Research Square

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The influence of direct oral anticoagulants on thrombin generation on Ceveron TGA

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Research Article

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Abstract

Introduction: Monitoring of direct oral anticoagulants (DOACs) with calibrated anti-Xa assay is limited by the high intra- and interindividual variation of the test results. Thrombin generation (TG) is a global hemostatic assay that reflects the patient´s individual coagulation status. The aim of this study was to investigate the influence of DOACs on TG measured with a fully automated assay system.

Methods: All consecutive patients under apixaban and rivaroxaban coming to the outpatient coagulation center MVZ Limbach, Magdeburg, Germany between October 2017 and April 2020 were included. DOAC plasma levels were correlated with TG assessed using the fully automated Ceveron TG analyser.

Results: A total of 703 rivaroxaban and 252 apixaban containing plasma samples were included. There was a significant correlation between DOAC plasma levels and all TG parameters except for lag time regarding apixaban. Time to peak and peak thrombin followed an exponential regression curve, while this was linear for the endogenous thrombin potential (ETP). Apixaban showed a lower correlation coefficient for all TG parameters compared to rivaroxaban and thrombin generation was less influenced by apixaban than rivaroxaban at plasma levels > 100 ng/mL. The sensitivity and negative predictive value of normal TG parameters for the prediction of DOAC plasma levels < 30 ng/mL was > 85%.

Conclusion: The present data show a moderate predominantly non-linear correlation between TG parameters and plasma levels of apixaban and rivaroxaban. Rivaroxaban has a stronger effect on TG than apixaban.

Introduction

Direct oral anticoagulants (DOACs) are currently the most widely used antithrombotic drugs in patients with atrial fibrillation (AF) and venous thromboembolism (VTE). The main advantage of DOACs over vitamin K antagonists (VKA) is the fixed dosing regimen without routine laboratory monitoring. However, measurement of the DOAC effect might be necessary in certain situations such as bleedings, thromboembolism on therapy or prior to reversal of anticoagulation [1,2].

Calibrated tests have been introduced to measure the concentration of DOACs in patient's plasma [2]. However, this does not necessarily correlate with the global hemostatic status of the patient [3].

The thrombin generation assay (TGA) is a global hemostatic assay to measure the tendency of a plasma sample to form thrombin after the initiation of the coagulation cascade [4]. It has initially been developed by Hemker and colleagues [5] and reflects the total thrombin kinetic [6]. Several assays for the measurement of thrombin generation (TG) have been introduced in the last years. The main disadvantages of these assays are the poor standardization and the high inter- and intralaboratory variations [7] resulting in the need for standardization [8,9] and automatization. Currently available fully automated TGA include the ST Genesia system (Diagnostica Stago) and the Ceveron TGA (Technoclone).

There are limited data in the literature on thrombin generation measured with the Ceveron TGA in patients on DOACs. Published data about the effect of DOACs on TG were mainly carried out on the semi-automated calibrated thrombin generation assay (CAT) [10–13] and the ST Genesia system [14–17]. In a recent survey among laboratories working with TG assays, CAT was used by 56%, while Ceveron Alpha only by 4% [18]. However, the main advantage of

Ceveron TGA is the combined measurement of standard cagulation assays and thrombin generation in the same device, making it a promising tool for a coagulation laboratory.

The aim of this study is to investigate TG parameters measured with the Ceveron TGA in unselected real-world patients on steady-state DOAC treatment.

Materials And Methods:

In this retrospective study, all consecutive patients treated with DOACs presenting for their routine check-up between October 2017 and April 2020 to the outpatient coagulation center MVZ Limbach Magdeburg, Germany were included. Patients with newly diagnosed thrombosis at the time of presentation and patients with positive lupus anticoagulant were excluded due to inconsistent thrombin generation in these patients.

Blood sampling and laboratory analysis

Blood was drawn by venipuncture at the time of presentation and there was no standardized time between DOAC intake and blood sampling. Blood was collected in sodium citrate-containing tubes using a standardized procedure. Blood samples were than centrifuged at 4000rpm for 20 min (2500 x g) to prepare platelet poor plasma and analyzed within two hours of blood sampling. DOAC plasma levels were measured using the Innovance® Heparin anti-Xa assay (Siemens Healthineers, Erlangen, Germany) and the STA®-Apixaban and STA®-Rivaroxaban calibrators and controls (Diagnostica Stago, Asnières-sur-Seines, France) on a COAG 360 analyzer (Siemens Healthineers, Erlangen, Germany).

