Supplementary Material

Supplementary Methods

Inter-assay variability

We quantified inter-assay variability using the relative standard deviation or coefficient of variance (CV) which was determined by repeated point-of-care ecarin clotting time (POC-ECT) measurements conducted with normal citrated plasma (CP), re-thawed CP plasma samples taken during the study from dabigatran-treated patients, and commercially available dabigatran calibrator plasma (Hyphen BioMed, Neuville-sur-Oise, France). We performed a set of 34 measurements, each with normal plasma and two levels of dabigatran calibrator plasma, as well as a set of 16 measurements each with two different re-thawed patient CP samples. The CV was calculated for each set of measurements by dividing their standard deviation by the arithmetic mean and multiplying the result by 100%.

Interaction with heparins and direct oral Xa-inhibitors

For the assessment of sensitivity to unfractionated heparin (UFH), six CP samples from different patients were re-thawed, re-measured with POC-ECT and then spiked with one, two and four international units of UFH (Heparin-Natrium Braun Multi, B. Braun, Melsungen, Germany) per mL (see Supplementary Table 3).

In order to check for interaction with direct oral factor Xa-inhibitors and low molecular weight heparin (LMWH), we individually spiked samples of commercially available dabigatran calibrator plasma (Hyphen BioMed, Neuville-sur-Oise, France) with apixaban, edoxaban, rivaroxaban or LMWH calibrator plasma (dabigatran, edoxaban: Hyphen BioMed, Neuville-sur-Oise, France; apixaban, rivaroxaban, and LMWH: Technoclone, Vienna, Austria). POC-ECT was measured for apixaban, edoxaban, rivaroxaban or LMWH calibrator plasma, a 1:1 dilution of apixaban, edoxaban, rivaroxaban or LMWH calibrator plasma with normal plasma, a 1:1 dilution of dabigatran calibrator plasma of different concentration levels with normal plasma, and a 1:1 mixture of each level of dabigatran calibrator plasma with apixaban, edoxaban, rivaroxaban or LMWH calibrator plasma (see Supplementary Table 4).

Monitoring of dabigatran reversal with idarucizumab

To verify whether anticoagulation reversal is correctly reflected by POC-ECT, we performed POC-ECT measurements during in-vitro reversal of dabigatran using idarucizumab

(Praxbind®, Boehringer Ingelheim, Germany), a recombinant antibody fragment which disables the anticoagulant effect of dabigatran on factor IIa by binding to dabigatran specifically and irreversibly. We separated the re-thawed CP samples taken during the SPOCT-DOAC I study into four aliquots of 250 µL and spiked them with different doses of idarucizumab which we diluted with normal plasma using a 1:500 dilution for a low and an intermediate concentration plasma sample, as well as a 1:250 dilution for another intermediate and a high dose sample (see Supplementary Table 5). Idarucizumab dilutions were added in doses of 0, 10, 25 and 50 µL and normal plasma was added to yield a total sample volume of 300 µL. POC-ECT, as well as the calibrated laboratory-based Biophen Direct Thrombin Inhibitor assay (BDTI; Hyphen BioMed, Neuville-sur-Oise, France), were performed on each sample.

