol (mg/dL)	>40 (male) >45 (female)
Triglycerides (mg/dL)	<180

Normal value for routine laboratory data of Policlinico Umberto 1 Hospital

Laboratory test	Reference values
RBC (10^6/µL)	4.30-5.90 (male) 3.50-5.10 (female)
Hb (g/dL)	13-17 (male) 12-16 (female)
WBC (10^9/L)	4-10
Lymphocytes (10^9/L)	1-3.2
Lymphocytes (%)	19-48
Neutrophils (10^9/L)	2.20-6.60
Neutrophils (%)	40-74
Platelets (10^9/L)	150-450
NLR	
PLR	
INR	0.8-1.2
aPTT	
ATIII (%)	80-120
D-dimer (µg/L)	0-550
Fibrinogen (mg/dL)	200-400
CRP (mg/L)	28-72
Procalcitonin(ng/mL)	0.02-0.064
Albumin (g/L)	3.5-5.2
LDH (U/L)	135-225
Myoglobin (µg/L)	
Glucose (mg/dL)	74-106
Cholesterol Tot (mg/dL)	80-200
HDL (mg/dL)	>55
LDL (mg/dL)	50-100
Triglycerides(mg/dL)	30-200

Supplementary Table 1. Stroke clinical characteristics

	All patients (N=85)	**Stroke with Cancer (n=33)**	**Stroke without Cancer (n=32)**	**Cancer without Stroke (n=20)**	**P value**
Onset on awakening (%)	7/65 (10.8)	2/33 (6.1)	5/32 (15.6)	-	0.258
Unknown onset (%)	14/65 (21.5)	9/33 (27.3)	5/32 (15.6)	-	0.253
HB at admission, mean (SD)	81.5 (16.1)	84.1 (16.4)	79.4 (15.8)	-	0.281
SBP, mean (SD)	141.2 (22.7)	138.1 (24.9)	143.8 (20.9)	-	0.353
DBP, mean (SD)	78.4 (11.6)	78.9 (12.4)	78.1 (11.14)	-	0.801
BT, mean (SD)	36.0 (0)	36.0 (0)	36 (0)	-	1.0
HGT, mean (SD)	125.5 (30.0)	121.8 (25.5)	129.5 (34.4)	-	0.401
NIHSS at admission, median (IQR)	13.0 (7-18)	11.0 (4-13.75)	15.50 (9-20)	-	**0.015**
NIHSS at discharge, median (IQR)	3 (1-13)	6.0 (2-28.50)	2 (0-13)	-	0.072
Onset-to-IVT time (min), median (IQR)	112.0 (50.50-139.0)	0 (0-93.50)	1211.50 (62.0-149.50)		**0.021**
Onset-to-MT (min), median (IQR)	166.0 (136.0-274.0)	220.0 (1130.0-390.0)	156.0 (1137.0-248.25)	-	0.271
Onset-to-blood sample (min), median (IQR)	1440.0 (870.0-2280.0)	1665 (346.5-2760.0)	1440 (990-1800)	-	0.779
Onset-to-blood sample (hours), median (IQR)	25.0 (22.75-41.50)	39.0 (27.50-66.50)	25 (22.50-33.0)	-	**0.032**
Recanalization treatment (%)	49/65 (75.4)	18/33 (54.5)	31/32 (96.9)	-	**<0.001**
- IVT alone	9/65 (13.8)	0	9/32 (28.1)		**<0.001**
- MT alone	32/65 (49.2)	16/33 (48.5)	16/32 (50.0)		0.903
- IVT+MT	14/65 (21.5)	2/33 (6.1)	12/32 (37.5)		**0.002**
MT procedure (%)				-	0.482
- Thromboaspiration	19/43 (44.2)	8/18 (44.4)	11/25 (44.0)		
- Stent retrieving	1/43 (2.3)	1/18 (5.6)	0		
- Thromboaspiration + stent retrieving	23/43 (53.5)	9/18 (50.0)	14/25 (56.0)		
Occluded vessel (%)				-	0.319
- M1	23/50 (46.0)	9/21 (42.0)	14/29 (48.3)		
- M2	11/50 (22.0)	5/21 (23.8)	6/29 (20.7)		
- ICA	2/50 (4.0)	0	2/29 (4.0)		
- Tandem (ACI+M1)	7/50 (14.0)	3/21 (14.3)	7/29 (14.0)		
- Basilar	2/50 (4.0)	0	2/29 (4.0)		
- PCA	1/50 (2.0)	0	1/29 (2.0)		
- Vertebral	1/50 (2.0)	1/21 (4.8)	1/29 (2.0)		
- M1-M2	2/50 (4.0)	2/21 (9.5)	2/29 (4.0)		
- M2-M3	1/50 (2.0)	1/21 (4.8)	1/29 (2.0)		
Extracranial ICA stenosis <50% (%)	5/61 (8.2)	3/29 (10.3)	2732 (6.3)	-	0.662
Extracranial ICA stenosis 50-70% (%)	1/61 (1.6)	0	1/32 (3.1)	-	1.0
Extracranial ICA stenosis >70% (%)	3/61 (4.9)	1/29 (3.4)	2/32 (6.3)	-	1.0
Collateral circulation status (%)				-	0.342
- good	9/39 (23.1)	5/16 (31.3)	4/23 (17.4)		
- moderate	18/39 (46.2)	8/16 (50.0)	10/23 (43.5)		
- poor	12/39 (30.8)	3/16 (18.8)	9/23 (39.1)		

SBP, systolic blood pressure, DBP, diastolic blood pressure; BT body temperature; HGT hemo gluco test; NIHSS, National Institutes of Health Stroke Scale; IVT, intravenous Thrombolysis; MT mechanical thrombectomy; PCA posterior cerebral artery; ICA Internal Carotid Artery.

Supplementary Table 2. Stroke radiological characteristics

	All patients (N=85)	**Stroke with Cancer (n=33)**	**Stroke without Cancer (n=32)**	**Cancer without Stroke (n=20)**	**P value**
CT-Angiography (%)	40/53 (75.5)	20/28 (71.4)	20/25 (80.0)	-	0.469
CTP (%)	38/53 (71.7)	18/28 (64.3)	20/25 (80.0)	-	0.205
MRI (%)	38/53 (71.7)	21/28 (75.0)	17/25 (68.0)	-	0.572
MRP (%)	28/53 (52.8)	14/28 (50.0)	14/25 (56.0)	-	0.662
Lesion volume (cm3), median (IQR)	5.80 (0.50-23.40)	10.90 (1.73-28.15)	3.50 (0.30-16.70)	-	0.091
Vessel hyperdensity at baseline (%)	20/36 (55.6)	7/18 (38.9)	13/18 (72.2)	-	**0.044**
Clot length (mm), mean (SD)	15.4 (7.7)	13.4 (6.75-18.40)	17.4 (7.3)	-	0.136
Basal thrombus (HU),				-	
-median (IQR)	40.0 (31.0-49.0)	31.0 (24.50-38.0)	47.0 (41-55.50)		**<0.001**
-mean (SD)	45.7 (29.4)	32.2 (9.9)	58.6 (35.7)		**0.006**
Thrombus (after contrast medium) (HU),				-	
-median (IQR)	60.0 (53.0-96.0)	61.0 (51.0-124.0)	57.0 (52.75-74.25)		0.372
-mean (SD)	84.3 (66.2)	85.7 (46.1)	83.1 (82.3)		0.910
Thrombus HU (proximal),				-	
-median (IQR)	244.0 (200.0-282.0)	244.0 (179.0-278.0)	243.50 (201.50-290.50)		0.428
-mean (SD)	237.4 (57.5)	227.5 (58.7)	246.72 (56.34)		0.329
Thrombus HU (medium),				-	
-median (IQR)	60.0 (46.0-90.0)	61.0 (44.50-84.0)	59.50 (45.50-93.50)		0.947
-mean (SD)	79.7 (58.9)	78.6 (53.6)	80.7 (65.06)		0.919
Thrombus HU (distal),				-	
-median (IQR)	91.5 (611.25-187.5)	127.0 (72.25-216.0)	85.0 (57.0-173.50)		0.408
-mean (SD)	122.71 (76.45)	136.25 (85.17)	110.67 (67.96)		0.338

Supplementary Table 3. Stroke outcomes

	All patients (N=85)	**Stroke with Cancer (n=33)**	**Stroke without Cancer (n=32)**	**Cancer without Stroke (n=20)**	**P value**
TICI (%)				-	0.356
- 0	2/44 (4.5)	2/19 (10.5)	0		
- 1	0	0	0		
- 2a	2/44 (4.5)	1/19 (5.3)	1/25 (4.0)		
- 2b	8/44 (18.2)	2/19 (10.5)	6/25 (24.0)		
- 2c	5/44 (11.4)	3/19 (15.8)	2/25 (8.0)		
- 3	27/44 (61.4)	11/19 (57.9)	16/25 (64.0)		
Complications during hospital stay (%)	12/57 (21.1)	8/26 (30.8)	4/31 (12.9)	-	0.099
Hemorrhagic transformation (%)	14/62 (22.6)	6/30 (20.0)	8/32 (25.0)	-	0.638
- PH1	5/7 (71.4)	3/3 (100.0)	2/4 (50.0)		0.429
- PH2	2/7 (28.6)	0	2/4 (50.0)		
Remote ICH (%)	3/63 (4.8)	2/31 (6.5)	1/32 (3.1)	-	0.613
SICH (%)	2/62 (3.2)	1/30 (3.3)	1/32 (3.1)	-	1.0
Intra-hospital death (%)	11/61 (18.0)	9/32 (28.1)	2/29 (6.9)	-	**0.031**
mRS at 3 months (%)				-	0.074
- 0	11/59 (18.6)	3/30 (10.0)	8/29 (27.6)		
- 1	8/59 (13.6)	2/30 (6.7)	6/29 (20.7)		
- 2	3/59 (5.1)	3/30 (10.0)	0		
- 3	4/59 (6.8)	1/30 (3.3)	3/29 (10.3)		
- 4	4/59 (6.8)	2/30 (6.7)	2/29 (6.9)		
- 5	4/59 (6.8)	2/30 (6.7)	2/29 (6.9)		
- 6	25/59 (42.4)	17/30 (56.7)	8/29 (27.6)		
Death at 3 months (%)	25/59 (42,4)	17/30 (56.7)	8/29 (27.6)	-	**0.024**
Lesion volume (cm3), median (IQR)	5.80 (0.50-23.40)	10.90 (1.73-28.15)	3.50 (0.30-16.70)	-	0.091