Thrombin generation was performed using the photo-optical Ceveron TGA (Technoclone, Vienna, Austria) with the RC high reagent and dedicated control plasmas according to the manufacturer's instructions. The concentration of thrombin is measured with a fluorescent peptide substrate which is catalyzed by thrombin to release a fluorophore. Coagulation is initiated through the addition of recombinant human tissue factor at a high picomolar concentration lapidated in phospholipid micells containing phosphatidylcholine and phosphatidylserine as previously described [19]. The final concentrations are 40 µL plasma sample in 20 µL buffer and 40 µL fluorogenic substrate activated by 15 µL RC high trigger reagent and 35 µL of a 25 mN calcium chloride solution. The RC high reagent was used because it allows better discrimination at low plasma levels in anticoagulated patients and is recommended by the manufacturer for this purpose. The rate of thrombin generation is measured over time resulting in a thrombin formation curve. Laboratory tests were externally validated by the biannual participation in the ECAT system. The coefficient of variation (CV) of the method was < 5% for the intra-assay reproducibility and < 10% for the inter-assay reproducibility for all values except the velocity index (supplementary tables 1 and 2).

Statistical analysis

Descriptive data are given as median with interquartile range (IQR) in brackets if not otherwise indicated. Comparison of groups was performed with t-test for normally distributed data and given as mean ± standard deviation (SD). For not normally distributed data, the Mann-Whitney U test was used and data given as median with IQR. The level of significance was set at $p < 0.05$. Spearman's correlation coefficient r and p values were determined for the correlation between DOAC levels and TG parameters. For the analysis of outliers, the TG values were grouped according to their DOAC plasma levels: 50–100, 101–150, 151–200, 201–300 and > 300 ng/mL and analysed separately for apixaban and rivaroxaban containing plasma samples. DOAC plasma levels < 50 were excluded as these probably correspond to the trough levels and are more influenced by the hypercoagulable state than by the

DOAC effect. Outliers were defined as TG values that are more than 1.5 times the IQR above the third or below the first quartile of each group of DOAC plasma level according to Tukey`s fences method [20]. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the combination of TG parameters within the normal range to predict DOAC levels < 50 ng/mL or < 30 ng/mL were calculated and receiver operator curves (ROC) computed. Statistical analyses were performed using the software SPSS version 26 (SPSS Inc, Chicago, IL) and Microsoft Office Excel.

Ethical considerations

This study was approved by the Ethics committee of the University of Leipzig (reference 420/19-ek) and conducted according to the declaration of Helsinki.

Results:

A total of 806 patients were identified, of whom 193 were treated with apixaban, 15 with dabigatran, 74 with edoxaban, and 524 with rivaroxaban. Due to the very low number of patients on dabigatran and edoxaban, only data from patients on apixaban and rivaroxaban were further analysed.

Median age of the remaining 717 patients was 61 (range 20–90, IQR 52–73) years and 392 were female. There were two plasma samples from different time points available in 238 patients, making a total of 955 plasma samples (252 apixaban and 703 rivaroxaban) included into the study. Median age was 61 (IQR 52–73) years in the apixaban group and 61 (IQR 52–73) years in the rivaroxaban group. One patient was switched from rivaroxaban to apixaban. This patient was excluded in the patient's characteristics displayed in Table 1.

Table 1
Patient characteristics.

		Apixaban		Rivaroxaban		
		n	%	n	%	p
Anamnesis	Female gender	108/193	55.9	284/524	54.2	0.675
	Family history of VTE	43/193	22.4	144/524	27.5	0.159
	History of stroke	9/193	4.7	15/524	2.9	0.234
	Myocardial infarction	6/193	3.1	10/524	1.9	0.334
	Arterial embolism	6/193	3.1	4/524	0.8	0.027
	Renal insufficiency	16/193	8.3	47/524	9.0	0.776
	Obesity	24/193	12.4	72/524	13.7	0.649
	History of cancer	19/193	9.8	40/524	7.6	0.339
	Active cancer	4/193	2.1	10/524	1.9	1.000
	Diabetes mellitus	11/193	5.7	46/524	8.8	0.176
	Smoking	25/193	13.0	68/524	13.0	0.993
	Oral contraceptive	4/193	2.1	5/524	1.0	0.260
Thrombophilia	Antithrombin deficiency	4/193	2.1	10/524	1.9	1.000
	Protein C deficiency	2/193	1.0	7/524	1.3	1.000
	Protein S deficiency	5/193	2.6	9/524	1.9	0.541
	Factor V-Leiden mutation	34/193	17.6	113/524	21.6	0.281
	Prothrombin-G20210A-Mutation	12/193	6.2	39/524	7.4	0.447
	Combined thrombophilia	3/193	1.6	13/524	2.5	0.578
Reason for anticoagulation	Chronic DVT	139/193	72.0	440/524	84.0	< 0.001
	Recurrent DVT	27/193	14.0	94/524	17.9	0.210
	Pulmonary embolism	97/193	50.3	218/524	41.6	0.038
	Recurrent pulmonary embolism	7/193	3.6	24/524	4.6	0.578
	Recurrent VTE	34/193	17.6	110/524	21.0	0.317
	Atrial fibrillation	23/193	11.9	30/524	5.7	0.005

Reference cohort

For the determination of reference values, plasma samples from a cohort of 50 healthy subjects coming to the outpatient coagulation center MVZ Limbach Magdeburg, Germany for thrombophilia testing because of a family

history of hereditary thrombophilia were analyzed. These subjects were not on anticoagulation, had no thrombophilia and no history of thromboembolism.

