Received: 20 November 2019 | Revised: 12 January 2020 | Accepted: 2 February 2020

DOI: 10.1002/rth2.12332

ORIGINAL ARTICLE

rpth research & practice in thrombosis & haemostasis

Inhibition of thrombin generation 12 hours after intake of direct oral anticoagulants

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Funding information
Stago provided Genesia analyzer and reagents

Handling Editor: Suzanne Cannegieter

Abstract

Background: The residual antithrombotic activity 12 hours after intake of direct oral anticoagulants (DOACs) is of clinical relevance in the setting of bleeding or urgent surgery.

Objective: To evaluate the effects of DOACs on thrombin generation 12 hours after DOAC intake in comparison to baseline and a healthy control group.

Methods: Eighty patients were recruited, 20 patients for each approved DOAC: apixaban, edoxaban, rivaroxaban, and dabigatran. The patients were either to be put on anticoagulation for the first time or had stopped taking oral anticoagulation for at least 48 hours. Blood plasma was sampled before (baseline) and 12 hours after starting DOAC for quantification of drug levels and thrombin generation assayed using an automated system (ST Genesia). Sixty-one blood donors served as control group.

Results: The factor Xa inhibitors significantly increased lag time (137%-219%) and reduced thrombin peak (47%-76%) and velocity index (17%-44%) after 12 hours compared to baseline. Dabigatran showed prolongation of lag time to 133% and time to peak to 119%. All patients had residual antithrombotic activity, with reduced thrombin generation parameters 12 hours after DOAC intake compared to baseline and to the healthy control group. This effect remained significant in patients with low residual DOAC plasma levels <50 ng/mL.

Conclusion: Thrombin generation remains reduced 12 hours after DOAC intake. While thrombin peak is particularly modified by factor Xa inhibitors, all DOACs prolong the lag time and time to thrombin peak. In the setting of bleeding or urgent surgery, the automated thrombin generation assay may assist in decision making and antidote administration.

KEYWORDS

anticoagulants/therapeutic use, blood coagulation, dabigatran/therapeutic use, factor Xa inhibitors/therapeutic use, thrombin/analysis, thrombin/drug effects

Michael Metze and Christian Pfrepper contributed equally to this work.

Essentials

- Knowledge of residual antithrombotic activity (AA) 12 hours after direct oral anticoagulant (DOAC) intake is scarce.
- AA was analyzed using thrombin generation (TG) and compared to patient's baseline and healthy controls.
- TG measured 12 hours after DOAC intake was reduced in comparison to baseline and control.
- TG remains reduced even in patients with low drug levels <50 ng/mL.

1 | INTRODUCTION

Permanent or temporary antithrombotic therapy is increasingly prescribed with the aging of the population in industrialized countries. Approximately 1 in 6 patients will require perioperative anticoagulation management including urgent surgery every year.[1] Furthermore, about 1% of the patients need treatment for acute DOAC-associated bleeding.[2] Therefore, the assessment of the residual antithrombotic activity is crucial for clinical decision making. Antithrombotic activity is usually defined by drug plasma level. However, global hemostasis tests such as thrombin generation (TG) assays are becoming increasingly important. These tests allow assessment of the biological effect on the coagulation system, and measure hyper-and hypocoagulability.[3]

Standard laboratory assays (prothrombin time, activated partial thromboplastin time) correlate too little with plasma levels or the biological effect of a certain agent, as well as with the bleeding risk of the patient.[4-6] Residual plasma levels can be measured by chromogenic assays calibrated to the anti-IIa or anti-Xa activity. However, those tests are insensitive to low levels of factor Xa inhibitors (eg, 20-30 ng/mL).[5] This makes them useful for qualitative decision, whether the patient has residual DOAC activity or not. In patients on dabigatran, the thrombin time is very sensitive to low dabigatran concentrations (eg, 10 ng/mL) and may rule out residual activity.[5] TG assays that allow a detailed assessment of the intrinsic coagulation activity have not been validated in patients taking DOACs.

The Subcommittee on Control of Anticoagulation of the ISTH recommends that a DOAC concentration >50 ng/mL in patients with serious bleeding, might be sufficiently high to warrant administration of an antidote. In those patients requiring urgent intervention associated with a high bleeding risk, antidote administration should be considered if the drug concentration exceeds 30 ng/mL.[7] However, additional clinical and experimental data are required to support this expert-based recommendation.

Finally, reliable data on the residual plasma level of a DOAC and its biological effect 12 hours after the last dose are lacking. Taking renal clearance into consideration, the DOAC plasma concentration may be estimated based on pharmacological data (12 hours equates to about 1.5 half-life values), but the biological effect of the inhibition of the clotting system after 12 hours compared to the baseline is currently unknown. This information could provide important clinical guidance in patients with bleeding or requiring urgent or emergency surgery.