Supplementary Table 4. Cancer characteristics

	All patients (N=85)	Stroke with Cancer (n=33)	Stroke without Cancer (n=32)	Cancer without Stroke (n=20)	P value
Primary tumor (%)			-		0.154
- lung	19/53 (35.8)	10/33 (30.3)		9/20 (45.0)	
- colon	3/53 (5.7)	3/33 (9.1)		0	
- rectal	4/53 (7.5)	4/33 (12.1)		0	
- stomach	4/53 (7.5)	3/33 (9.1)		1(20 (5.0)	
- pancreas	3/53 (5.7)	3/33 (9.1)		0	
- breast	10/53 (18.9)	4/33 (12.1)		6/20 (30.0)	
- bladder	5/53 (9.4)	4/33 (12.1)		1/20 (5.0)	
- ovary	1/53 (1.9)	1/33 (3.0)		0	
- prostate	3/53 (5.7)	1/33 (3.0)		2/20 (10.0)	
- gallbladder	1/53 (1.9)	0		1/20 (5.0)	
Remote metastasis (%)	37/53 (69.8)	21/33 (53.6)	-	16/20 (80.0)	0.208
Metastasis (%)			-	-	-
- absent	12/33 (36.4)	12/33 (36.4)			
- single	12/33 (36.4)	12/33 (36.4)			
- multiple	9/33 (27.3)	9/33 (27.3)			
Metastasis location			-		0.247
- bone	7/21 (33.3)	4/15 (26.7)		3/6 (50.0)	
- cerebral	1/21 (4.8)	1/15 (6.7)		0	
- lymphnodes	2/21 (9.5)	2/15 (13.3)		0	
- liver	3/21 (14.3)	3/15 (20.0)		0	
- peritoneum	1/21 (4.8)	1/15 (6.7)		0	
- lung	2/21 (9.5)	2/15 (13.3)		0	
Ongoing treatment (%)	32/53 (60.4)	20/33 (60.6)	-	12/20 (60.0)	0.965
- Chemotherapy	17/33 (51.5)	11/21 (52.4)		6/12 (50.0)	0.096
- Radiotherapy	3/33 (9.1)	3/21 (14.3)		0	
- Immunotherapy	4/33 (12.1)	4/21 (19.0)		0	
- Chemoth+Radioth	2/33 (6.1)	1/21 (4.8)		1/12 (8.3)	
- Other combinations	7/33 (21.2)	2/21 (9.5)		5/12 (41.7)	
Previous treatments (%)	26/51 (51.0)	15/31 (48.4)	-	11/20 (55.0)	0.645
- Chemotherapy	8/30 (26.7)	4/17 (23.5)		4/13 (30.8)	0.684
- Radiotherapy	6/30 (20.0)	3/17 (17.6)		3/13 (23.1)	
- Chemoth+Radioth	8/30 (26.7)	6/17 (35.3)		2/13 (15.4)	
- Other combinations	8/30 (26.7)	4/17 (23.5)		4/13 (30.8)	
Surgery (%)	25/52 (48.1)	15/33 (45.5)	-	10/19 (52.6)	0.618
Cerebral metastasis (%)	2/53 (3.8)	1/33 (3.0)	-	1/20 (5.0)	1.0
Disease duration (months), median (IQR)	17.0 (2.25-41.25)	15.0 (2-31)	-	36.0 (5.0-66.0))	0.272
Treatment start-to-enrollment time (months), median (IQR)	15.0 (1.50-29.0)	17.0 (2-30.0)	-	4.0 (0.75-21.25)	0.275
Treatment end-to-enrollment time (months), median (IQR)	7.50 (0.13-28.50)	6.50 (0.38-45.0)	-	7.5 (0-13.25)	0.512
Surgery-to-enrollment time (months), median (IQR)	24.0 (6.38-48.75)	20.50 (4.13-46.0)	-	30.0 (13.75-68.25)	0.349

Stroke-cerebral metastasis (months), mean (SD)	0.28 (1.49)	0.29 (1.51)	-	-	-
TICI (%) **- 0** **- 1** **- 2a** **- 2b** **- 2c** **- 3**	 2/44 (4.5) 0 2/44 (4.5) 8/44 (18.2) 5/44 (11.4) 27/44 (61.4)	 2/19 (10.5) 0 1/19 (5.3) 2/19 (10.5) 3/19 (15.8) 11/19 (57.9)	 0 0 1/25 (4.0) 6/25 (24.0) 2/25 (8.0) 16/25 (64.0)	-	0.356

Supplementary Table 5. Demographic and clinical characteristics of AIS and AIS-Cancer patients recruited at the Istituti Ospedalieri of Cremona

	All patients (N=14)	**Stroke with cancer (n=5)**	**Stroke without cancer (n=9)**	**P value**
Age, mean (SD)	82.6 (6.9)	80 (5.0)	84.11 (7.6)	0.305
Sex (female), %	6 (42.9)	1 (20.0)	5 (55.6)	0.301
Pre-stroke mRS (%)				0.543
- 0	9 (64.3)	4 (80.0)	5 (55.6)	
- 1	2 (14.3)	0	2 (22.2)	
- 2	1 (7.1)	0	1 (11.1)	
- 3	2 (14.3)	1 (20.0)	1 (11.1)	
- 4	0	0	0	
- 5	0	0	0	
Smoking (%)				1.0
- No	9 (64.3)	3 (60.0)	6 (66.7)	
- Yes	5 (35.7)	2 (40.0)	3 (33.3)	
- Past	0	0	0	
Alcohol use (%)	0			
Obesity (%)	1 (7.1)	1 (20.0)	0	0.357
HT (%)	12 (85.7)	4 (80.0)	8 (88.9)	1.0
DM (%)	3 (21.4)	1 (20.0)	2 (22.2)	1.0
AF (%)	4 (28.6)	1 (20.0)	3 (33.3)	1.0
Dyslipidemia (%)	5 (35.7)	2 (40.0)	3 (33.3)	1.0
HF (%)	1 (7.1)	1 (20.0)	0	0.357
CAD (%)	1 (7.1)	1 (20.0)	0	0.357
AMI (%)	0			
Peripheral artery disease (%)	0			
Previous DVT (%)	0			
Previous stroke/TIA/other (%)	4 (28.6)	2 (40.0)	2 (22.2)	0.580
- stroke	4 (28.6)	2 (40.0)	2 (22.2)	
- TIA	0	0	0	
Chronic inflammatory disease (%)	0			
Autoimmune disease (%)	0			
Thrombocitopenia (%)	0	0		
Thrombocytosis (%)	1 (7.1)	1 (11.1)	0	1.0
Other hematological diseaases (%)	2 (14.3)	2 (40.0)	0	0.110
Coagulopathy (%)	0			
Anticoagulant therapy (%)	1 (7.1)	0	1 (11.1)	1.0
- Apixaban	0		0	
- Edoxaban	1 (7.1)		1 (11.1)	
- Acenocoumarol	0		0	
- Warfarin	0		0	
- Enoxaparin sodium (anticoagulant doses)	0		0	
	0		0	
- Enoxaparin sodium (prophylactic doses)	0		0	
	0		0	
Antiplatelet therapy (%)	4 (28.6)	1 (20.0)	3 (33.3)	1.0
- Aspirin (ASA)	2 (14.3)	0	2 (22.2)	
- Clopidogrel	2 (14.3)	1 (20.0)	1 (11.1)	
- ASA+Clopidogrel	0	0	0	
Lipid lowering therapy (%)	1/13 (7.7)	1 (20.0)	0	0.385
Glucose lowering therapy (%)	1/12 (8.3)	0	1/8 (12.5)	11.0
Insulin (%)	1/13 (7.7)	0	1 (11.1)	1.0

Antihypertensive therapy (%)	10/13 (76.9)	3/4 (75.0)	7 (77.8)	1.0
- ACE inhibitors (%)	2/12 (16.7)	2/4 (50.0)	0	0.091
- Sartans (%)	3/12 (25.0)	0	3/8 (37.5)	0.491
- Beta-blockers (%)	3/12 (25.0)	1/4 (25.0)	2/8 (25.0)	1.0
- Calcium channel-blockers (%)	4/12 (33.3)	1/4 (25.0)	3/8 (37.5)	1.0
- Diuretics (%)	3/13 (23.1)	1/4 (25.0)	2 (22.2)	1.0
Non steroid antinflammatory drugs (%)	0			
Corticosteroids (%)	0			

AMI, acute myocardial infarct; ASA, acetylsalicylic acid; AF, atrial fibrillation; BMI, body mass index; CAD, coronary artery disease; DM, diabetes mellitus; DVT, deep venous thrombosis; HF, heart failure; HT, hypertension; mRS, modified Rankin Scale; PPI, proton pump inhibitors; TIA, transient ischemic attack.