Median age of the reference population was 62 (IQR 56–66) years and 25 (50.0%) were females. Mean (2.5th -97.5th percentile) TG parameters of this cohort were: lag time 4.5 (3.0-6.6) min; TTP 9.8 (7.4–13.1) min; peak thrombin 314 (177–465) nmol/L; velocity index 63 (22–123) nmol/min; ETP 3207 (1946–3874) nmol/L*min.

Correlation between DOAC plasma levels and thrombin generation parameters

There was a significant correlation between DOAC plasma levels and thrombin generation parameters except for the lag time with apixaban. The correlation between DOAC plasma levels and the TG parameters TTP, peak thrombin and ETP in all included patients is shown in Fig. 1.

The correlation beween DOAC plasma levels and TG parameters was higher for rivaroxaban compared to apixaban in patients with AF, patients with thrombophilia and in the subgroup of thrombophilia patients with Factor-V-Leiden-Mutation and Prothrombin-G20210A-Mutation. In addition, there was a higher correlation between DOAC plasma levels and TG parameters in patients with thrombophilia, especially in patients with Prothrombin-G20210A-Mutation, compared to patients with AF (supplementary table 3).

Comparison of TG parameters at different drug levels

For the comparison of TG parameters at different drug concentrations, drug levels were divided into the subgroups < 20 ng/mL, 21–50 ng/mL, 51–100 ng/mL, 101–200 ng/mL, 201–300 ng/mL and > 300 ng/mL. Patients treated with rivaroxaban showed a significant increase in the TG parameters lag time and TTP and a drop in peak thrombin, velocity index and ETP between all subsequent drug level categories except for the highest drug levels. Patients on apixaban showed these differences only between the drug levels 21–50 ng/mL and 51–100 ng/mL.

Rivaroxaban-treated patients had a longer TTP compared to patients on apixaban at all plasma levels > 20 ng/mL. Peak thrombin, velocity index and ETP were lower and lag time longer in patients on rivaroxaban at drug levels between 100 and 300 ng/mL. The comparison of thrombin generation between rivaroxaban and apixaban is given in Table 2 and the p-values for the comparison of the TG parameters between different drug levels in the supplementary table 4.

Table 2

Thrombin generation parameters in apixaban and rivaroxaban treated patients at different drug levels. Values are given as median and interquartile range in brackets.

		n	Drug level	Lag time	TTP, min	Peak thrombin	VI	ETP
< 20 ng/mL	Apixaban	41	0 (0–13)	4.6 (3.8–5.2)	9.3 (8.2–10.8)	360 (256–460)	70 (42–124)	2896 (2633–3511)
	Rivaroxaban	186	10 (0–14)	4.7 (4.0-5.6)	9.6 (7.9–11.6)	324 (238–469)	69 (37–132)	3122 (2645–3476)
	p		0.310	0.397	0.475	0.807	0.793	0.518
21–50 ng/mL	Apixaban	27	39 (32–45)	4.5 (3.9–5.4)	9.8 (8.6–11.4)	279 (192–348)	55 (31–86)	2961 (2648–3332)
	Rivaroxaban	93	33 (27–42)	5.0 (4.3–5.8)	11.1 (9.4–13.3)	224 (170–360)	36 (22–82)	2745 (2415–3121)
	p		*0.005*	0.140	*0.018*	0.194	0.085	*0.048*
51–100 ng/mL	Apixaban	81	71 (61–83)	5.3 (4.1–6.2)	11.9 (10.2–13.5)	164 (114–214)	26 (16–38)	2285 (1660–2796)
	Rivaroxaban	72	73 (61–85)	5.4 (4.4–6.6)	13.5 (11.4–15.8)	172 (123–219)	22 (13–34)	2402 (1980–2757)
	p		0.946	0.437	*< 0.001*	0.473	0.207	0.208
101–200 ng/mL	Apixaban	69	140 (117–163)	5.1 (4.1–6.1)	11.2 (9.6–13.0)	170 (108–232)	29 (16–48)	2247 (1621–2892)
	Rivaroxaban	98	153 (123–178)	6.3 (5.5–7.4)	16.2 (14.0-19.6)	123 (86–164)	12 (7–18)	1806 (1302–2388)
	p		*0.042*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*0.001*
201–300 ng/mL	Apixaban	24	239 (210–274)	4.9 (3.6–6.1)	11.7 (8.9–16.6)	141 (86–178)	22 (10–38)	1970 (1509–2608)