2 | METHODS

2.1 | Study design and participants

The study was approved by the ethics commission of the University of Leipzig (reference numbers: 207/16-ek and 438/17-ek) and conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all study participants before inclusion.

We enrolled patients to be newly started on a DOAC as well as those in whom the DOAC was discontinued for at least 48 hours for elective coronary angiography or planned cardiac device implantation. We enrolled 20 consecutive patients for each of the following 4 anticoagulants: apixaban, edoxaban, rivaroxaban, and dabigatran. Exclusion criteria were age < 18 years, lack of informed consent, current pregnancy and lactation, autoimmune disease, severe anemia (hemoglobin < 8 g/dL or <5.0 mmol/L), or sepsis.

Additionally, we enrolled 61 consecutive healthy adult blood donors as controls. Exclusion criteria were age < 18 years, hormone replacement therapy, oral contraceptives, antithrombotic medication, pregnancy, or lactation. Demographic data including age, sex, and blood group were obtained.

2.2 | Patient characteristics

The following details were recorded in each patient: age, sex, weight, height, creatinine clearance calculated using the Modification of Diet in Renal Disease equation, comedications, indication and type of DOAC, and hours of discontinuation.

2.3 | Sample collection, storage, and processing

2.3.1 | Blood sampling and storage

Blood samples were obtained from each patient at the following time points: before intake of the anticoagulant ($t_{0\ h}$) and 12 hours after drug intake ($t_{12\ h}$). All patients were taking the recommended dose: 5 mg apixaban, 60 mg edoxaban, 20 mg rivaroxaban, and 150 mg dabigatran, respectively. Blood samples were drawn according to current standards[8,9] using 5 mL 9NC S-Monovette tubes (Sarstedt, Germany). The samples were processed within 1 hour of the blood draw. Samples were centrifuged at 1500 *g* for

2 × 10 minutes at 20°C. Platelet-free aliquots were then immediately stored at −80°C. The aliquots were only thawed at the time of analysis.

2.4.1 | DOAC concentration measurements

All DOAC concentrations were measured using commercial assays, dedicated calibrators, and controls according to the manufacturer's recommendations: diluted Hemoclot thrombin time with the corresponding calibrator (Hyphen Biomed, Neuville-sur-Oise, France); factor Xa inhibitors: STA-liquid anti-Xa (Diagnostica Stago, Parsippany, NJ, USA) with drug-specific calibrators of apixaban, edoxaban, and rivaroxaban (Diagnostica Stago). All measurements apart from TG were performed using the BCS analyzer (Siemens Healthineers, Erlangen, Germany).

2.4.2 | Thrombin generation

TG measurements of all patients and controls were performed using the ST-Genesia Thrombin Generation System (Diagnostica Stago) with the reagent STG-DrugScreen[10] according to manufacturer's instructions. The measurement principle is similar to the semiautomated calibrated automated thrombogram (CAT)[11] with differences in temperature control and calibration.[12] The ST Genesia uses dedicated reagents, calibrators, reference plasmas, and quality controls. STG-DrugScreen contains a mixture of phospholipids and recombinant transferrin at a relatively high picomolar concentration and uses human thrombin in buffer for calibration. Detailed information has been previously described.[10,12] The following parameters were measured: lag time, peak thrombin, time to peak, endogenous thrombin potential (ETP), and velocity index. Incubation was carried out at 37°C for 5 minutes.

2.5 | Statistical Analysis

Descriptive statistics are given as either mean ± standard deviation (SD) or median with interquartile range (IQR) for quantitative variables and numbers (percentages) for qualitative data. Groups were tested using either *t*-test when normally distributed or Mann-Whitney *U*-test otherwise. A *P* value < .05 was considered statistically significant. Computations were performed using the Prism 7.0 statistical package (GraphPad Software, San Diego, CA, USA).

3 | RESULTS

3.1 | Demographics

We included 43 male and 37 female patients. The descriptive characteristics of the patients, associated comorbidities, and creatinine clearance are shown in Table 1. The subgroups according to DOAC intake were similar without significant differences. The patients in the dabigatran group were slightly younger and had better renal function. There were 52 patients (65%) who received a new DOAC prescription. Sixty patients (75%) were taking a DOAC for atrial fibrillation, 18 patients (23%) for venous thromboembolism, and 2 patients (3%) for ventricular thrombi.

A full data set with plasma levels, global coagulation parameters, and TG results was available for 79 patients. The 12-hour sample was not available for 1 patient in the edoxaban group because the patient was discharged prematurely.