Supplementary Table 6. Stroke clinical characteristics of AIS and AIS-Cancer patients recruited at the Istituti Ospedalieri of Cremona

	All patients (N=14)	**Stroke with cancer (n=5)**	**Stroke without cancer (n=9)**	**P value**
Onset on awakening (%)	2 (14.3)	0	2 (22.2)	0.505
Unknown onset (%)	2 (14.3)	0	2 (22.2)	0.505
HB at admission, mean (SD)	75.5 (18.2)	90.5 (11.2)	68.0 (16.6)	0.036
SBP, mean (SD)	151.2 (20.5)	149.5 (19.4)	152.0 (22.1)	0.849
DBP, mean (SD)	77.8 (12.6)	83.0 (16.8)	75.4 (10.6)	0.340
BT, mean (SD)	36.2 (0.5)	36,3 (0.3)	36.3 (0.6)	1.0
HGT, mean (SD)	128.9 (31.7)	126,3 (31.0)	130.1 (33.8)	0.849
NIHSS at admission, median (IQR)	18 (13-21)	13 (12.25-19)	19 (115.50-22.0)	0.162
NIHSS at discharge, median (IQR)	7 (1.25-18.0)	6 (0-….)	14 (1.50-18.0)	0.350
Onset-to-IVT time (min), median (IQR)	83.0 (0-120.50)	32.5 (0-107.75)	110.50 (101.0-…) (n=2)	0.348
Onset-to-MT (min), median (IQR)	171.0 (153.0-270.0)	220.0 (140-555)	165 (151.50-268.50)	0.465
Onset-to-blood sample (min), median (IQR)	73.0 (49.0-134.0)	73.0 (34-152.5)	81.50 (56.50-193.0)	0.465
Onset-to-blood sample (hours), median (IQR)	1 (1-3)	1 (n=1)	1.0 (1.0-4.0)	0.488
Recanalizationn treatment (%) **- IVT alone** **- MT alone** **- IVT+MT**	 0 9 (64.3) 5 (35.7)	 0 3 (60.0) 2 (40.0)	 0 6 (66.7) 3 (33.3)	 - 1.0 1.0
Occluded vessel (%) **- M1** **- ACI+M1** **- Tandem (ACI+M1)** **- Vertebral+Basilar**	 7 (50.0) 3 (21.4) 3 (21.4) 1 (7.1)	 3 (50.0) 1 (20.0) 1 (20.0) 0	 4 (44.4) 2 (22.2) 2 (22.2) 1 (11.1)	0.867
Extracranial Carotid stenosis (%) **- <50%** **- 50%-70%** **- >70%**	 6 (42.8) 2 (14.3) 3 (21.4)	 3 (60.0) 0 1 (20.0)	 3 (33.3) 2 (22.2) 2 (22.2)	 0.580 0.505 1.0

Supplementary Table 7. Stroke outcomes of AIS and AIS-Cancer patients recruited at the Istituti Ospedalieri of Cremona

	All patients (N=14)	**Stroke with cancer (n=5)**	**Stroke without cancer (n=9)**	**P value**
TICI (%)				0.713
- 0	0	0	0	
- 1	0	0	0	
- 2a	1 (7.1)	0	1 (11.1)	
- 2b	1 (7.1)	0	1 (11.1)	
- 2c	2 (14.3)	1 (20.0)	1 (11.1)	
- 3	10 (71.4)	4 (80.0)	6 (66.7)	
TICI 2b-3 (%)	13 (92.9)	5 (100.0)	8 (88.9)	1.0
Complications during hospital stay (%)	1 (7.1)	1 (20.0)	0	0.357
Hemorrhagic transformation (%)	6 (42.9)	2 (40.0)	4 (44.4)	1.0
- H1	2 (33.3)	0	2 (50.0)	
- H2	1 (16.7)	0	1 (25.0)	
- PH1	3 (50.0)	2 (100.0)	1 (25.0)	
- PH2	0	0	0	
Remote ICH (%)	0			
SICH (%)	0			
Intra-hospital death (%)	2 (14.3)	2 (40.0)	0	0.110
mRS at 3 months (%)				0.382
- 0	3 (21.4)	1 (20.0)	2 (22.2)	
- 1	2 (14.3)	0	2 (22.2)	
- 2	0	0	0	
- 3	1 (7.1)	1 (20.0)	0	
- 4	3 (21.4)	1 (20.0)	2 (22.2)	
- 5	2 (14.3)	0	2 (22.2)	
- 6	3 (21.4)	2 (40.0)	1 (11.1)	
mRS 0-2 at 3 months (%)	5 (35.7)	11 (20.0)	4 (44.4)	0.580
Death at 3 months (%)	3 (21.4)	2 (40.0)	1 (40.0)	0.505
Lesion volume (cm3), median (IQR)	36.20 (36.0-36.60)	17,60 (6.10-192.20)	22.30 (6.50-1106.55	1.0

Supplementary Table 8. Cancer characteristics of patients recruited at the Istituti Ospedalieri of Cremona

	All patients (N=14)	Stroke with cancer (n=5)	Stroke without cancer (n=9)	P value
Primary tumor (%)				0.050
- lung	1 (7.1)	1 (20.0)	0	
- colon	1 (7.1)	1 (20.0)	0	
- rectal	0	0	0	
- stomach	1 (7.1)	1 (20.0)	0	
- pancreas	0	0	0	
- breast	3 (21.4)	1 (20.0)	2 (22.2)	
- genito-urinary	1 (7.1)	1 (20.0)	0	
- bladder	0	0	0	
- ovary	0	0	0	
- prostate	0	0	0	
- gallbladder	0	0	0	
Remote metastasis (%)	1/7 (14.3)	1/5 (20.0)	0	1.0
Metastasis location				0.357
- bone	0	0	0	
- cerebral	0	0	0	
- lymphnodes	0	0	0	
- liver	1 (7.1)	1 (20.0)	0	
- peritoneum	0	0	0	
- lung	0	0	0	
Ongoing treatment (%)	2/7 (28.6)	2/5 (40.0)	0	1.0
- Chemotherapy	1/2 (50.0)	1/2 (50.0)		
- Hormone therapy	1/2 (50.0)	1/5 (50.0)		
- Radiotherapy	0	0		
- Immunotherapy	0	0		
- Chemoth+Radioth	0	0		
- Other combinations	0	0		
Previous treatments (%)	3/7 (42.9)	1/5 (20.0)	2/2 (100.0)	0.143
- Chemotherapy	1/3 (33.3)	1/1 (100.0)	0	
- Radiotherapy	1/3 (33.3)	0	1/2 (50.0)	
- Radiotherapy + Hormone therapy	1/3 (33.3)	0	1/2 (50.0)	
- Chemoth+Radioth	0	0	0	
- Other combinations	0	0	0	
Surgery (%)	6/7 (85.7)	4/5 (80.0)	2/2 (100.0)	1.0
Cerebral metastasis (%)	0			
Disease duration (months), median (IQR)	9 (1-80.75)	9.0 (1.0-80.75)	-	
Treatment start-to-enrollment time (months), median (IQR)	17.50 (0-…) (n=2)	17.50 (n=2)	-	
Treatment end-to-enrollment time (months), median (IQR)	60.0 (n=1)	60.0 (n=1)	-	
Surgery-to-enrollment time (months), median (IQR)	102.0 (9-210) (n=5)	14.0 (n=3)	210.0 (1168.0-…) (n=2)	0.083
Stroke-cerebral metastasis (months), mean (SD)	-	-	-	

Supplementary Table 9. Routine blood examination of patients recruited at the Istituti Ospedalieri of Cremona

	All patients (N=14)	**Stroke with cancer (n=5)**	**Stroke without cancer (n=9)**	**P value**
Red Blood Cells	3.94 (0.36)	3.97 (0.35)	3.92 (0.38)	0.828
Hemoglobin	11.2 (1.6)	11.0 (1.3)	11.38 (1.83)	0.691
White Blood cells	10.69 (2.24)	11.49 (2.38)	10.24 (2.17)	0.340
Lymphocytes (absolute number)	1.29 (0.53)	1.08 (0.34)	1.41 (0.60)	0.287
Lymphocytes (%)	12.18 (4.48)	9.32 (1.63)	13.77 (4.83)	0.073
Neutrophils (absolute number)	8.56 (1.96)	9.62 (1.71)	7.97 (1.92)	0.136
Neutrophils (%)	80.0 (6.81)	84.2 (2.8)	77.66 (7.34)	0.083
Platelets	167 (68.1)	296.8 (104.2)	250.4 (35.1)	0.236
INR	1.09 (0.09)	1.13 (0.06)	1.06 (0.10)	0.165
aPTT	0.95 (0.11)	0.97 (0.10)	0.93 (0.12)	0.551
D-dimer	5.74 (6.78)	8.93 (9.82)	2.55 (0.81)	0.457
Fibrinogen	344.17 (124.19)	479.5 (125.16)	276.50 (46.55)	0.035
ESV	32.4 (22.3)	42.0 (18.9)	26.0 (23.6)	0.293
CRP	14.51 (30.16)	35.2 (45.7)	3.04 (4.29)	0.052
Procalcitonin	0.25 (0.53)	0.49 (0.84)	0.08 (0.04)	0.257
LDH	243 (143.2)	327.4 (226.1)	196.1 (29.9)	0.101
Glucose levels	139.9 (32.5)	125.6 (44.2)	147.9 (23.3)	0.233
Total cholesterol	170.3 (36.4)	139.3 (8.1)	181.9 (36.2)	0.082
LDL cholesterol	121.5 (36.1)	105.4 (20.5)	131.5 (41.2)	0.219
HDL cholesterol	41.3 (10.31)	34.7 (9.1)	43.8 (10.1)	0.204
Triglycerides	109.8 (40.8)	87.2 (23.9)	123.9 (44.1)	0.118

Values are expressed as mean (SD)

