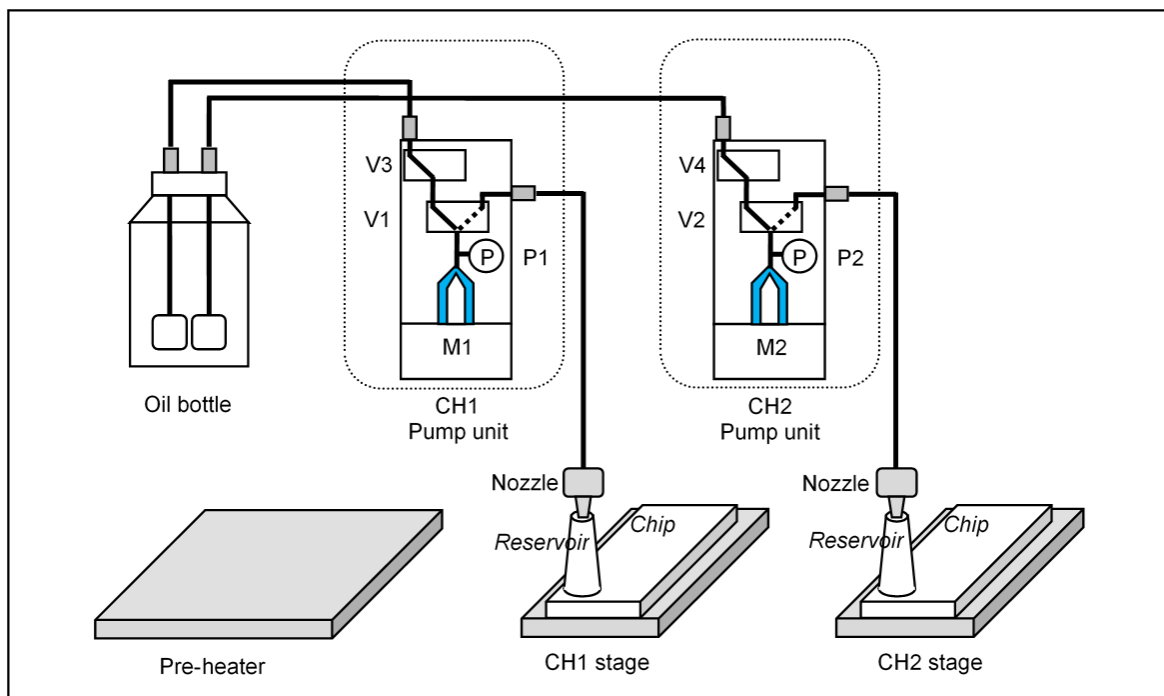


Supplementary material

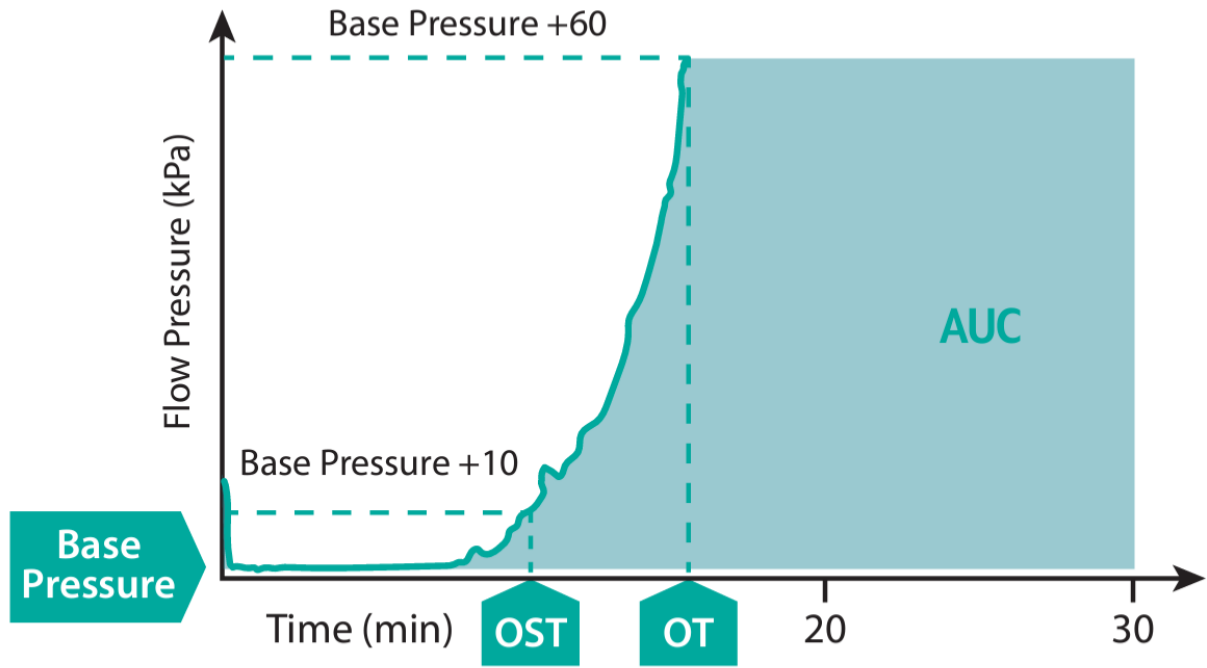
Supplement to:

Total Thrombus-Formation System in patients with peripheral artery disease