Abbreviations: n: number of plasma samples, TTP: time to peak, VI: Velocity index, ETP: endogenous thrombin potential

		n	Drug level	Lag time	TTP, min	Peak thrombin	VI	ETP
	Rivaroxaban	117	240 (221–275)	7.1 (6.0-8.3)	18.1 (15.3–21.0)	102 (70–140)	9 (6–15)	1580 (1116–2125)
	p		0.548	*< 0.001*	*< 0.001*	*0.007*	*0.001*	*0.013*
> 300 ng/mL	Apixaban	9	329 (313–404)	5.3 (4.3–6.4)	12.3 (10.5–14.7)	106 (83–133)	15 (8–30)	1651 (1355–2077)
	Rivaroxaban	136	377 (337–422)	7.6 (6.1–9.3)	19.6 (16.2–23.6)	99 (63–142)	9 (4–15)	1533 (956–2106)
	p		0.141	*0.001*	*< 0.001*	0.724	0.026	0.306

Abbreviations: n: number of plasma samples, TTP: time to peak, VI: Velocity index, ETP: endogenous thrombin potential

Description of outliers

The outliers in peak thrombin were further analysed in order to determine the reason for the high peak thrombin in these patients. There were more outliers in apixaban compared to rivaroxaban treated patients (18 outliers in 16 apixaban vs. 21 outliers in 20 rivaroxaban treated patients, p = 0.003; OR 2.5, 95% CI: 1.3–4.6). Indication for anticoagulation was VTE in 35 patients with concomitant AF in eight. One patient had a prior VTE on anticoagulation with rivaroxaban and another patient had pulmonary embolism three months after cancer surgery. His peak thrombin was within the expected range for the DOAC plasma level another seven months later. Four of the patients with pulmonary embolism (16.7%) had right heart failure which resulted in resuscition in two of them. Only one patient had no history of VTE but AF with history of stroke, resuscitation and coronary artery by-pass. There was a higher incidence of AF (22.9% vs. 3.6%, p < 0.001) and a trend to a higher incidence of pulmonary embolism (60.0% vs. 45.0%, p = 0.082) in VTE patients with at least one outlier compared to VTE patients without outliers. Age, sex and thrombophilia did not influence outliers in patients with VTE (supplementary table 5).

Outliers showed a higher factor VIII activity (240% [IQR 208–287%] vs. 218 % [178–255%], p = 0.006) and a trend to a higher factor IX activity (142% [IQR 124–160%] vs. 134% [IQR 117–151%], p = 0.097) but there was no difference in thrombin time, fibrinogen, factor II, D-dimer and prothrombin fragments.

Bleeding and thrombotic events at follow up

A total of 351 patients who had a follow up visit between April 1st 2020 and May 1st 2021 were analysed. During this time period four thrombotic events occurred. One patient with AF suffered a stroke under anticoagulation with rivaroxaban, one patient had retinal vein thrombosis under rivaroxaban and two patients had DVT after discontinuation of anticoagulation. None of these patients had peak thrombin above the median for their DOAC plasma level. One intracerebral bleeding occurred in a patient under apixaban. Peak thrombin was at the 56th percentile for the DOAC plasma level.

Prediction of normal values

The sensitivity, specificity, positive and negative predictive value and the area under the curve of the ROC for TG parameters within the normal range to predict a DOAC level < 30ng/mL are shown in Table 3 and for the prediction of DOAC plasma levels < 50ng/mL in the supplementary table 6. The resulting ROC curves for the prediction of DOAC levels < 30ng/ml are shown in Fig. 2 and for the prediction of DOAC levels < 50ng/ml in the supplementary Fig. 1.

Table 3
Sensitivity, specificity, positive (PPV) and negative predictive value (NPV) for the prediction of DOAC plasma levels < 30 ng/mL in patients with TG parameters within the normal range.