Supplemetary Table 10. Routine laboratory data at T0

	AIS/cancer n=33	AIS n=32	Cancer n=20	p-value
RBC (10^6/µL)	4.15 (0.79)	4.24 (0.53)	3.93 (0.57)	0.248
Hb (g/dL)	11.5 (2.0)	12.6 (1.9)	11.8 (1.9)	**0.045**
WBC (10^9/L)	9.98 (2.97)	9.18 (2.49)	10.50(7.13)	0.393
Lymphocytes(10^9/L)	1.48 (1.49)	1.92 (0.99)	1.09 (0.50)	**<0.001**
Lymphocytes (%)	13.75 (10.65)	21.78 (10.74)	12.93(8.28)	**<0.001**
Neutrophils (10^9/L)	8.06 (2.98)	6.42 (2.31)	8.45 (6.78)	**0.033**
Neutrophils (%)	77.7 (12.6)	69.0 (12.2)	77.8 (12.0)	**0.001**
Platelets (10^9/L)	239 (134)	257 (74)	255 (153)	0.560
NLR	7.59 (4.27)	4.35 (3.20)	9.28 (7.39)	**<0.001**
PLR	215.83 (137.69)	164.98 (84.77)	285.09(210.52)	0.055
INR	1.14 (0.22)	1.09 (0.15)	1.15 (0.38)	0.577
aPTT	1.93 (5.48)	0.89 (0.14)	0.96 (0.26)	0.606
D-dimer (µg/L)	2104.43 (1627.20)	1557.59	2110.40 (1764.77)	0.772
Fibrinogen (mg/dL)	448 (117)	390 (106)	770 (981)	**<0.001**
CRP (mg/L)	11.97 (23.14)	2.06 (3.19)	7.02 (6.52)	**0.001**
Procalcitonin (ng/mL)	0.38 (0.62)	0.07 (0.03)	0.04 (–)	0.258
Albumin (g/L)	7.7 (12.3)	4.1 (0.4)	3.4 (0.4)	0.114
LDH (U/L)	394 (234)	229 (72)	307 (148)	0.038

Myoglobin (µg/L)	42 (–)	47 (38)	83 (61)	0.931
Glucose (µg/dL)	124 (29)	130 (32)	120 (24)	0.492

Values are expressed as mean (SD)

Supplementary Table 11. Routine laboratory data at T1

	AIS/Cancer n=33	AIS n=32	Cancer n=20	p-value
RBC (10^6/µL)	4.29 (1.71)	4.15 (0.58)	3.84 (0.75)	0.410
Hb (g/dL)	10.96 (1.72)	12.36 (1.77)	11.42 (1.99)	**0.025**
WBC (10^9/L)	8.99 (3.23)	9.36 (3.22)	10.70 (6.71)	0.938
Lymphocytes(10^9/L)	1.19 (0.65)	1.55 (0.70)	1.08 (0.46)	**0.018**
Lymphocytes (%)	17.02 (16.15)	17.68 (7.98)	12.75 (7.35)	**0.048**
Neutrophils (10^9/L)	7.00 (3.02)	6.93 (3.14)	8.80 (6.47)	0.841
Neutrophils (%)	72.58 (17.82)	73.38 (9.50)	78.24 (10.33)	0.177
Platelets (10^9/L)	209.22 (136.57)	262.17 (116.63)	241.70 (139.87)	0.197
NLR	7.56 (4.61)	5.33 (3.48)	9.34 (6.91)	**0.026**
PLR	208.50 (138.31)	208.32 (166.01)	250.37 (196.69)	0.493
INR	1.11 (0.12)	1.16 (0.23)	1.22 (0.27)	0.674
aPTT	0.96 (0.15)	1.00 (0.25)	0.97 (0.20)	0.990
ATIII (%)	92.12 (13.51)	84.98 (15.01)	93.00 (24.04)	0.763
D-dimer (µg/L)	3642 (1464)	2657 (1271)	2033 (1676)	0.183
Fibrinogen (mg/dL)	441.75 (121.96)	341.88 (109.51)	484.18 (78.95)	**0.035**
CRP (mg/L)	8.29 (6.88)	2.88 (3.79)	7.39 (6.59)	**<0.001**
Procalcitonin(ng/mL)	0.08 (0.07)	0.05 (0.03)	0.12 (–)	0.382
Albumin (g/L)	6.66 (11.02)	7.75 (11.83)	4.76 (6.10)	0.170

LDH (U/L)	391 (249)	230 (90)	271 (244)	0.100
Myoglobin (µg/L)	21 (–)	102 (124)	– (–)	0.143
Glucose (mg/dL)	101 (22)	116 (54)	94 (31)	0.210
Cholesterol Tot (mg/dL)	165 (32)	173 (44)	142 (44)	0.313
HDL (mg/dL)	45.9 (15.5)	46.4 (14.8)	50.5 (31.4)	0.696
LDL (mg/dL)	101 (26)	109 (44)	71 (43)	0.124
Triglycerides(mg/dL)	105.29 (39.95)	114.00 (49.44)	107.33 (41.11)	0.777

Values are expressed as mean (SD)

Supplementary Table 12. Laboratory data: biomarkers

	N	AIS/Cancer	N	AIS	N	Cancer	p-value
Factor VIII	20	181.90 (146.55–254.60)	23	190.50 (148.70–223.30)	18	153.60 (139.00–272.30)	0.900
WF:RCO	20	232.00 (192.75–330.90)	23	212.00 (166.20–253.60)	18	287.20 (202.40–316.80)	0.234
vWFAg	20	212.70 (177.85–312.10)	23	202.70 (157.00–249.10)	18	263.15 (187.80–343.00)	0.361
IL-1β con.	24	480.55 (439.48–547.64)	20	506.45 (409.34–581.00)	19	483.07 (442.69–622.65)	0.933
IL-6 conc.	24	350.02 (314.63–437.52)	20	417.27 (297.86–462.05)	19	393.50 (295.51–534.09)	0.436
VEGF conc.	23	27.91 (16.12–91.60)	20	28.46 (16.14–93.86)	17	45.77 (30.59–67.69)	0.659
ICAM-1 conc.	24	152.86 (126.08–209.61)	20	187.87 (133.47–223.40)	19	154.98 (110.26–205.66)	0.406
VCAM-1conc.	24	2514.06(2155.67–3211.94)	20	2583.42 (2211.64–2958.22)	19	2077.68 (1823.43–2763.35)	0.139
C CL4-PF4conc.	24	555.42 (299.52–856.07)	20	305.88 (139.84–597.10)	19	526.91 (336.66–1119.72)	0.076
CD40 conc.	24	21.20 (11.17–45.52)	20	17.54 (10.13–34.23)	19	41.41 (15.46–95.07)	0.102
P-selectin conc.	26	17.78 (14.32–22.44)	19	19.56 (13.22–21.88)	18	15.35 (13.14–17.28)	0.181
E-selectin conc	26	5334.03 (4258.62–7200.34)	19	5773.81 (3206.03–8350.08)	18	5194.61 (3973.13–6787.93)	0.692

Values are expressed as median (IQR)

Supplementary Table 13. Laboratory data: biomarkers. Sensitivity analysis (no pre-stroke anti-thrombotic therapies)

	AIS/Cancer n=17	AIS n=16	Cancer n=16	p-value
Factor VIII	169.4 (141.8–279.7)	187.7 (148.7–201.8)	153.6 (136.28–272.3)	0.748
WF:RCO	230 (151.83–296.9)	212.3 (153.2–253.6)	280.35 (200.03–332.33)	0.332
vWFAg	193.95 (149.5–329.63)	192.3 (152.2–235.4)	255.75 (185.95–327.4)	0.340
Lag Time	4.33 (3.58–6.23)	4 (3.67–5)	6.42 (5–7.743)	**<0.001 1 vs 3 p=0.047; 2 vs 3 p=0.003**
ETP	1267.65 (1117.47–1406)	1181.32 (925.26–1327.61)	602.55 (383.65–951.27)	**<0.001 1 vs 3 p=0.001; 2 vs 3 p=0.004**
Peak	185.02 (172.5–239.49)	219.87 (123.11–303.08)	64.87 (36.22–124.07)	**<0.001 1 vs 3 p=0.004; 2 vs 3 p=0.002**
ttPeak	8 (7–9.65)	7.5 (5.83–9)	10.67 (8.8–11.96)	**<0.001 1 vs 3 p=0.025; 2 vs 3 p=0.004**
Start Tail	26.34 (23.13–30.61)	24.17 (21.67–28.17)	33.08 (27.21–38.83)	**<0.001 1 vs 3 p=0.044; 2 vs 3 p=0.013**
Velocity Index	51.37 (44.74–78.43)	66.52 (32.18–120.81)	16.58 (6.8–35.08)	**0.010 2 vs 3 p=0.021**
IL-1β	472.67 (445.22–528.81)	479.39 (384.2–531.21)	471.93 (392.99–516.03)	0.665
IL-6	333.61 (317.22–435.45)	420.3 (281.13–469.54)	393.5 (295.51–534.09)	0.340
VEGF	25.52 (15.92–101.84)	37.96 (21.08–136.65)	39.9 (29.52–63.48)	0.311
ICAM-1	138.49 (125.08–175.06)	193.12 (172.92–259.02)	150.2 (108.84–167.75)	**0.008 1 vs 2 p=0.051; 2 vs 3 p=0.042**
VCAM-1	2602.85 (2216.64–3349.02)	2783.96 (2211.64–3008.07)	2015.33 (1823.43–2761.53)	**0.042 1 vs 3 p=0.051**
C CL4-PF4	506.34 (285.22–769.46)	152 (90.05–399.25)	593.99 (421.43–1119.72)	0.067
CD40	15.88 (10.3–35.75)	14.85 (8.93–41.72)	41.41 (13.7–128.18)	0.374
P-selectin	17.73 (13.3–23.78)	19.29 (12.63–20.14)	15.36 (12.64–17.59)	0.175