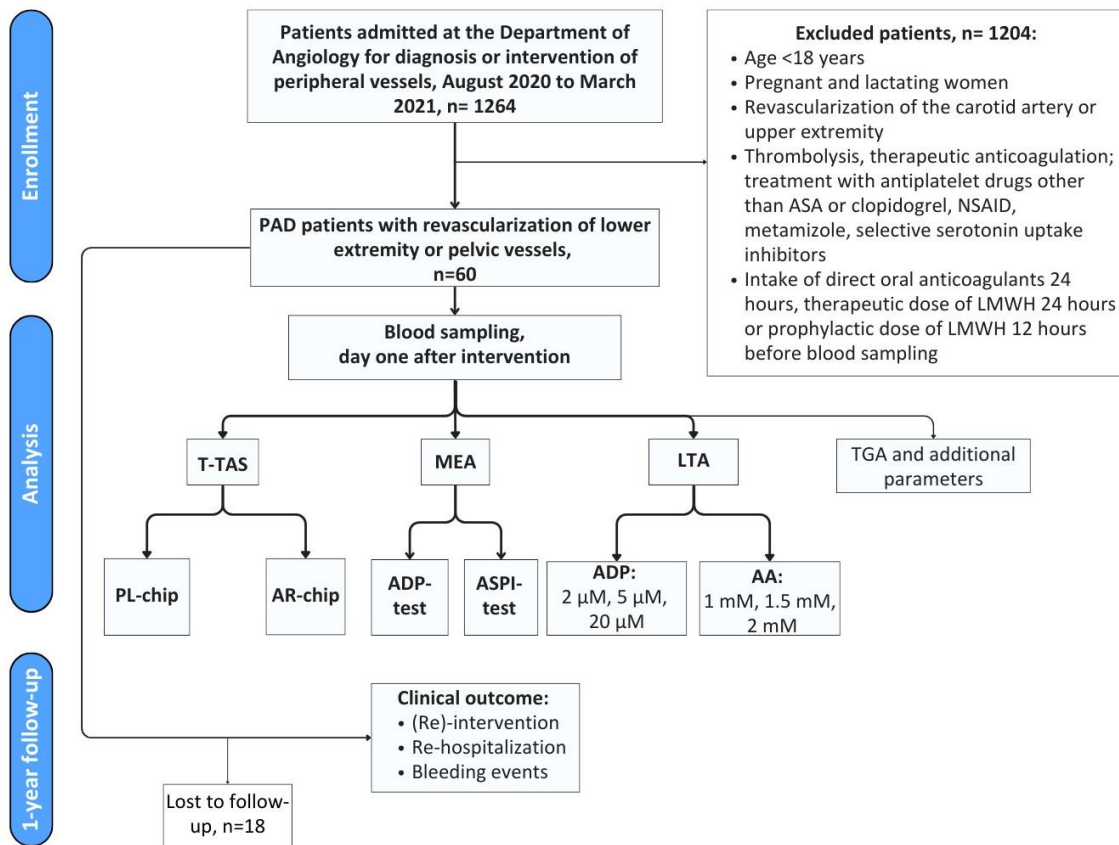
Christian Pfrepper, Careen Franke, Michael Metze, Maria Weise, Annelie Siegemund, Roland Siegemund, Martin Federbusch, Reinhard Henschler, Sirak Petros, Manuela Konert