The control group was recruited by the blood donation service and consisted of men and women without known history of disease or medication. The mean age of the control group was 43 ± 15 years, and included 38 men (mean age, 42 ± 15 years) and 23 women (mean age, 44 ± 16.3 years).

3.2 | Drug plasma levels

Of the 28 patients who had stopped their DOAC for 48 hours, 9 patients had residual plasma levels at baseline (28 ± 19 ng/mL). Two patients had a plasma levels >50 ng/mL (63 and 57 ng/mL), the remaining 26 patients had a plasma levels <30 ng/mL. Mean plasma levels 12 hours after DOAC intake were apixaban, 42 ± 13 ng/mL;

TABLE 1 Demographic characteristics of the DOAC patients and healthy control group

	Age	CrCl	AF/VTE	Hypertension (%)	Diabetes (%)	CAD (%)	DAPT (%)
Apixaban	72 ± 10	66 ± 18	85%/15%	80	50	65	35
Edoxaban	73 ± 10	65 ± 15	95%/5%	85	40	20	5
Rivaroxaban	72 ± 13	67 ± 18	60%/40%	75	30	35	20
Dabigatran	59 ± 17	78 ± 19	60%/40%	55	25	30	20
Total	69 ± 14	69 ± 18	75%/25%	73	36	38	20
Control group	43 ± 15	No history of disease or medication					

Note: Data are expressed as mean ± standard deviation.

Abbreviations: AF, atrial fibrillation; CAD, coronary artery disease; CrCl, creatine clearance; DAPT, dual antiplatelet therapy; VTE, venous thromboembolism.

TABLE 2 Residual drug plasma levels at baseline after at least 48 h of discontinuation and 12 h after DOAC intake

	Baseline $t_{0\,h}$ (n = 28)		After 12 h $t_{12\,h}$ (n = 79)	
	<50 ng/mL	<30 ng/mL	<50 ng/mL	<30 ng/mL
Apixaban	100%	100%	80%	25%
Edoxaban	95%	95%	16%	11%
Rivaroxaban	95%	95%	10%	0%
Dabigatran	100%	100%	65%	50%
Total	98%	98%	43%	22%

Note: Data are expressed as percentage referring to 100% of those patients who had stopped their DOAC for 48 h (n = 28). The reminder of 52 patients had new DOAC treatment. Therefore, no residual drug level was measured. The second column comprises measurements of all patients after 12 h, while the measurement of 1 patient taking edoxaban was not obtained.

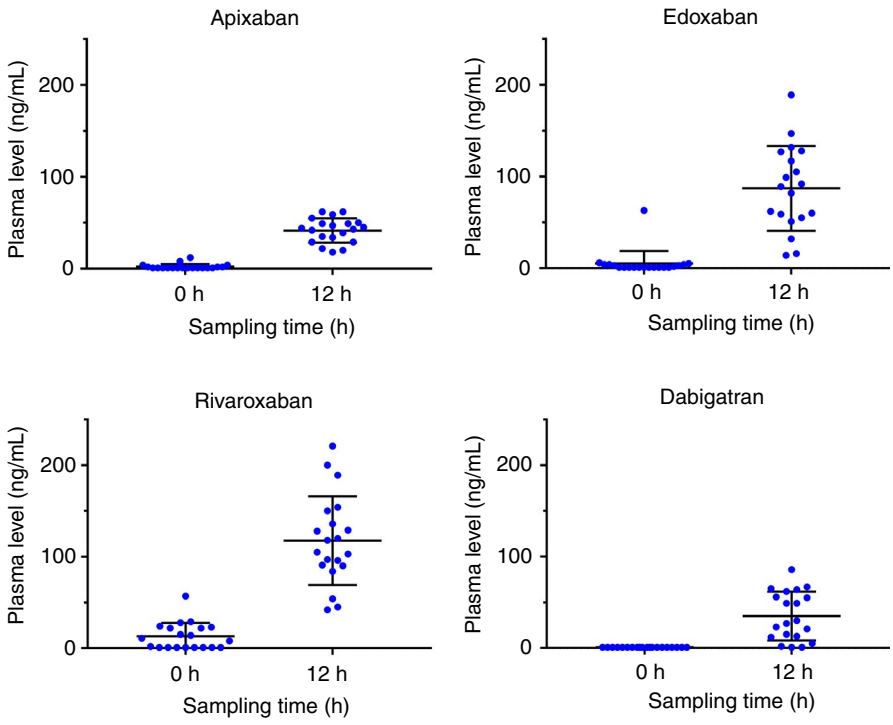

FIGURE 1 Plasma levels of the different DOACs at baseline ($t_{0\,h}$) and 12 h ($t_{12\,h}$) after intake. DOACs, direct oral anticoagulants

edoxaban, 83 ± 49 ng/mL; rivaroxaban, 118 ± 48 ng/mL; and dabigatran, 35 ± 26 ng/mL (Table 2, Figure 1).