Values are expressed as median (IQR)

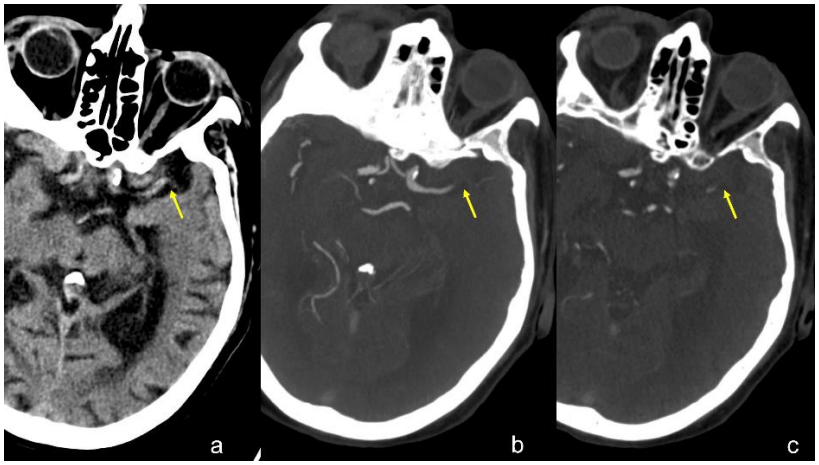
Supplementary Table 14. Platelets (no anti-thrombotic therapies)

	AIS/Cancer n=13	AIS n=14	Cancer n=14	p-value
PTL-Apopt. **-mean (SD)** **-median (IQR)**	4.79 (7.42) 1.8 (0.44–6.94)	1.55 (1.3) 1.02 (0.58–2.46)	3.57 (4.1) 1.45 (0.43–5.85)	0.253
PTL-Nec. **-mean (SD)** **-median (IQR)**	1.58 (3.14) 0.45 (0.2–1.61)	0.52 (1.16) 0.1 (0.05–0.32)	0.48 (0.75) 0.22 (0.1–0.39)	0.297
PTL AV+ **-mean (SD)** **-median (IQR)**	5.07 (3.87) 3.9 (2.54–6.13)	5.17 (2.52) 4.54 (3.24–7.31)	6.69 (5.19) 5.41 (2.67–8.85)	0.531
PTL CD62 **-mean (SD)** **-median (IQR)**	23.71 (13.41) 23.51 (12.68–32.7)	26.29 (9.53) 24.99 (21.12–33.01)	25.27 (16.42) 25.9 (11.2–38.98)	0.895
PLT PAC-1 **-mean (SD)** **-median (IQR)**	0.79 (0.49) 0.65 (0.4–1.3)	0.79 (0.66) 0.49 (0.32–1.18)	0.76 (1.03) 0.38 (0.1–1.05)	0.993
PLT CD62/PAC-1 **-mean (SD)** **-median (IQR)**	2.93 (3.76) 1.4 (0.92–2.72)	2.75 (2.9) 2.1 (1.05–2.93)	4.5 (5.33) 2.76 (1.34–5.1)	0.511
HTF1 **-mean (SD)** **-median (IQR)**	4.72 (1.24) 4.49 (3.81–6.09)	4.33 (1.59) 4.96 (2.55–5.47)	5.95 (3.84) 4.83 (3.68–6.4)	0.332

Supplementary Table 15. Platelet Microvesicles (no anti-thrombotic therapies).

	AIS/Cancer n=13	AIS n=14	Cancer n=14	p-value
MV CD62 **-mean (SD)** **-median (IQR)**	10.63 (10.02) 8.57 (2.65–15.3)	12.55 (8.44) 13.5 (4.28–18.98)	13.35 (16.32) 8.66 (3.59–14.68)	0.867
MV AV **-mean (SD)** **-median (IQR)**	25.71 (15.04) 27.6 (14.58–36.48)	31.23 (18.09) 34.51 (18.67–43.7)	30.2 (19.23) 35.2 (12.3–41.4)	0.744
MV CD62/AV **-mean (SD)** **-median (IQR)**	9.89 (7.64) 8.32 (3.7–15.21)	18.43 (13.84) 17.5 (6.7–29.5)	13.07 (12.66) 6.35 (4.16–18.6)	0.236

A



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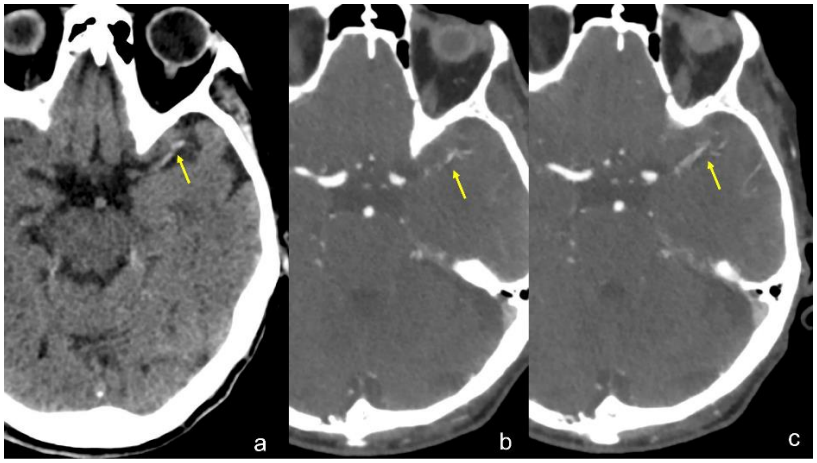
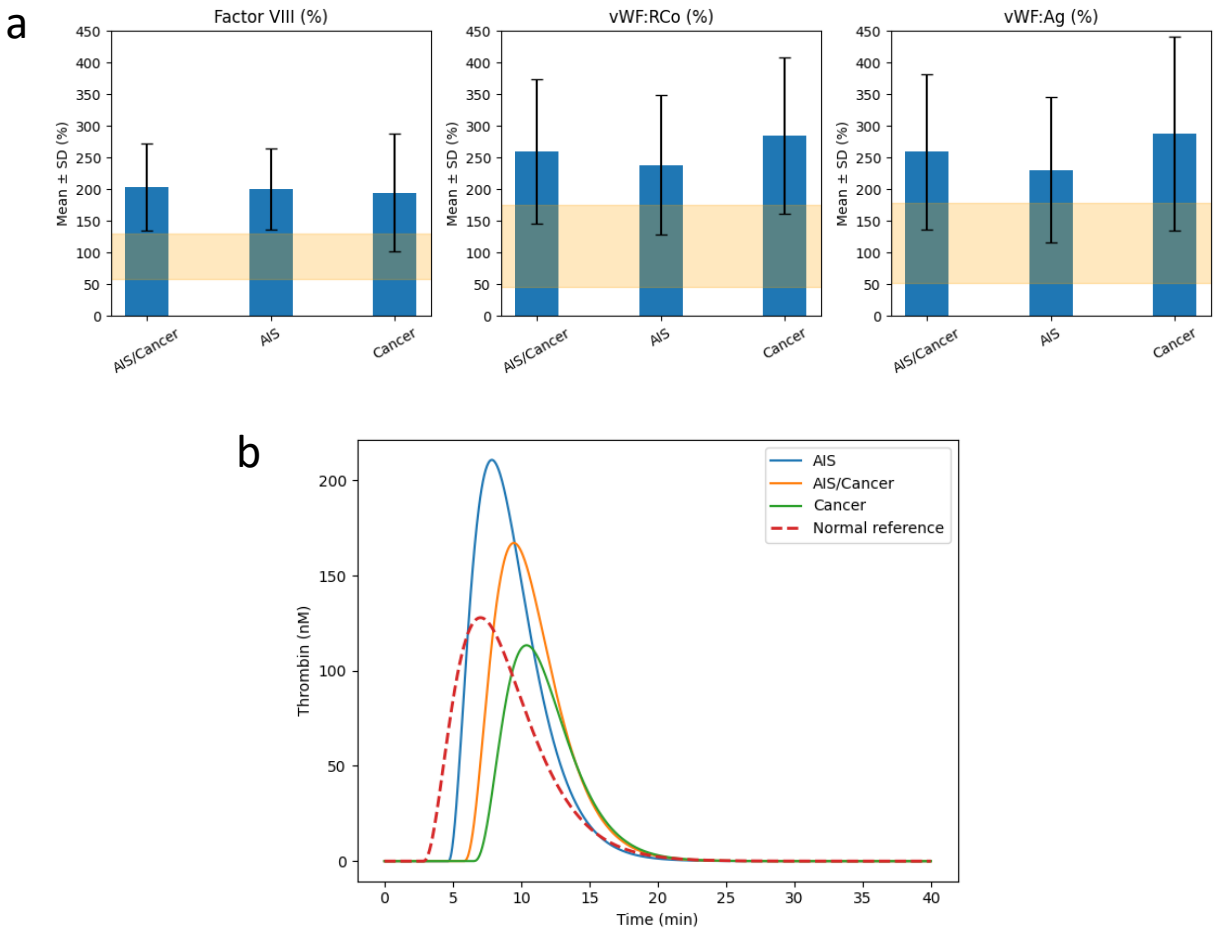
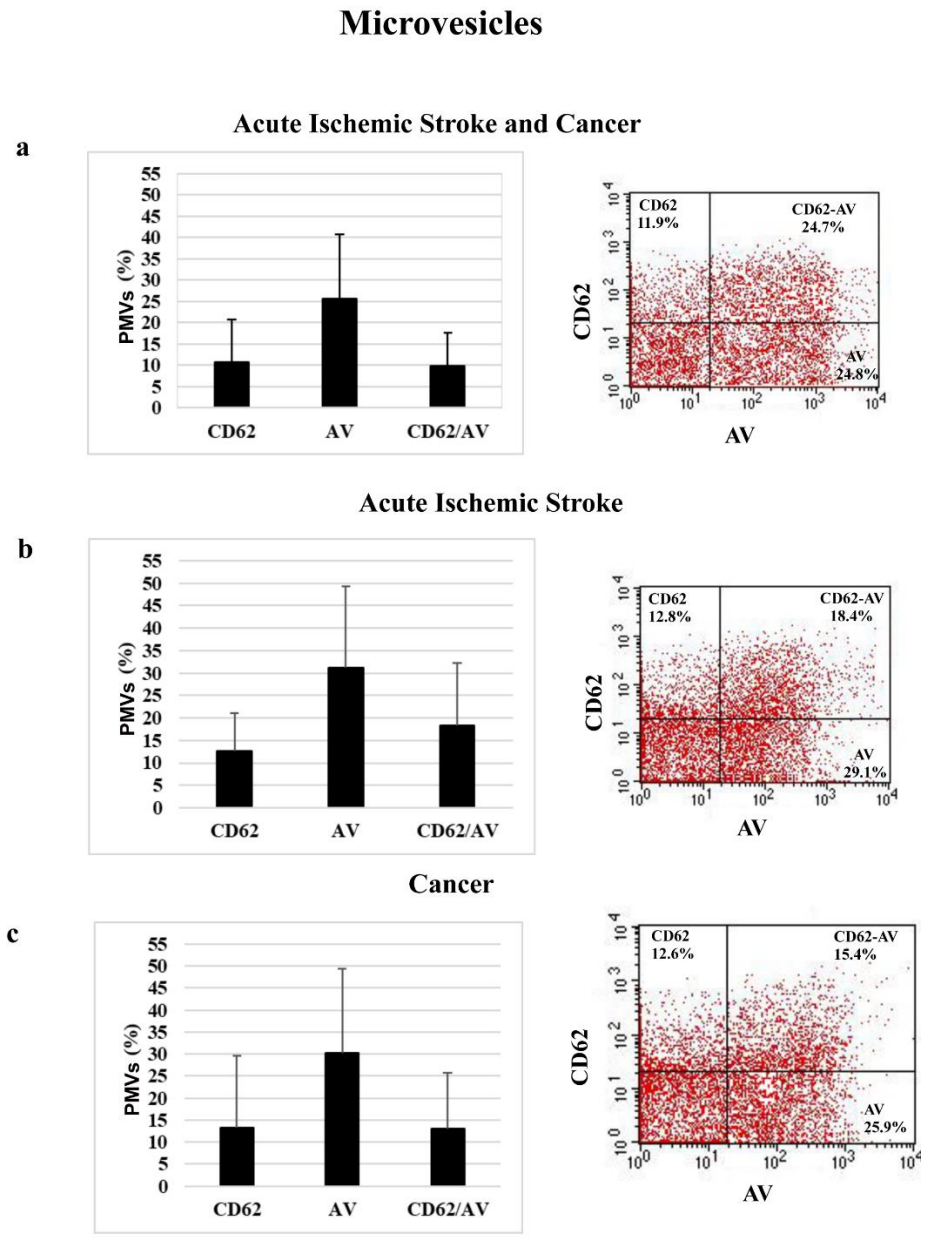