Supplementary figure 1: Technical structure of the T-TAS system

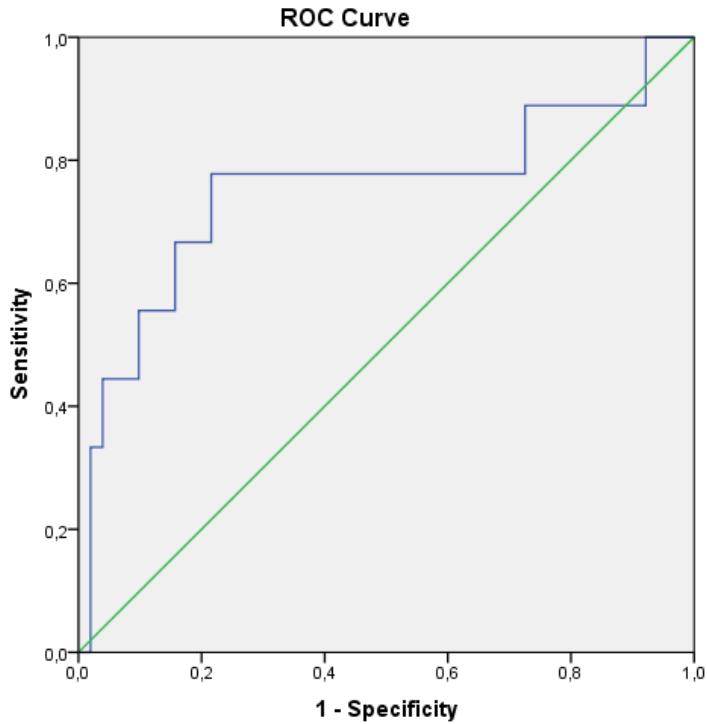


Supplementary figure 2: Diagram of an AR-Chip measurement, Abbreviation: OST, occlusion start time; OT, occlusion time; AUC, area und the curve.



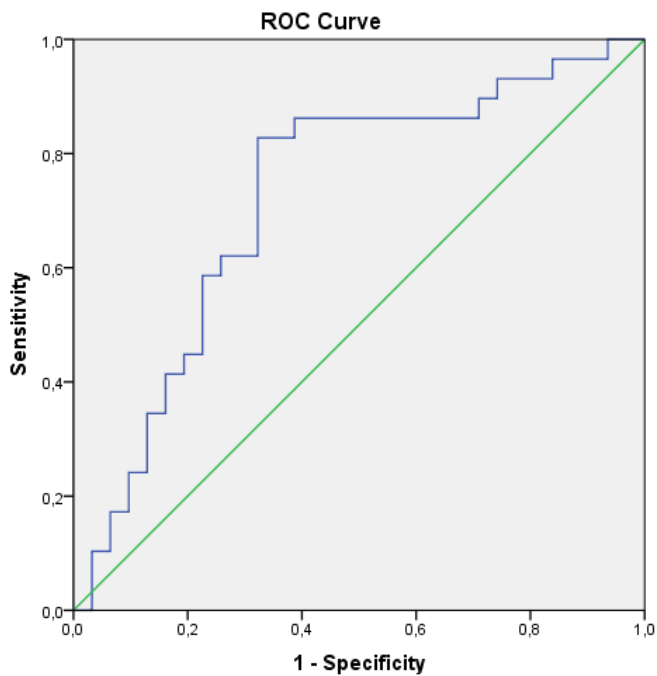
Supplementary figure 3: Flow chart of the study design.