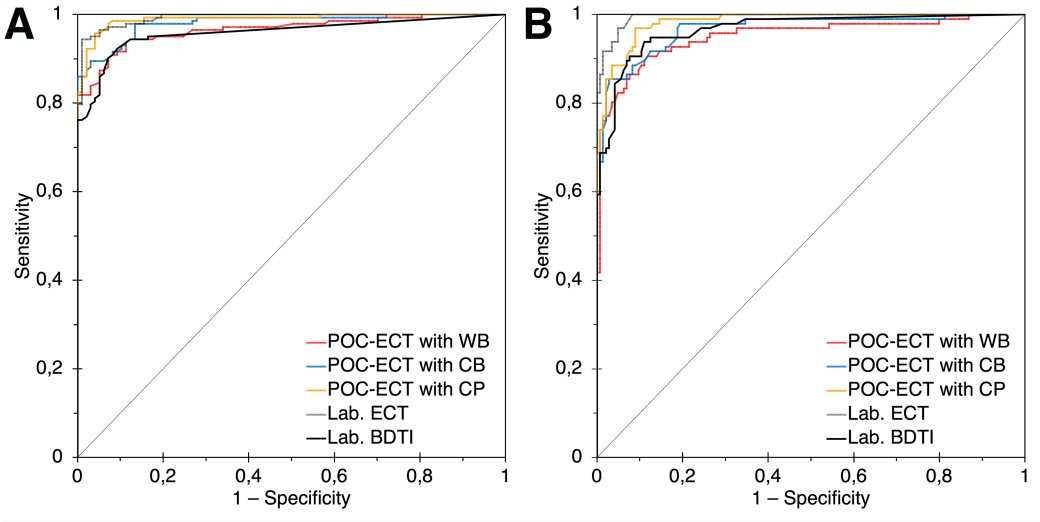
Supplementary Results

Supplementary Table I: Baseline laboratory results

Laboratory parameters	Results	Reference range
WBC count [1/µL]	7,980 ± 2,354	3,800 –10,300
RBC count [10^6/µL]	4.3 ± 0.6	4.2 – 6.2
Haematocrit [%]	37.4 ± 4.3	42.0 – 52.0
Haemoglobin [g/dL]	12.8 ± 1.7	14.0 – 18.0
Platelet count [10^3/µL]	216 ± 55	150 – 450
PT activity (Quick) [%] *	89 ± 14 / 97 ± 12 / 83 ± 13	70 – 120
INR *	1.1 ± 0.1 / 1.0 ± 0.1 / 1.1 ± 0.1	<1.2
aPTT [s] *	26 ± 6 / 23 ± 2 / 31 ± 6	≤40
Fibrinogen [mg/mL]	309 ± 104	170 – 410
D-Dimers [µg/mL]	2.1 ± 3.3	≤0.5
Anti-Xa activity [IU/mL]	<0.1 ± 0.0	<0.1
Creatinine [mg/dL]	0.8 ± 0.2	0.6 – 1.1
eGFR (MDRD) [mL/min x 1.73 m^2]	86 ± 28	>60
eGFR (CKD-EPI) [mL/min x 1.73 m^2]	78 ± 15	>60
eGFR (Cockcroft-Gault) [mL/min x 1.73 m^2]	93 ± 40	>60
Urea [mg/dL]	35 ± 15	12 – 46
Total protein [g/dL]	6.6 ± 0.7	6.5 – 8.5
Albumin	3.3 ± 0.5	3.0 – 5.0
CRP [mg/dL]	1.75 ± 3.41	≤0.5
Procalcitonin [ng/mL]	0.08 ± 0.03	≤0.1
ALT [IU/L]	30 ± 42	≤50
AST [IU/L]	32 ± 20	≤50
γ-GT [IU/L]	63 ± 75	≤60
CHE [kIU/L]	6.9 ± 1.8	4.9 – 12.0

All results displayed as mean ± standard deviation (SD); * values are given for whole cohort (N = 40) / prior to the initiation of dabigatran treatment, n = 20 / ongoing dabigatran treatment, n = 20

WBC = white blood count, RBC = red blood count, PT = prothrombin time, INR = international normalized ratio, aPTT = activated partial thromboplastin time, eGFR = estimated glomerular filtration rate, CRP = C-reactive protein, AST = aspartate transaminase, ALT = alanine transaminase, γ-GT = gamma-glutamyl transferase, CHE = cholinesterase.



Supplementary Figure I: Receiver operating characteristics curves for the detection of dabigatran plasma levels >30 ng/mL (A) and >50 ng/mL (B) using point-of-care ecarin clotting time (POC-ECT) with whole-blood (WB), citrated blood and citrated plasma, as well as of laboratory-based ECT (Lab. ECT) and laboratory-based Biophen Direct Thrombin Inhibitor assay (Lab. BDTI) (compare values in Table 3).