Supplementary Figure 1. Neuroradiological features of arterial clots. A. Representative brain NCCT scan at admission showing occlusion of the left MCA in an AIS patient without cancer (yellow arrow in a). CTA confirmed the vessel occlusion by demonstrating absence of contrast opacification and lack of visible flow within the vessel (yellow arrows in b and c). **B.** Representative NCCT scan of a patient in the AIS-Cancer group showing a hyperdense thrombus sign, consistent with an acute RBC-rich intraluminal clot (yellow arrow in a) in the left MCA. Partial contrast permeability within the thrombus is visible on CTA, suggesting increased thrombus friability and incomplete vessel occlusion (yellow arrows in b and c). NCCT, non-contrast computed tomography; MCA, middle cerebral artery; CTA, computed tomography angiography; RBC, red blood cells.



Supplementary Fig. 2. Endothelial activation and thrombin generation analysis. a. Plasma levels of factor VIII, von Willebrand factor (vWF) and vWF:Ristocetin Cofactor activity (WF:RCo) were comparable across the three groups although above the upper limit of the normal reference range **b.** Reconstructed calibrated automated thrombography (CAT) curves are shown for patients with AIS-Cancer, AIS, and Cancer without stroke. The dashed line indicates the normal reference range derived from established cut-off values. Curves were reconstructed from group mean CAT parameters and scaled to match the reported thrombin peak values. AIS patients exhibit an accelerated and amplified thrombin generation profile, characterized by an earlier and higher peak, whereas Cancer patients display delayed and attenuated thrombin generation with reduced peak height and prolonged kinetics. AIS-Cancer patients show an intermediate thrombin generation phenotype, suggesting combined modulation of coagulation dynamics by acute cerebrovascular ischemia and malignancy. Curves are shown for visualization purposes and do not represent individual CAT tracings.

vWF, von Willebrand factor; vWF:RCo, vWF:Ristocetin Cofactor

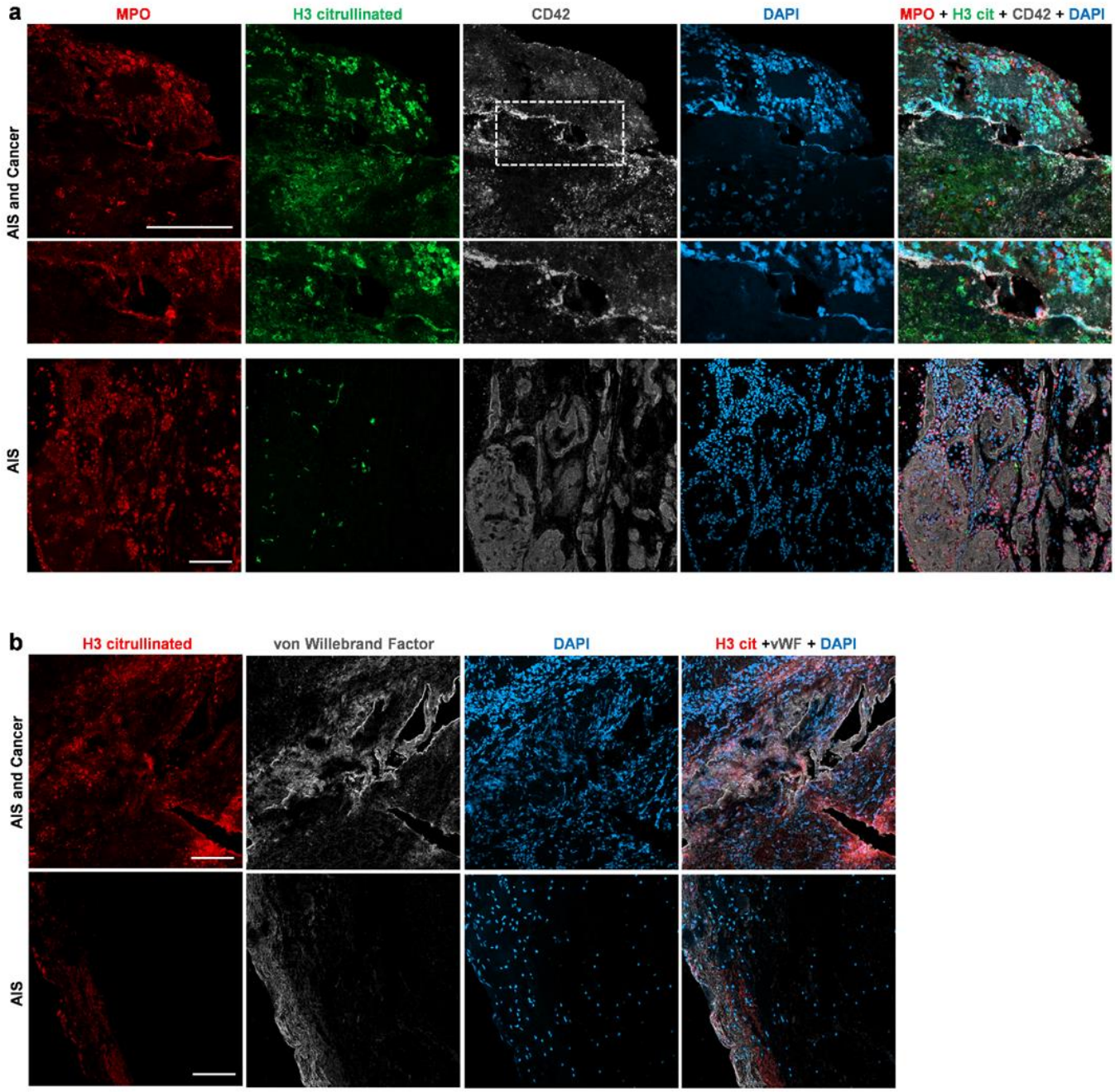


Supplementary Figure 3. Phenotypic characterization of circulating PMVs by flow cytometry.
PMVs were analyzed for CD62 expression and AV binding.