Abbreviations: AA, arachidonic acid; ADP, adenosine diphosphate; ASPI-test, acetylsalicylic acid-test; LTA, light transmission aggregometry; MEA, multiple electrode aggregometry; PAD, peripheral artery disease; TGA, Thrombin generation assay; T-TAS, Total Thrombus-formation Analysis System.



Supplementary figure 4. ROC-Curve for the area under the curve of the PL-chip (AUC-PL) considering MEA as reference.

Abbreviations: AUC-PL, area under the curve of the platelet-chip; MEA, multiple electrode aggregometry; ROC, receiver operating characteristic.



Supplementary figure 5. ROC-Curve for the area under the curve of the PL-chip (AUC-PL) considering LTA as reference.

Abbreviations: AUC-PL, area under the curve of the platelet-chip; MEA, multiple electrode aggregometry; ROC, receiver operating characteristic.

Supplementary table 1. Reference ranges for OST and OT of blood donors for the PL- and AR-chip. All healthy subjects reached OST and OT

	PL-chip (n=30)	AR-chip (n=30)
Mean OST (reference range), sec	140 (93-203)	594 (341-1578)
Mean OT (reference range), sec	335 (236-537)	656 (423-1258)

Reference ranges are determined as 2.5th -97.5th percentile.

Abbreviations: AR-chip, atheroma chip; OST, occlusion start time; OT, occlusion time; PL-chip, platelet chip.

Supplementary table 2. OST and OT in PAD patients the PL- and AR-chip

	PL-chip	AR-chip
Median (IQR) OST, sec	152 (IQR 258-433)	454 (IQR 355-387)
Number of samples reaching OST, n (%)	36 (60%)	58 (97%)
Mean (IQR) OT, sec	310 (IQR 258-433)	540 (IQR 446-692)
number of samples reaching OT, n (%)	5 (8%)	58 (97%)

Abbreviations: AR-chip, atheroma chip; OST, occlusion start time; OT, occlusion time; PL-chip, platelet chip; IQR, interquartile range.

Supplementary table 3. Correlation of MEA- and LTA-tests with AUC-PL and AUC-AR.

Parameter		Correlation with AUC-PL (p-value)	Correlation with AUC-AR (p-value)
MEA	ADP-test	0.500 (<0.001)	0.394 (0.002)
	ASPI-test	0.449 (<0.001)	0.342 (0.008)
MA LTA	ADP 20 µmol/L	0.603 (<0.001)	0.005 (0.971)
	ADP 5 µmol/L	0.459 (<0.001)	0.069 (0.608)
	ADP 2 µmol/L	0.103 (0.433)	0.183 (0.166)
	AA 1 mmol/L	0.135 (0.304)	0.437 (0.001)
	AA 1.5 mmol/L	0.072 (0.584)	0.358 (<0.005)
	AA 2 mmol/L	0.012 (0.929)	0.278 (0.033)

Correlation coefficient r is Spearman's rho.

Abbreviations: AA, arachidonic acid; ADP, adenosine diphosphate; ASPI-test, acetylsalicylic acid-test; AUC-AR, area under the curve of the AR-chip; AUC-PL, area under the curve of the PL-chip; LTA, light transmission aggregometry; MA, maximum aggregation; MEA, multiple electronic aggregometry.

Supplementary table 4. Number of aggregation tests outside the target range and comparison of the area under the curve (AUC) of the PL chip for patients inside or outside the target range, including double low responders in each single aggregation test.