Assay	DOAC	Sensitivity		PPV		Specificity		NPV		ROC
		n	%	n	%	n	%	n	%	AUC
Lag time	Apixaban	42/44	95.5	42/218	19.3	32/208	15.4	32/34	94.1	0.622
	Rivaroxaban	199/222	89.6	199/460	43.3	219/480	45.6	219/242	90.5	0.781
TTP	Apixaban	41/44	93.2	41/196	20.9	53/208	25.5	53/56	94.6	0.723
	Rivaroxaban	190/222	85.6	190/307	61.9	363/480	75.6	363/395	91.9	0.880
Peak	Apixaban	39/44	88.6	39/132	29.5	115/208	55.3	115/120	95.8	0.819
	Rivaroxaban	196/222	88.3	196/319	61.4	357/480	74.4	357/383	93.2	0.893
Velocity index	Apixaban	41/44	93.2	41/164	25.0	85/208	40.9	85/88	96.6	0.789
	Rivaroxaban	197/222	88.7	197/327	60.2	350/480	72.9	350/375	93.3	0.893
ETP	Apixaban	41/44	93.2	41/179	22.9	70/208	33.7	70/73	95.9	0.739
	Rivaroxaban	205/222	88.7	205/429	47.8	256/480	53.3	256/273	93.8	0.857

Abbreviations: AUC: area under the curve, ROC: receiver operator curve

Thrombin generation in patients with thrombophilia

Thrombin generation in patients with drug levels < 30 ng/ml

Patients with thrombophilia had a shorter lag time (4.3 [IQR 3.7–5.1] vs. 4.8 [4.2–5.8] min, p = 0.001), a shorter TTP (9.3 [IQR 7.4–11.1] min vs. 9.8 [IQR 8.3–11.8] min, p = 0.033) and a trend to a higher ETP (3204 [IQR 2657–3558] nmol/L*min vs. 2999 [IQR 2599–3423] nmol/L*min, p = 0.072). Lag time was shorter in patients with Protein S deficiency (3.4 [2.9–3.7] min vs. 4.8 [IQR 4.2–5.8] min, p = 0.001) and in patients with Factor-V-Leiden-Mutation (4.4 [3.8-5.0] min vs. 4.8 [IQR 4.2–5.8] min, p < 0.005). Patients with Prothrombin-G20210A-Mutation a higher ETP (3456 [IQR 3102–3790] vs. 3013 [IQR 2602–3423], p = 0.002) when only blood samples with a DOAC level < 30 ng/mL were analyzed.

Thrombin generation in patients with drug levels > 30 ng/ml

Apixaban treated patients with thrombophilia had a higher velocity index at plasma levels between 101–200 ng/mL compared to those without thrombophilia (37 [IQR 20–72] nmol/min vs. 21 [IQR 14–41] nmol/min, p = 0.049) and a trend to a shorter lag time at plasma levels between 51–100 ng/mL (5.0 [IQR 3.9–5.7] min vs. 5.6 [4.5–6.4] min, p = 0.058). In addition, patients with a Prothrombin-G20210A-Mutation on apixaban had a shorter time to peak at

plasma level between 101–200 ng/mL (7.3 [IQR 6.2–11.3] min vs. 11.3 [9.7–15.0] min, p = 0.038) compared to patients without thrombophilia.

Apart from a shorter lag time at a drug level between 201–300 ng/mL and a Factor-V-Leiden-Mutation (6.6 [IQR 5.3–7.5] vs. 7.3 [6.0-8.5] min, p = 0.052), thrombin generation parameters were not influenced by thrombophilia in rivaroxaban treated patients.

TTP was shorter in apixaban compared to rivaroxaban treated patients with thrombophilia at drug level of 51–100 ng/mL (11.4 [IQR 9.9–12.3] min vs. 13.9 [IQR 11.2–16.8] min, p = 0.007). In addition, lag time and TTP were shorter and peak thrombin and ETP higher in apixaban (n = 20) compared to rivaroxaban (n = 32) treated patients with thrombophilia at DOAC plasma concentrations between 101–200 ng/mL

Discussion

In this study the effect of DOACs on TG measured with the fully automated Ceveron TGA was evaluated in a large real-life cohort of patients treated with apixaban and rivaroxaban.

Correlation of thrombin generation and DOAC plasma levels

The correlation of plasma drug levels with thrombin generation parameters was higher for rivaroxaban than for apixaban, although this correlation was generally moderate. Higher correlations were reported using CAT in healthy volunteers after a single DOAC dose [10] and in DOAC treated patients using the DrugScreen® reagent with the ST Genesia system [14]. These three TG systems use a mixture of phospholipids and tissue factor at a picomolar concentration for activation and thrombin for calibration. As the exact concentrations in ST Genesia are subject to the manufacturer`s discretion, no final conclusion can be drawn if the different activator and calibrator sets are the reason for the different behaviour of the DOACs in these systems.