3.3 | TG in DOAC patients

3.3.1 | Factor Xa inhibitors

Apart from ETP, all TG parameters were significantly influenced by the DOAC (Figure 2A-C). The prolongation of the lag time and reduction of thrombin peak were more pronounced in rivaroxaban and edoxaban (drugs based on once-daily intake) compared to apixaban (twice daily). The recovery of lag time and thrombin peak to baseline was not complete 12 hours after intake but higher in patients taking apixaban (137% from baseline) compared to rivaroxaban or edoxaban (200%-219% from baseline, Table 3). All patients had significant residual reduction of TG at 12 hours, suggesting a sustained antithrombotic effect.

3.3.2 | Dabigatran

The TG for the dabigatran patients showed a significant prolongation of the lag time and a shift of the time to peak to the right, while preserving the thrombin peak and ETP at the plasma peak and after 12 hours. Lag time and the time to peak did not recover after 12 hours (Figure 2D).

Table 3 shows the results of comparisons of coagulation parameters at baseline ($t_{0\,h}$) compared to those 12 hours after intake ($t_{12\,h}$). This effect was always associated with elevated plasma levels.

The ETP measurements in the dabigatran group were significantly higher than in the group of factor Xa inhibitors. Mean age in the dabigatran group was significantly younger, due to the inclusion of