Test		Outside target range, n (%)	AUC-PL inside target, median (IQR)	AUC-PL outside target, median (IQR)	p
MEA	ASPI-test ¹	6 (10.0)	69 (19-158)	221 (133-380)	0.023
	ADP-test ¹	5 (8.3)	70 (19-165)	237 (90-412)	0.034
	ADP and ASPI-test	2 (3.3)	69 (18-142)	237, 425*	0.008
LTA	ADP 20 μ M ¹	28 (46.7)	31 (11-92)	148 (70-208)	<0.001
	AA 2 mM ¹	10 (16.7)	69 (19-167)	120 (55-191)	0.362
	AA and ADP	8 (13.3)	32 (11-113)	161 (70-222)	0.440

* Absolute values. ¹ Including cases of single low response plus cases of double low response.

Abbreviations: AA, arachidonic acid; ADP, adenosine diphosphate; ASPI-test, acetylsalicylic acid-test; AUC-PL, area under the curve of the PL-chip; IQR, interquartile range; LTA, light transmission aggregometry; MEA, multiple electronic aggregometry.

Supplementary table 5. Median values of additional hemostatic parameter and thrombin generation assay and their correlation (Spearman's rho) with AUC-PL and AUC-AR.

Parameter		Median	IQR	Correlation with AUC-PL, (p-value)	Correlation with AUC-AR, (p-value)
d-dimers (mg/L)		0.97	0.65-1.51	0.168 (0.200)	0.379 (0.003)
aPTT (s)		30.5	28.6-32.6	0.105 (0.427)	0.133 (0.315)
Fib (g/L)		5.9	5.3-6.3	0.038 (0.776)	0.021 (0.879)
FVIII (%)		183	160-197	0.114 (0.384)	0.095 (0.472)
vWF:Ag (%)		230	200-263	0.082 (0.535)	0.078 (0.557)
vWF:Ac (%)		247	198-317	0.091 (0.488)	0.212 (0.106)
Prothrombin fragments (pmol/L)		322	220-433	0.033 (0.803)	0.051 (0.699)
Lp(a) (nmol/L)		22.3	20-119	0.126 (0.409)	0.051 (0.740)
Thrombin generation	Lagtime (min)	2.7	2-3	0.011 (0.934)	0.073 (0.581)
	ETP (nMxmin)	2136	1878-2315	0.023 (0.864)	0.013 (0.924)
	Peak (nM)	371	341-401	0.092 (0.483)	0.100 (0.452)
	Time to peak (min)	5.4	4.7-6.3	0.06 (0.646)	0.019 (0.452)
	Velocity index (nM/min)	137	109-154	0.071 (0.590)	0.052 (0.698)

Abbreviations: aPTT, activated partial thromboplastin time; AUC-PL, area under the curve of the PL-chip; AUC-AR, area under the curve of the AR-chip; ETP, endogenous thrombin potential; FVIII, factor VIII activity; IQR, interquartile range; Lp(a), lipoprotein (a); vWF:Ac, von Willebrand factor activity; vWF:Ag, von Willebrand factor antigen.

Supplementary table 6. Number of cases inside and outside the target range in MEA and LTA for a) both ADP and AA/ASPI-tests b) ASPI/AA-tests c) ADP-tests.

A		MEA ASPI- and ADP-test (n)		
		Above cut-off	Below cut-off	Total (n)
LTA AA- and ADP-test (n)	Above cut-off	5	24	29
	Below cut-off	4	27	31
Total (n)		9	51	60
B		MEA ASPI-test (n)		
		Above cut-off	Below cut-off	Total (n)
LTA AA-test (n)	Above cut-off	3	7	10
	Below cut-off	3	47	50
Total (n)		6	54	60
C		MEA ADP-test (n)		
		Above cut-off	Below cut-off	Total (n)
LTA ADP-test (n)	Above cut-off	3	25	28
	Below cut-off	2	30	32
Total (n)		5	55	60

Abbreviations: AA, arachidonic acid; ADP, adenosine diphosphate; ASPI-test, acetylsalicylic acid-test; LTA, light transmission aggregometry; MEA, multiple electronic aggregometry.