Supplementary Table II: Heparin sensitivity

	POC-ECT [s] (two measurements per sample)						
Added heparin	Normal plasma	Sample 1B4 275 ng/mL*	Sample 4B1 105 ng/mL*	Sample 9B3 207 ng/mL*	Sample 6B5 263 ng/mL*	Sample 11A4 96 ng/mL*	Sample 23A4 193 ng/mL*
none	29	283	196	304	362	118	221
	27	316	167	290	328	133	236
1 U/mL	29	282	193	293	403	128	239
	25	279	182	278	386	125	226
2 U/mL	28	304	192	315	401	126	247
	31	274	184	276	422	122	249
4 U/mL	28	313	192	272	350	141	277
	24	309	162	292	312	134	219

* dabigatran plasma concentration as determined by ultra-performance liquid chromatography/tandem mass spectrometry

Sensitivity of point-of-care ecarin clotting time (POC-ECT) to the presence of therapeutic and supra-therapeutic concentrations of unfractionated heparin (UFH) was determined by spiking re-thawed patient citrated plasma samples. Note that there is no significant change of POC-ECT with increasing addition of UFH.

Supplementary Table III: Effect of direct oral Xa-inhibitors and low molecular weight heparin (LMWH) on point-of-care ecarin clotting time (POC-ECT)

Added substance	Concentration of added substance	POC-ECT [s]				
			added to			
		Added substance alone	**Normal plasma**	**Dabigatran calibrator 1 (43 ng/mL)**	**Dabigatran calibrator 2 (257 ng/mL)**	**Dabigatran calibrator 3 (489 ng/mL)**
normal plasma	N/A	23 / 25	33 / 34	46 / 54	136 / 126	289 / 242
apixaban	458 ng/mL	28 / 24	26 / 33	45 / 48	148 / 131	258 / 249
edoxaban	425 ng/mL	39 / 37	32 / 35	70 / 54	202 / 141	303 / 294
rivaroxaban	462 ng/mL	26 / 26	27 / 26	49 / 53	143 / 134	259 / 305
LMWH *	1.99 IU/mL	29 / 28	38 / 31	63 / 53	154 / 149	239 / 210

*LMWH: low molecular weight heparin

Influence of oral Xa-inhibitors and low molecular weight heparin (LMWH) on point-of-care ecarin clotting time (POC-ECT): POC-ECT remains stable when apixaban, edoxaban, rivaroxaban or LMWH calibrator plasma is added to normal citrated plasma or commercially available calibrator plasma containing different levels of dabigatran. Note that there is a significant increase of POC-ECT only with increasing dabigatran levels (from left to right), but no significant increase through the addition of apixaban, edoxaban, rivaroxaban or LMWH. All dilutions (apart from "added substance alone") were performed in a 1:1 fashion, leading to a 50% decrease of both apixaban, edoxaban, rivaroxaban or LMWH, and dabigatran in the final solution.

Supplementary Table IV: In-vitro dabigatran reversal with idarucizumab

Sample	Original concentration [ng/mL] * (volume 250 µL)	Calculated concentration (after addition of NP and/or DI†) [ng/mL] (volume 300 µL)	**POC-ECT [s]** (calibrated laboratory-based BDTI [ng/mL]) Addition of			
			50 µL of NP	10 µL of DI (1:500†) and 40 µL of NP	25 µL of DI (1:500†) and 25 µL of NP	50 µL of DI (1:500†)
39B2	72	60	**102** (69)	**53** (19)	**25** (0)	**29** (0)
40B4	128	106	**169** (137)	**140** (98)	**81** (50)	**22** (0)
			50 µL of NP	10 µL of DI (1:250†) and 40 µL of NP	25 µL of DI (1:250†) and 25 µL of NP	50 µL of DI (1:250†)
39B3	134	112	**143** (133)	**92** (66)	**27** (0)	**32** (0)
34B4	246	205	**249** (245)	**227** (192)	**82** (58)	**30** (0)

* dabigatran plasma concentration as determined by ultra-performance liquid chromatography/tandem mass spectrometry †dilution (diluted idarucizumab, DI) was achieved with normal plasma (NP)

In-vitro reversal of dabigatran using idarucizumab (Praxbind®, Boehringer Ingelheim, Germany); a recombinant antibody fragment which binds to dabigatran specifically and irreversibly, thus disabling its anticoagulant effect on factor IIa. Re-thawed citrated plasma samples were separated into four aliquots of 250 µL and spiked with different doses of diluted idarucizumab (as indicated); normal plasma was added in order to yield a total sample volume of 300 µL. Note that point-of-care ecarin clotting time (POC-ECT) decreases in parallel to laboratory-based Biophen Direct Thrombin Inhibitor assay (BDTI) with increasing doses of idarucizumab accurately indicating the reversal of the anticoagulant effect of dabigatran.