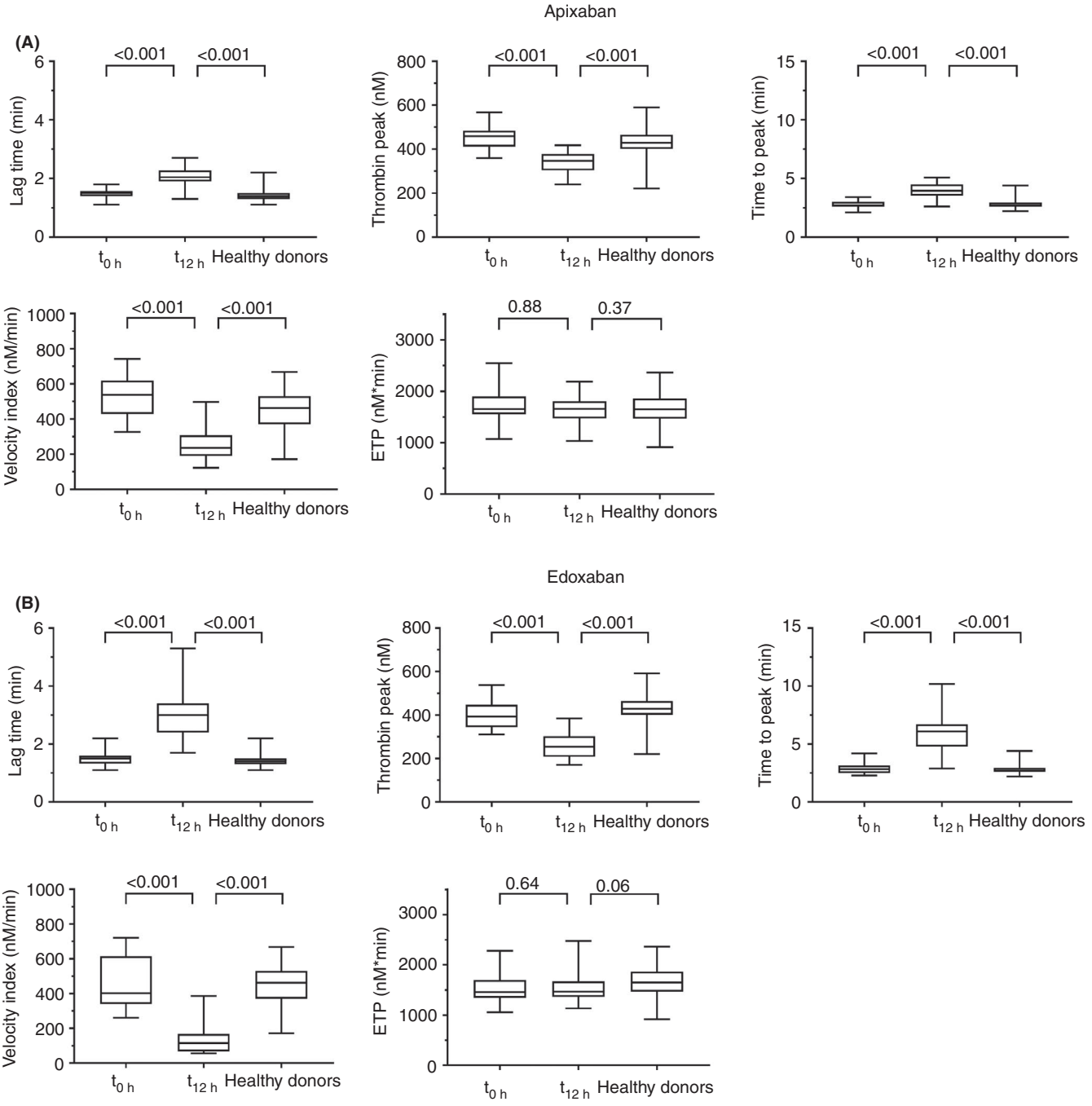

FIGURE 2 A, Apixaban group: thrombin generation parameters at baseline ($t_{0\,h}$), 12 h after drug intake ($t_{12\,h}$) compared to baseline and controls (numbers represent *P* values from Mann-Whitney *U*-test). B, Edoxaban group: thrombin generation parameters at baseline ($t_{0\,h}$), 12 h after drug intake ($t_{12\,h}$) compared to baseline and controls (numbers represent *P* values from Mann-Whitney *U*-test). C, Rivaroxaban group: thrombin generation parameters at baseline ($t_{0\,h}$), 12 h after drug intake ($t_{12\,h}$) compared to baseline and controls (numbers represent *P* values from Mann-Whitney *U*-test). D, Dabigatran group: thrombin generation parameters at baseline ($t_{0\,h}$), 12 h after drug intake ($t_{12\,h}$) compared to baseline and controls (numbers represent *P* values from Mann-Whitney *U*-test)

younger women with acute venous thromboembolism and therefore hypercoagulable state.

3.4 | TG in healthy blood donors

The detailed TG parameters of the control group are given in Table 3.

3.5 | Comparison of DOAC patients and healthy blood donors

TG parameters in patients taking factor Xa inhibitors, especially lag time and thrombin peak, were significantly reduced 12 hours after DOAC intake compared to that of the donors and baseline levels, except for ETP (Figure 2A-C). In dabigatran patients, only the lag time