Another reason for the different results might be the fact that in our study a large cohort of real-life patients was included and that there was no standardized time interval between DOAC intake and blood sampling. Artang and colleagues analysed plasma samples from a homogenous cohort of healthy male volunteers with a mean age of 41 years at 3 time points after the intake of the DOAC. Although the semiautomated CAT system was used, the correlation between DOAC plasma levels and TG parameters was very high. In the cohort of patients reported with the ST Genesia system, fifty percent of the apixaban and 60% of the rivaroxaban treated patients were newly started on DOACs or had a discontinuation of the drugs for more than 48h [14]. In those patients, blood was sampled at 3 time points after the intake of the DOAC. It is known that the individual base-line TG level has a high inter- and intraindividual variability [3,21,22]. The same DOAC plasma level in two different patients does not neccessarly translate into the same TG values [3]. Therefore, the higher correlation between the drug levels and the TG parameters reported with the CAT and the ST Genesia systems [10,14] may have been caused, at least in part, by the higher amount of blood samples taken from the same patient on the same day.

Comparison of the effect of apixaban and rivaroxaban on thrombin generation

The different behaviour of the DOACs regarding the correlation between drug levels but also the different sensitivity of the TG parameters for an increasing DOAC dose can be shown in our analysis. The higher dose response relationship of rivaroxaban compared to apixaban for the reduction of the peak thrombin has been described previously [12,13]. In our analysis, TG parameters continuously decreased with higher rivaroxaban plasma levels, while

apixaban exhibited a significant decrease in TG only at plasma levels between 20–100 ng/mL. The highest dose relationship was seen for both substances for peak thrombin but the effect on the lag time and time to peak was much more pronounced with rivaroxaban than with apixaban. The steep decrease in TG parameters at plasma levels < 100 ng/mL has been recently described by Evrard and colleagues with the CAT system [11]. In that analysis, the inhibition of peak thrombin at 100 ng/mL was comparable between apixaban and rivaroxaban but apixaban had a much lower effect on the time to peak as we have shown for the ceveron TGA in our cohort.

Prediction of normal values

The sensitivity analysis for the prediction of low plasma levels in patients with TG parameters within the reference range showed a high sensitivity and NPV meaning that almost all patients with low drug levels have a normal TG level and almost all patients with a low TG level (lag time and TTP above combined with peak thrombin and ETP below the reference range) have high plasma levels. In contrast, specificitiy and PPV were comparably low showing that high plasma levels are not necessarily correlated with a low TG level and normal TG parameters are found in patients with higher plasma levels too.

Translating this finding into clinical reality means, if one relies on low DOAC plasma levels for not giving an antidote to a patient prior to urgent intervention or in case of bleeding, this patient will most probably have a normal TG level and will not bleed. In contrast, if one relies on high DOAC plasma levels for giving an antidote, this may translate into giving an antidote to a large number of patients with normal TG parameters who might not have needed the antidote. Several studies in non-anticoagulated patients have shown that patients with a low TG level have a higher bleeding risk, while patients with a high TG level have a higher risk of thrombosis [19,23,24]. Anticoagulation lowers the TG level in a dose and substance specific manner and as a result, patients with a higher baseline TG level will have a higher TG level after DOAC intake [3] leading to a low predictability of DOAC plasma level by TG parameters and vice versa. As the cohort of patients in this study mainly consists of patients on anticoagulation for VTE, the baseline TG level of some of these patients is higher compared to the reference cohort (Fig. 1). These patients will have a higher TG level under standard DOAC therapy as well. Unfortunately, we had only a very limited number of patients with AF in our cohort so we could not perform a direct comparison between patients with VTE and AF.

Normal TG parameters in apixaban treated patients have a slightly higher sensitivity and NPV for the prediction of low plasma levels compared to rivaroxaban but PPV and specificity were much better for rivaroxaban. This observation has been shown for the fully automated ST Genesia system as well [14] and is caused by the higher substance specific reduction of TG by rivaroxaban [3]. The fact that specificity and PPV are lower in the TGA ceveron compared to the ST Genesia system [14] might be explained by the different activating agents and calibrators, but it could also be because of the different patient cohorts analysed in both studies.

Outliers and events at follow up

Most patients with plasma samples defined as outliers had VTE mainly with pulmonary embolism combined with a significant rate of right heart failure and resuscitation. In addition, there was a higher coincidence of AF and one patient had prior recurrent VTE under anticoagulation. Factor VIII activity was higher in those plasma samples but age, sex and thrombophilia did not have a significant impact. This shows that these patients are high risk patients defined by clinical characteristics rather than thrombophilia who have a highly upregulated peak thrombin even under anticoagulation. Nevertheless, only one of these patients had recurrent VTE at follow up and that was a provoked PE after cancer surgery. In contrast, patients with thrombotic events at follow up had TG values within the expected range for the DOAC plasma level, but there were only two events under anticoagulation (one stroke and one

retinal vein thrombosis) and no DVT or PE in these patients. However, these findings have to be interpreted with caution, because our study was not powered for the prediction of thrombotic events due to the limited number of patients, the short observation time and the fact that the time interval between DOAC intake and blood sampling is unknown. Previous studies have shown a predictive value of TG for the identification of patients at risk for recurrent VTE after discontinuation of anticoagulation [19,25] and more recently for the prediction of first VTE in elderly patients [26]. However, further studies including more patients with a longer follow up are needed to see whether TG might become a tool for the prediction of thrombotic events when measured under stable anticoagulation.