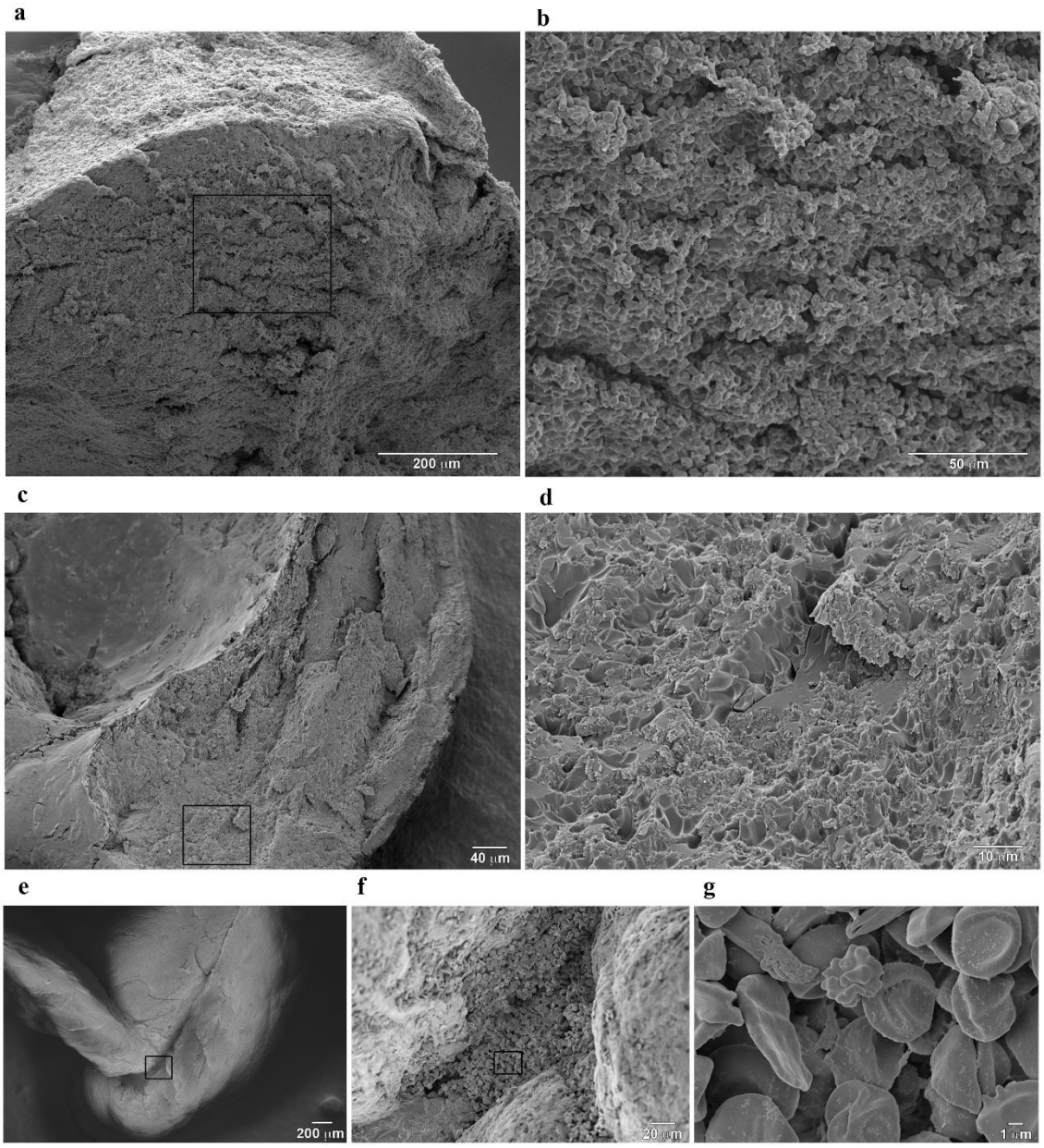
Left panels show histograms indicating the percentage of $CD62^+$, $CD62^+/AV^+$, and AV^+ PMVs in patients with AIS-Cancer (a), AIS (b) and Cancer (c). Values represent mean ± SD from 17 patients with AIS-Cancer, 16 with AIS and 16 with Cancer.

Right panels show representative dot plots illustrating the distribution of CD62 and AV expression on PMVs from one representative patient in each group.

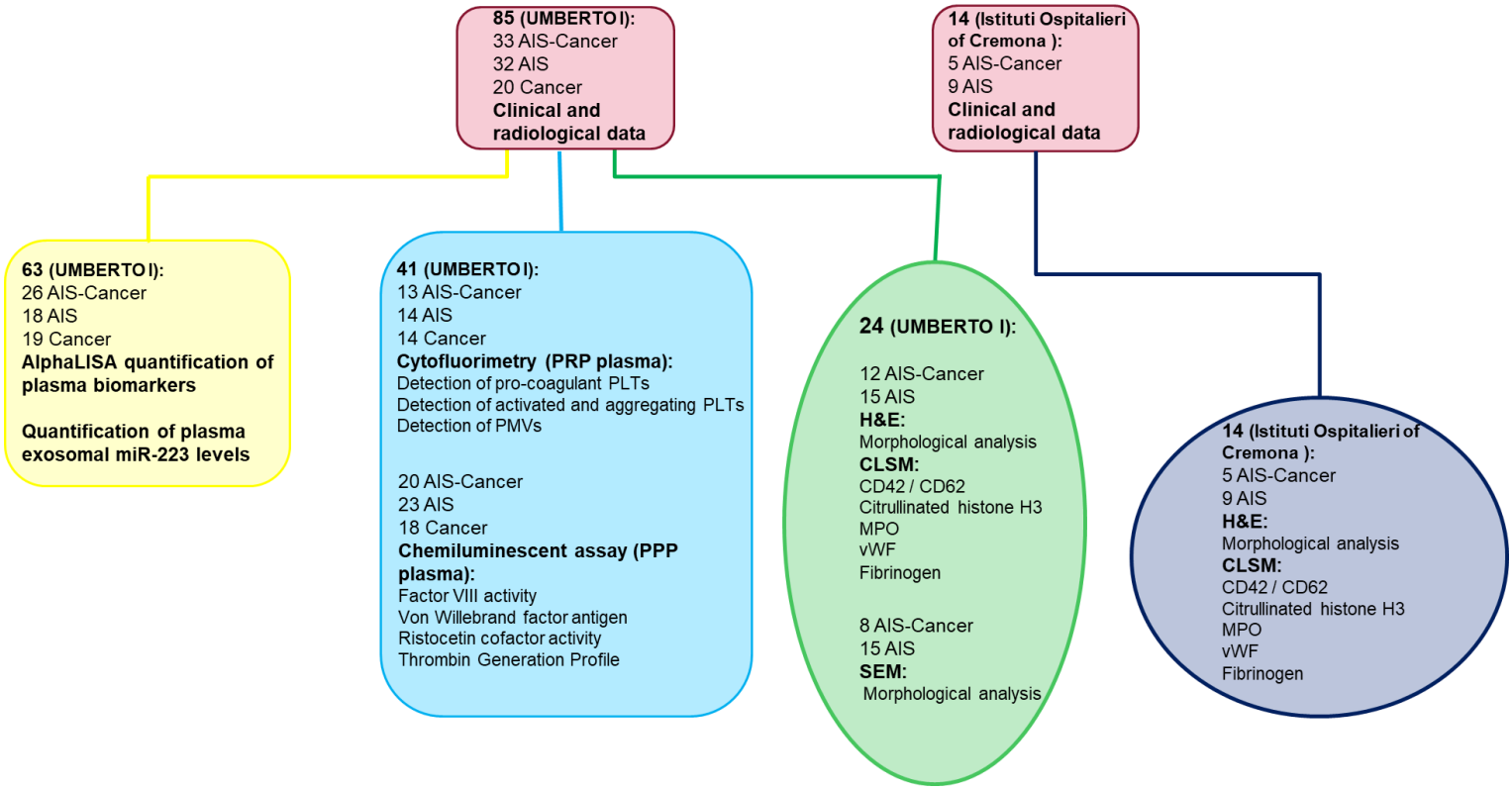
PMVs, platelets microvesicles.



Supplementary Fig. 4. a - b. Immunofluorescence staining of thrombus sections (5µm) retrieved from AIS-Cancer or AIS patients, stained for detection of CD42+ platelets (grey), histone H3 citrullinated (green) and MPO (red) to evaluate NETosis events (a), or double stained for (b) histone H3 citrullinated (red) in combination with vWF (grey). Nuclei are visualized in blue (DAPI). Separate channels and merged images are shown. Higher magnification images of NETosis are displayed on the panel **a**, showing the close interactions of extracellular DNA fibers with MPO and histone H3 citrullinated in thrombi from AIS-Cancer patients. In AIS thrombi NETosis frequency is low, even in those with extensive platelet-rich areas. Scale bars, 100 µm. Representative examples from 9 AIS and 8 AIS-Cancer thrombi are shown.



Supplementary Figure 5. **Wide areas of three different AIS thrombi characterized by a high prevalence of fibrin and erythrocytes in different morphological stages, related to different growing ages**. **a.** Break surface of a thrombus with a homogeneous matrix of polyedric erythrocytes **(b)** compressed by the fibrin fibers contraction. **c.** Break area of another thrombus displaying a smooth external shell and a core consisting of a compact matrix of fused undistinguishable erythrocytes **(d)** (frequently detected in the apical older growing areas). **e-f-g.** Increased magnifications of distal area of a thrombus composed predominantly by discoid erythrocytes or only lightly deformed ones by oxidative stress.



Supplementary Figure 6. Flow diagram of the sample selection and analysis.

Supplementary Methods

Blood collection and processing

Peripheral venous blood samples were collected into EDTA tubes and processed within 2 h of venipuncture. Samples were centrifuged at 1,600 × g for 15 min at room temperature to obtain platelet-poor plasma (PPP). Plasma aliquots were stored at −80 °C until analysis and thawed only once.

For coagulation assays, blood was collected into 3.2% sodium citrate tubes and centrifuged at 12,000 rpm to obtain PPP.

AlphaLISA quantification of plasma biomarkers

Plasma biomarker concentrations were measured using Amplified Luminescent Proximity Homogeneous Assay (AlphaLISA) kits (Revvity) according to the manufacturer's instructions. Briefly, 5 µl of plasma were incubated with biotinylated antibodies and acceptor beads, followed by addition of streptavidin-coated donor beads under low-light conditions. Plates were incubated at room temperature for 1 h.