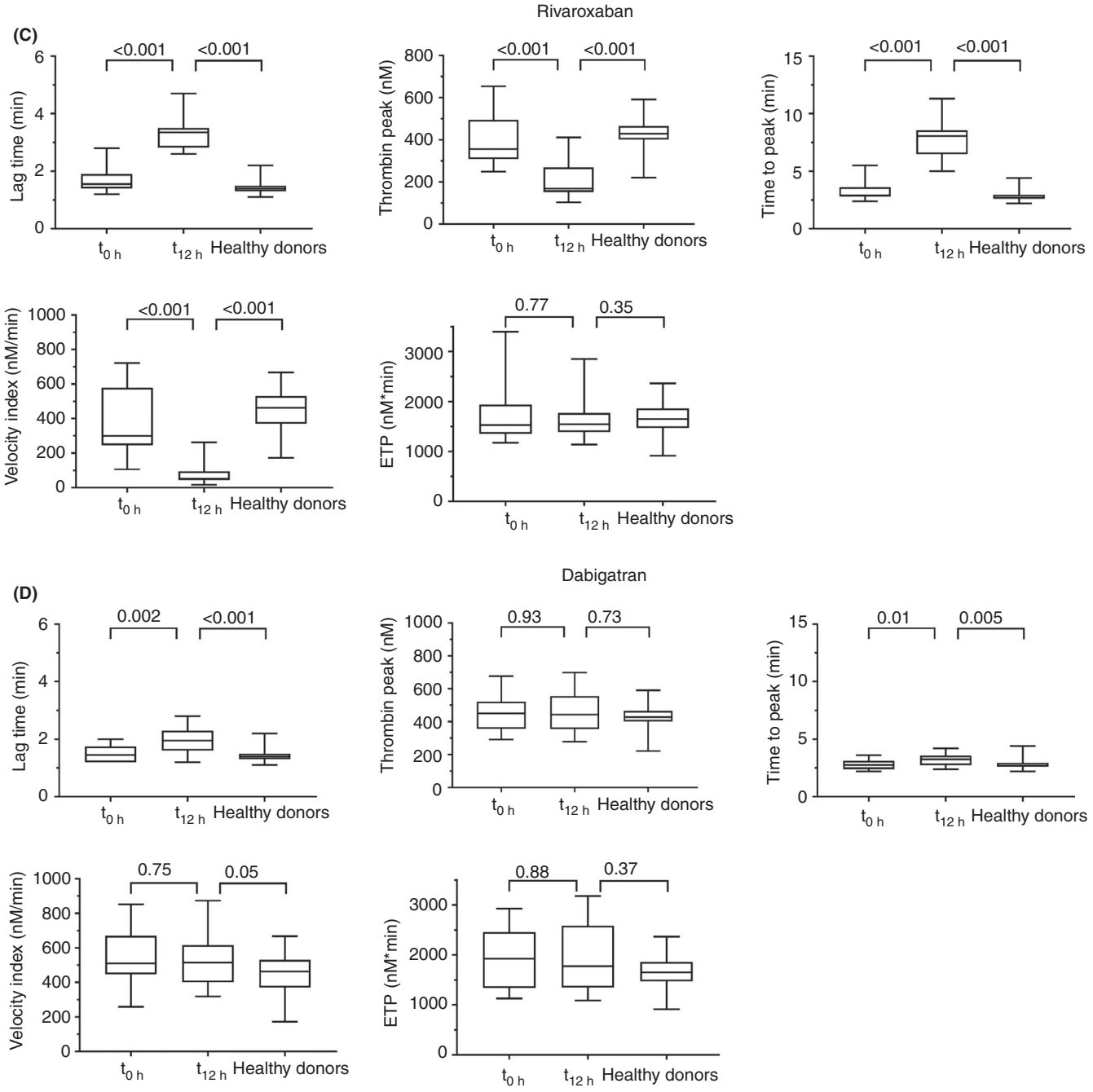

FIGURE 2 (Continued)

at 12 hours was significantly different from the patient's baseline and the lag time of the healthy donors.

3.6 | TG in patients with residual plasma levels < 50 ng/mL

After 12 hours, 34 of 80 DOAC patients (43%) had a drug plasma level <50 ng/mL (mean, 29 ± 16 ng/mL). The median TG parameters in this subgroup were lag time, 2.0 minutes (2-2.2); thrombin peak, 363 nmol/L (329-418); time to peak, 3.6 (3-4.1); velocity index, 327 nmol/L/min (229-490); and ETP, 1726 nmol/L (1435-1944). Apart from ETP, all TG parameters were significantly different compared to baseline values and to those of the controls ($P < .01$).

4 | DISCUSSION

We report on TG using a new automated system in patients taking DOACs in a real-world population. The main finding was that TG is significantly reduced 12 hours after DOAC intake compared to baseline as well as to those of healthy controls. Second, TG was still significantly reduced from baseline when only patients with DOAC plasma levels <50 ng/mL were considered.

TABLE 3 Effect of different fixed-dose DOACs on thrombin generation at baseline ($t_{0\,h}$) and 12 h after intake ($t_{12\,h}$) and thrombin generation parameters of healthy control group

	Lag time, min	Thrombin peak, nmol/L	Time to peak, min	Velocity index, nmol/L/min	ETP, nmol/L*min	Plasma level, ng/mL
Apixaban						
$t_{0\,h}$	1.5 (1.4-1.5)	459 (421-480)	2.7 (2.6-3.0)	538 (460-612)	1656 (1601-1893)	42 ± 13
$t_{12\,h}$	2.0 (1.9-2.3)	348 (306-372)	3.9 (3.6-4.5)	235 (196-303)	1659 (1474-1802)	
$t_{12\,h}/t_{0\,h}$	137%[a]	76%[a]	146%[a]	44%[a]	100%[b]	
Edoxaban						
$t_{0\,h}$	1.5 (1.4-1.6)	393 (346-441)	2.8 (2.5-3.1)	403 (344-607)	1458 (1344-1681)	83 ± 49
$t_{12\,h}$	3.0 (2.4-3.4)	247 (207-298)	6.1 (4.8-6.7)	109 (68-162)	1451 (1341-1670)	
$t_{12\,h}/t_{0\,h}$	200%[a]	63%[a]	214%[a]	27%[a]	99%[b]	
Rivaroxaban						
$t_{0\,h}$	1.5 (1.4-1.9)	356 (308-476)	2.9 (2.8-3.6)	300 (248-507)	1529 (1388-1901)	118 ± 48
$t_{12\,h}$	3.3 (2.8-3.5)	169 (155-223)	8.1 (6.6-8.4)	52 (45-93)	1547 (1433-1755)	
$t_{12\,h}/t_{0\,h}$	219%[a]	47%[a]	278%[a]	17%[a]	101%[b]	
Dabigatran						
$t_{0\,h}$	1.5 (1.2-1.7)	451 (357-521)	2.8 (2.4-3.0)	510 (451-668)	1921 (1399-2394)	35 ± 26
$t_{12\,h}$	2.0 (1.6-2.3)	444 (358-527)	3.3 (2.7-3.6)	515 (412-616)	1772 (1368-2561)	
$t_{12\,h}/t_{0\,h}$	133%[a]	98%[b]	119%[a]	101%[b]	92%[b]	
Healthy control group						
All donors	1.4 (1.3-1.5)	429 (402-465)	2.7 (2.6-2.9)	463 (371-529)	1648 (1482-1855)	
Men	1.4 (1.3-1.5)	422 (399-461)	2.8 (2.6-3.0)	454 (349-495)	1664 (1465-1848)	
Women	1.4 (1.3-1.5)	440 (412-474)	2.6 (2.5-2.9)	529 (439-569)	1611 (1482-1813)	