There are several limitations in this study. Plasma samples were taken only at one time point after the intake of the DOAC and the time after DOAC intake and the DOAC dose were not documented. As a result, we have no data about the individual kinetic of the DOAC plasma levels compared to the TG parameters. In addition, we included a rather inhomogenous cohort of thrombosis patients with and without thrombophilia and some patients with AF. However, the correlation between TG parameters and DOAC plasma levels was comparable between these subgroups of patients, so the effect of the inhomogeneity should be limited.

Summary

In summary, this study represents the largest analysis of the effect of apixaban and rivaroxaban on the TG measured with the fully automated Ceveron TGA. Rivaroxaban had a stronger effect on TG than apixaban but the correlation between DOAC plasma levels and TG parameters was only moderate. Thrombin generation assays may contribute to a better understanding of the hemostatic course of selected patients and possibly to treatment decisions, such as the administration of DOAC antidots. Clinical outcome studies are needed to further define the role of TG in the prediction of bleeding and thrombotic events in DOAC treated patients.

Declarations

Author Contributions:

CP was responsible for the design of the study, statistical analysis, and writing the manuscript. LCB was responsible for the design of the study, data collection, statistical analysis, and revising the manuscript. HB was responsible for the design of the study, and revising the manuscript. TS was responsible for the design of the study, laboratory analysis, and revising the manuscript. MM was responsible for revising the manuscript. SP and DF were responsible for the design of the study, and revising the manuscript, AS was responsible for the design of the study, laboratory analysis, and revising the manuscript.

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Competing Interests:

The authors have no competing interests.