Signals were detected using an EnVision Multimode Plate Reader (Revvity) in AlphaLISA mode. Raw luminescence values were converted to concentrations using a four-parameter logistic (4PL) standard curve generated for each plate[48].

Samples were randomly distributed across plates by an independent investigator to ensure balanced group allocation. All samples were analyzed in duplicate. Measurements with a coefficient of variation (CV) >30% were repeated. Quality-control samples were included on each plate to monitor inter-assay variability.

Exosomal miRNA isolation and quantification

Exosomes were isolated from plasma using the ExoQuick™ Plasma/Serum Exosome Precipitation Solution (System Biosciences), following the manufacturer's protocol[49]. Exosome pellets were resuspended in the provided buffer and processed immediately.

Total RNA, including small RNAs, was extracted using the miRNeasy Serum/Plasma Kit (Qiagen)[50]. Reverse transcription was performed using the miRCURY LNA RT Kit (Qiagen). Quantitative PCR was carried out on a QuantStudio™ 5 Real-Time PCR System (Applied Biosystems) using miRCURY LNA miRNA PCR assays specific for hsa-miR-223 and the miRCURY LNA SYBR Green PCR Kit (Qiagen).

Expression levels were calculated using the ΔCt method, with miR-16 used as the endogenous reference based on its stability in plasma-derived miRNA assays[51-53]. Relative expression was expressed as $2^{-}\Delta Ct$.

Platelet isolation and flow cytometry

Platelets were isolated from fresh blood collected in acid–citrate–dextrose (ACD, NIH formula A) tubes. Samples were centrifuged at 200 × g for 12 min at room temperature to obtain platelet-rich plasma (PRP). PRP was further centrifuged at 800 × g for 15 min to pellet platelets. For detection of procoagulant platelets, cells were stained with FITC-conjugated Annexin V (1:200; Abcam) and 0.05% trypan blue for 10 min at room temperature. Activated platelets were detected using PE-conjugated anti-CD62P (1:20; BD Biosciences), and platelet aggregation was assessed using FITC-conjugated PAC-1 (1:20; BD Biosciences). Staining was performed for 30 min on ice.

Platelet-derived microvesicles (PMVs) were isolated by centrifugation of PRP at 5,000 × g for 15 min at 4 °C to obtain PPP, followed by centrifugation at 20,000 × g for 20 min. PMVs were stained with PE-conjugated anti-CD62P and FITC-conjugated Annexin V.

Flow cytometry was performed using a FACSCalibur (BD Biosciences) equipped with a 488-nm laser. A minimum of 20,000 events was acquired per sample. Data were analyzed using standard gating strategies.

Coagulation assays

Factor VIII activity (FVIII:C) was measured using a one-stage clotting assay on a BCS XP analyzer (Siemens Healthineers). Von Willebrand factor antigen (VWF:Ag) and ristocetin cofactor activity (VWF:RCo) were measured using automated chemiluminescence on the AcuStar system (Werfen).

Thrombin generation was assessed using the calibrated automated thrombogram on a Fluoroskan Ascent fluorometer with Thrombinoscope software. The thrombin generation profile was obtained using a calibrated fluorogenic substrate and quantified through the following parameters:

- Lag Time (min): interval from reaction initiation to detectable thrombin generation
- Peak Thrombin (nM): maximum thrombin concentration reached during the reaction
- Endogenous Thrombin Potential (ETP, nM·min): area under the thrombin generation curve, representing total thrombin produced
- Time-to-Peak (ttPeak, min): time required to reach peak thrombin concentration

- Start Tail (min): time at which the curve returns to baseline after the peak
- Velocity Index (nM/min): rate of thrombin generation propagation, calculated as Peak/(ttPeak - Lag Time)

All measurements were performed in duplicate.

Confocal immunofluorescence microscopy

Thrombus fragments retrieved during mechanical thrombectomy from 41 patients with large-vessel occlusion stroke (17 AIS-Cancer and 24 AIS) were fixed in 4% buffered formalin and embedded in paraffin. Thrombi were sectioned at 5 μm by microtome, deparaffinized in xylene, rehydrated through graded ethanol and then stained for morphological analysis with the standard hematoxylin (Mayer's solution for hematoxylin Sigma Aldrich, Cat# MHS16) and eosin (alcoholic Eosin Y solution, Sigma Aldrich, Cat# HT110116l) technique. For immunofluorescence analysis sections were subjected to heat-induced epitope retrieval step by pH6 citrate buffer (Novus Biologicals) three times for 3 min in a microwave, were then washed with PBS-T (0.01% Tween 20) and blocked in PBS-BSA 3% for 45 min at 37 °C. The following primary antibodies (Abs) were used for immunolabeling at manufacturer-recommended dilutions: mouse anti human platelets CD42 (Huabio EM1902-15) or rabbit anti-CD62 (Invitrogen PA5-78162), rabbit anti-citrullinated histone H3 (Abcam 5103), goat anti-myeloperoxidase (R&D AF3667), sheep anti-von Willebrand factor (Abcam 11713) and rabbit anti-fibrinogen (DAKO A0080). The primary Abs were added in PBS-BSA3% and incubated overnight at 4°C, followed by incubation with the appropriate combination of secondary Abs (Alexa Fluor®-488 or -594 $F(ab)_2$ fragments of goat anti-rabbit or anti mouse IgG (H+L), Alexa Fluor®-647 Donkey anti sheep or Alexa Fluor®-594 Donkey anti goat, all from Life Technologies) plus DAPI (Thermo Fisher Scientific) for 60 minutes at r.t. Sections were finally mounted in Vectashield antifade mounting medium (Vector Laboratories). CLSM observations were performed with a Zeiss LSM980 apparatus, using a 20x or 40x-oil objective and excitation spectral laser lines at 405, 488, 594 and 647 nm. Image acquisition and processing were carried out using the Zeiss confocal software Zen 3.3 (Blue edition) and Adobe Photoshop CS5 software programs (Adobe Systems). Signals from different fluorescent probes were taken in sequential scan settings and colocalizations were visualized in merged images. Multiple regions, including central and peripheral areas of each thrombus, were analyzed and representative results are shown.

The number of DAPI positive nuclei was analyzed by ImageJ software (Fiji). For each thrombus, ten randomly selected fields of view (FOVs) were analyzed, excluding areas with

section artifacts or folds. Cellular density was calculated as nuclei per FOV and the mean value across the ten FOVs was used as the overall cellularity measure (N=5 both for AIS-Cancer or AIS thrombi).

The distribution of platelet-rich areas (CD42, CD62P), neutrophil infiltration (MPO), NETosis events (MPO/H3cit colocalization with extracellular DNA) and the spatial association between VWF, fibrinogen and platelet markers were qualitatively assessed by an investigator experienced in confocal microscopy and immunofluorescence analysis. Multiple sections per thrombus were reviewed, and discrepancies were resolved through joint reassessment.

Scanning electron microscopy (SEM)

Thrombi retrieved from large vessels of 8 AIS-Cancer patients and 15 AIS patients were fixed in paraformaldehyde 4%, glutaraldehyde 2.5% in Na-cacodilate 0.1M at 4°C and processed as described previously with some modifications[54]. HMDS dehydrated samples were gently split to expose the thrombus core, mounted on stubs with silver paint, and gold sputtered (20nm). Ultrastructural analyses were conducted by two high resolution Field Emission Scanning Electron Microscopies (Quanta INSPECT F – FEI/ThermoFisher and GeminiSEM450 – ZEISS). The wide surface of each sample was explored randomly (at least five different areas) detecting the apical, central and distal growing areas of thrombus, when recognizable.

In vitro platelet assays

To investigate the effect of soluble plasma mediators on platelet activation, an *in vitro* model was established in which platelets isolated from two healthy donor (HD) were incubated with plasma from AIS-Cancer, AIS and Cancer only patients. Experiments were conducted in two independent series using different patient samples for each clinical category. Whole blood from HD was collected in sodium citrate tubes and centrifuged at 900 rpm for 15 min without brake to obtain PRP. Then, it was centrifuged at 1200 rpm for 10 min to isolate the platelet pellet. The supernatant (platelet-poor plasma) was retained as a negative control, while the pellet was resuspended in PBS to a final volume of approximately 3.5 mL and divided into aliquots. Aliquots were centrifuged (3000 rpm for 6–8 min) to obtain compact platelet pellets. Depending on cell yield, pellets were resuspended in equal volumes of homologous donor plasma (control) or heterologous plasma from AIS-Cancer, AIS or Cancer only patients. After resuspension, half of the volume was immediately centrifuged (3000 rpm for 6–8 min) to obtain the 0-min time point, whereas the remaining aliquots were incubated at 37°C for 15 min. To assess PS exposure, platelets collected at 0 and 15 min were washed in Annexin V binding buffer and

incubated with Annexin V conjugate (1:50 dilution) for 15 min at 4°C. Fixation was performed with 4% paraformaldehyde for 20 min at room temperature, followed by washing for successive analysis.

For morphological analysis, platelet suspensions in paraformaldehyde were allowed to adhere to poly-L-lysine-coated slides for 2 h at room temperature and samples
were finally mounted in Vectashield antifade mounting medium (Vector Laboratories) and observed by CLSM. To quantify Annexin V-positive platelet aggregates, all clusters larger than 50µm across the entire slide were counted by microscopy for each experimental group.

Additional samples were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, let to adhere on polylysine treated glass coverslips and processed for SEM as described above.

Platelet aggregates were quantified by microscopy analysis of randomly selected fields. At least five high-magnification fields were analyzed for each sample, and aggregates, defined as clusters a size more than 50 µm, were counted.