Note: Data are expressed as mean ± standard deviation (plasma level) or median (thrombin generation) and interquartile range (25th-75th percentile). The change thrombin generation parameters in relation to baseline is given in percentage.

Abbreviations: DOACs, direct oral anticoagulants; ETP, endogenous thrombin potential.

[aa]$P < .001$.

[bb]Not significant (values were compared to the baseline using the paired *t*-test).

Less than 5% of the patients had a residual drug plasma level >30 ng/mL after discontinuation for 48 hours. This is consistent with the results of Wiesen et al[13] describing patients on rivaroxaban. Approximately 95% of the patients on DOACs were reported to have a residual drug plasma level <30 ng/mL after discontinuation for 49-72 hours,[14] while a discontinuation for <48 hours was associated with drug plasma levels >30 ng/mL in 36% of the DOAC patients.[15] The recent Perioperative Anticoagulant Use for Surgery Evaluation (PAUSE) study on patients with a normal creatinine clearance showed a residual activity <50 ng/mL in 90.5%-96.8% after the DOAC was discontinued for 48 hours.[1] Based on expert opinion, a residual plasma level of <30 or <50 ng/mL has been considered the acceptable preoperative anticoagulation level.[14] However, data providing insight into the biological effect on the coagulation system using thrombin generation at low DOAC plasma levels are scarce. Furthermore, the currently used anti-Xa assays to derive the drug plasma levels are not sensitive enough at levels <30 ng/mL. Dabigatran plasma levels <30 ng/mL can be calculated with high sensitivity using diluted thrombin time assays.[16] Therefore, automated TG assays may provide additional information at low residual plasma levels as shown in our study.

All DOACs significantly prolong the lag time and time to peak compared to baseline. The thrombin peak was markedly reduced by factor Xa inhibition, while dabigatran did not alter the thrombin peak. Most data on DOACs and TG are derived from studies with (mostly male) healthy volunteers or drug-spiked plasma samples. Artang et al[17] recruited 10 male healthy volunteers and measured a lag time prolongation and thrombin peak reduction with factor Xa inhibitors using CAT. In contrast to our results, where the change in the ETP was mild but not significant after 12 hours, those authors reported a significant reduction in ETP at plasma peak level after 2-4 hours. Dabigatran prolonged lag time and time to peak as well but did not significantly affect thrombin peak. Tripodi et al[18] reported a prolonged lag time and decrease in thrombin peak and ETP using apixaban-spiked plasma samples. As expected, the effect was more pronounced with thrombomodulin, activating the protein C system. Due to the significant effect of factor Xa inhibitors, thrombin peak has been suggested a better surrogate measure of the anticoagulant effect of factor Xa inhibitors.[19,20] Based on our data, time to peak may also be a good candidate for further investigation. Our measurements revealed differences in the strength of inhibition of TG parameters after 12 hours between different factor Xa inhibitors. Rivaroxaban and edoxaban (once-daily intake) were associated with greater and more sustained TG inhibition than apixaban (twice-daily intake). This effect on TG was

confirmed by Kreutz et al[21] in healthy volunteers using rivaroxaban and apixaban.

The effects on TG in warfarin-treated patients are different. Rigano et al[22] reported a stronger lag time prolongation (up to 10 minutes) and reduction of thrombin peak and ETP than in plasma with factor Xa inhibitors or dabigatran. Vitamin K antagonists combine the effects of thrombin and factor Xa inhibition, but no single TG parameter showed good correlation with the International Normalized Ratio. Thus, the influence of warfarin and DOAC on TG parameters cannot be compared in terms of a specific range. While warfarin patients exhibit a continuous anticoagulation throughout 24 hours, DOACs have introduced the concept "pulsed anticoagulation."