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Figures

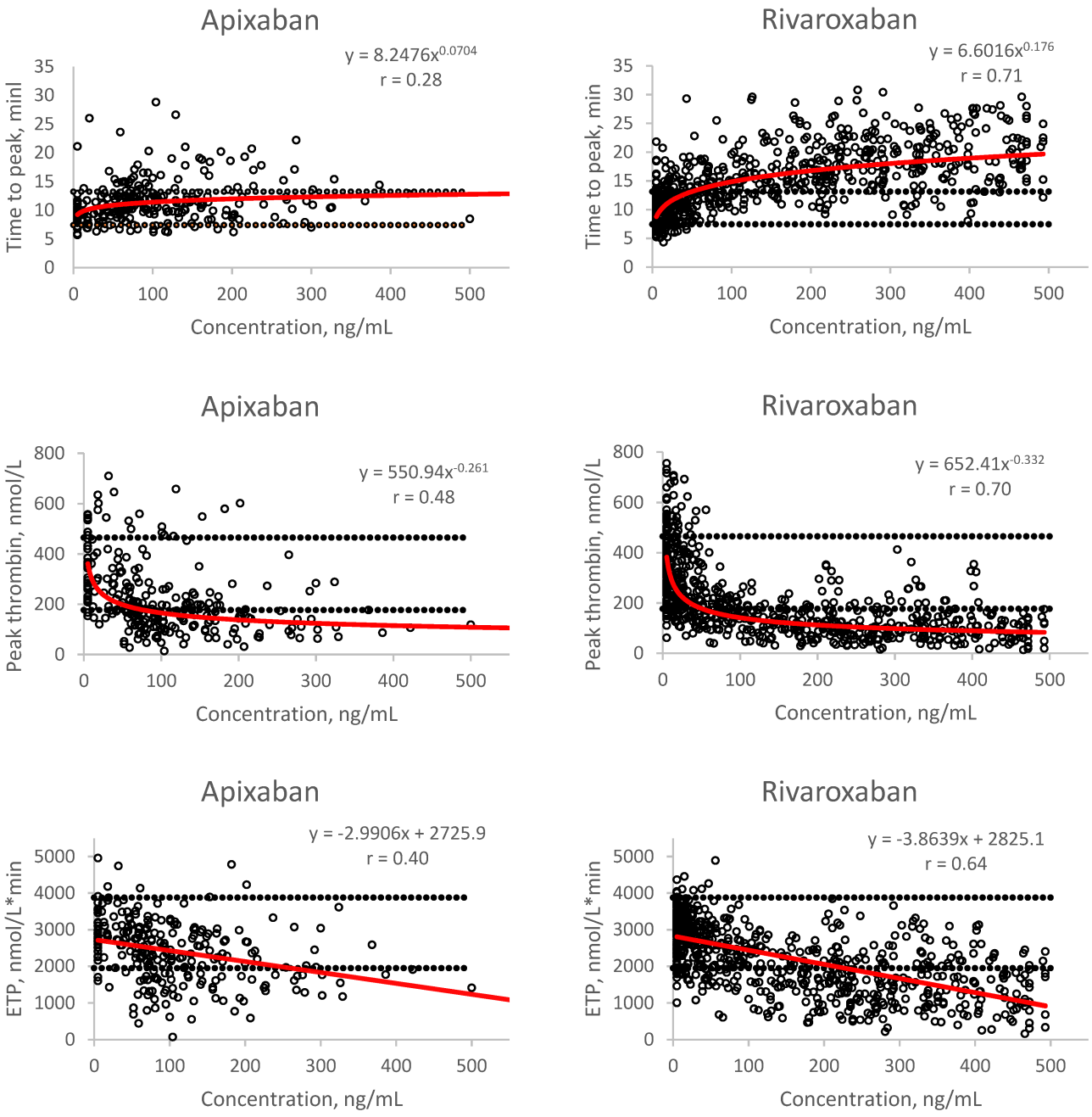

Figure 1

Correlation between direct oral anticoagulant (DOAC) plasma levels and the thrombin generation parameters time to peak, peak thrombin and endogenous thrombin potential (ETP). Solid line: regression curve with the correlation coefficient r; dotted lines: 2.5th and 97.5th percentile of the 50 healthy controls.

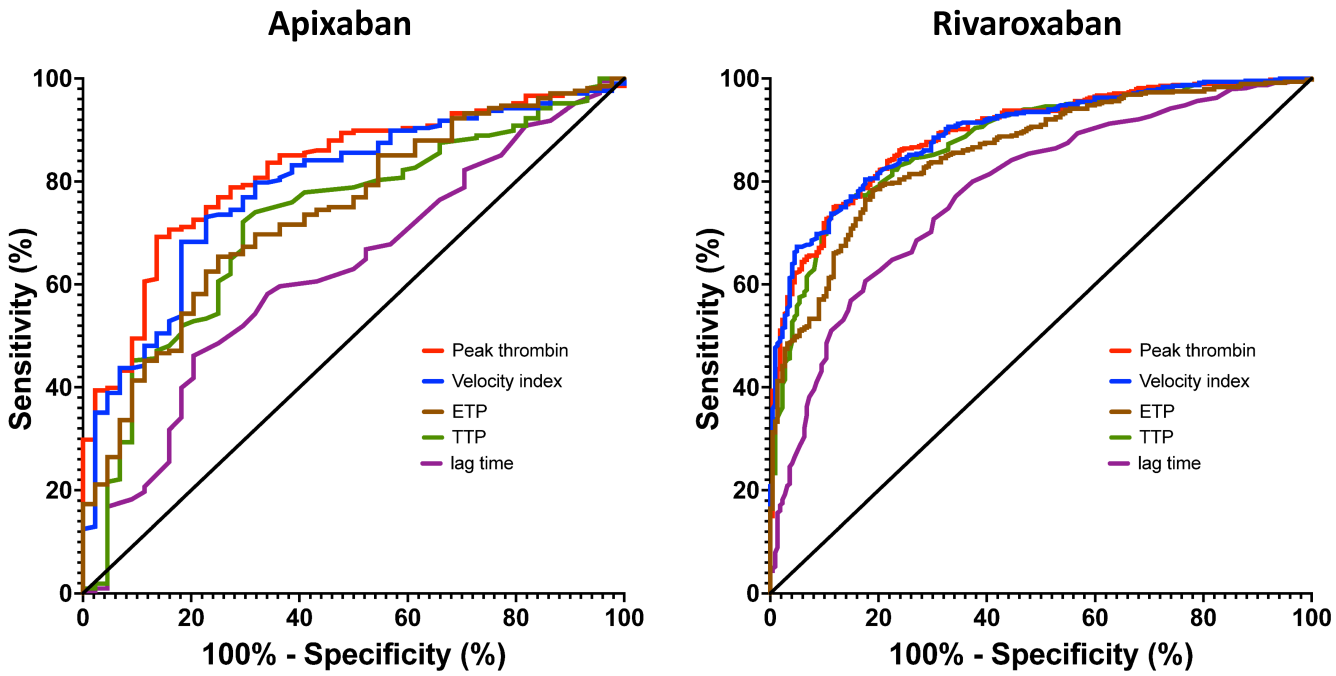

Figure 2

Receiver operater curve for the prediction of DOAC plasma levels < 30 ng/mL in patients with TG parameters within the normal range.

Supplementary Files

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- Supplementarymaterial.docx