While most studies focus on the TG change at DOAC peak levels, those patients with residual levels <50 ng/mL need further evaluation. Bertaggia-Calderara studied TG in 12 obese patients after 10 mg rivaroxaban for 24 hours and showed the same change in TG pattern as in our patients.[23] The median rivaroxaban plasma level 12 hours after drug intake was 28 ng/mL, which was accompanied by a significant lag time and time to peak prolongation and thrombin peak reduction. An in vitro investigation using edoxaban-spiked plasma samples showed that thrombin peak is reduced by almost 30% at low plasma levels of about 50 ng/mL.[24] This may indicate that a substantial amount of TG inhibition is still present at low plasma levels. Our small data sample of patients with a DOAC plasma level <50 ng/mL reveal that TG is significantly altered. Further research to translate this laboratory assay finding into clinical outcomes is required.

Data between different TG assays may not be comparable for lack of standardization.[25] TG assays differ regarding the substrate used, sample preparation, calibration, and data processing. Technical and preanalytical challenges precluded the widespread use of TG in clinical applications. Since 2018, the ST- Genesia is available as a fully automated, calibrated TG assay. Data on ST-Genesia with the STG-DrugScreen reagent are scarce, but a first study reported excellent interexperiment precision.[10] Currently, only one study compared TG measurements of CAT and ST-Genesia. The results of ST-Genesia differed from results obtained with the CAT in a cohort of patients with liver transplants.[26] Thus, ST-Genesia and CAT cannot be compared directly in terms of absolute measurements. Different concentrations in tissue factors in ST Genesia and CAT used for TG activation may explain different TG values.[27]

The changes in coagulation status assessed by TG are mainly defined by lag time, thrombin peak, time to peak, and ETP. To date, there are no published clinical outcome data regarding these parameters in patients on DOACs. TG gives more insight into the effect of DOACs on the coagulation status than classical coagulation parameters such as prothrombin time and anti-IIa/Xa assays. Since the result of the TG assay depends on the concentration of tissue factor, further research is required to establish a standard that may allow better prediction of risk for bleeding or thrombotic events.[28] Furthermore, defining reference ranges for TG parameters in patients taking DOACs may aid clinical decision making (eg, residual antithrombotic activity in surgery, monitoring of antidote treatment in bleeding or thrombolysis in stroke patients).

5 | LIMITATIONS AND STRENGTHS

To our knowledge, this is the first study presenting real-world pharmacokinetic and TG data in patients taking DOACs 12 hours after intake. We were able to show inhibition of TG after 12 hours as well as at low plasma levels <50 ng/mL compared to baseline. This study has certain limitations. The absolute amount of TG may not be representative because measurements were performed after 48 hours of discontinuation instead of steady state in a subgroup of our patient population. Pharmacokinetics is influenced by several factors, including absorption, distribution, metabolism, and excretion that may differ with age, comediation, and nutrition. TG may exhibit a circadian rhythm interfering with the results.[28,29] The age of the control group was significantly younger than the patient group and may not be representative, and should be interpreted with caution.

6 | SUMMARY

While the evidence of the antithrombotic treatment effect of DOACs is well defined by phase III studies and registries, the perioperative and periprocedural management of patients with residual plasma levels remains challenging. Our study shows reduced thrombin generation 12 hours after DOAC intake, which applies to the clinical situation of emergency surgery or bleeding. Furthermore, we can confirm that TG is reduced in the subgroup with a plasma level <50 ng/mL. Clinical studies are required to assess the drug-specific inhibition of TG at low plasma levels and the clinical impact on bleeding complications.

ACKNOWLEDGMENTS

We acknowledge support of this work by institutional resources of the University of Leipzig. We thank Stago for kindly providing the ST-Genesia analyzer and related reagents.

AUTHOR CONTRIBUTIONS

MM was responsible for study concept, data collection, statistical analysis, and writing the manuscript. CP was responsible for study concept, data collection, statistical analysis, and revising the manuscript. TK and SS did patient recruitment and blood sampling. RS and TS performed laboratory measurements. TS revised the manuscript. EE provided blood samples of the healthy donors. UL supported the study by institutional sources and revised the manuscript. SP was responsible for study concept, revised, and finally approved the manuscript.

RELATIONSHIP DISCLOSURE

MM received speaker honoraria (Bayer, Pfizer), consulting honoria (Bayer, Boehringer-Ingelheim, Daiichi Sankyo), and research grant by Daiichi Sankyo. CP received speaker honoraria (Pfizer) and grant by Stago (lab equipment). SP reports grant and nonfinancial support from Stago (lab equipment). There are no competing financial interests in relation to the work described. The other authors declare nothing to report.

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How to cite this article: Metze M, Pfrepper C, Klöter T, et al. Inhibition of thrombin generation 12 hours after intake of direct oral anticoagulants. *Res Pract Thromb Haemost*. 2020;4:610–618. https://doi.org/10.1002/rth2.12332