Evaluation of Coaguchek®Pro II coagulation testing device performance to assess direct oral anticoagulant action. The DOAC-CHECK study

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Consent to participate: Written informed consent was obtained from each patient before enrollment. The promoter of the study provided the measures to safeguard the subject's privacy and the protection of personal data according to the EU GDPR 2016/679 and Italian law.

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ABSTRACT

Direct oral anticoagulants (DOAC) measurement is recommended in specific conditions. A point-of-care testing should be used in emergency to qualitatively rule out relevant DOAC concentrations. The DOAC-CHECK Study aims to evaluate whether the use of CoaguChek® Pro II (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) coagulation testing device can provide reliable information in patients treated with DOAC. The study was carried out in two FCSA (Italian Federation of Thrombosis Centers) centers. We choose 3 different concentration thresholds for our analysis (30, 50 and 100 ng/mL) and by ROC curves the ideal cut-off point was selected to be the one that yielded a sensitivity of at least 95% associated with the highest possible specificity. 512 patients were enrolled. For Edoxaban and Rivaroxaban, both CoaguChek® Pro II prothrombin time (PT) and activated partial thromboplastin time (aPTT) tests showed a sensitivity >95% corresponding to satisfying specificity values; negative predictive values resulted in the range 90-100%. At variance, CoaguChek® Pro II PT and aPTT tests did not seem to be useful for identifying Apixaban and Dabigatran concentrations higher than the pre-defined thresholds. Our results suggest that CoaguChek® Pro II coagulation testing device can be used to qualitatively identify relevant concentrations of Edoxaban or Rivaroxaban, but not of Apixaban or Dabigatran.

INTRODUCTION

Direct oral anticoagulants (DOACs), including Dabigatran (anti-IIa agent), and Apixaban, Edoxaban, Rivaroxaban (all anti-Xa agents), have been licensed in many countries for prevention and treatment of cardioembolic complications in patients with non-valvular atrial fibrillation (NVAF), and for prophylaxis, acute-long-term, and extended treatment of venous thromboembolism (VTE). One of the most valued characteristics of these agents, that has been an important factor for their fast-increasing use worldwide, is that they are administered at fixed and unmonitored doses, different in relation to the clinical indications of patients. Randomized controlled trials included almost one hundred thousand NVAF or VTE patients and proved that the use of these agents at fixed-dose was safer (especially for reduction

of intracranial hemorrhage occurrence) and as effective as dose-adjusted vitamin K antagonists (VKAs). Although DOACs do not need routine monitoring, some situations or specific patient conditions may require an assessment of anticoagulation levels, which is currently possible by using laboratory tests.[1,2] Unfortunately, for several reasons including the need of expert and dedicated laboratory staff, these tests have insufficiently been implemented in clinical laboratories, resulting in a generally lower than required measurement of DOAC activity levels. The lack of promptly available DOAC assessment is particularly important in the setting of urgent/emergent conditions, in which clinicians should face not only the scarcity of laboratories able to perform the specific tests but also, what is even more important, the time needed to receive results timely useful for the necessary clinical decisions. Point-of-care testing (POCT), performed where clinical care is delivered, can provide significant benefits regarding improvement of time to test results and would be particularly helpful in case of urgently needed surgery, operative procedures, or treatments (thrombolysis after stroke). Unfortunately, POCT specifically designed to measure DOAC activity are not available at present.[3] In contrast, POCT designed to measure VKAs or heparin activity by performing prothrombin time (PT) and activated partial thromboplastin time (aPTT) are available.[4] However, these tests do not accurately assess the levels of DOAC-induced anticoagulation.[5] Since the clinical decisions in some conditions do not require a precise measurement of DOAC activity but only to verify that the anticoagulant activity is below prespecified cutoff levels to allow safe clinical procedures, it is possible that the currently available POCT may be useful to this scope.

The present study aimed to evaluate whether CoaguChek® [Trademark of Roche] Pro II coagulation testing device (Roche Diagnostics International, Rotkreuz, Switzerland) use can provide reliable information in DOAC treated patients; in particular, we aimed to determine the diagnostic accuracy of this POCT to qualitatively rule out relevant DOAC concentrations (concentrations that are considered safe for surgery and thrombolysis) in real-life patients. The study promoted by Fondazione Arianna Anticoagulazione, was a national multi-center, prospective, observational and no-profit trial with blinded end-point assessment.

MATERIALS AND METHODS

Anonymized source data and study protocol will be made available to other researchers on request to the first Author.

Standard protocol approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and in compliance with the protocol. Independent review board approval was obtained prior to all study-related activity from the Ethics Committee of both Cremona and Vicenza hospitals. Written informed consent was obtained from each patient before enrollment. The promoter of the study provided the measures to safeguard the subject's privacy and the protection of personal data according to the EU GDPR 2016/679 and Italian law.

Setting and eligibility

The study was conducted in two tertiary care facilities affiliated to FCSA (Italian Federation of Thrombosis Centers): the Haemostasis and Thrombosis Center of Cremona hospital and the Department of Hematology of Vicenza hospital. Between October 2020 and May 2021, 512 outpatients treated with a DOAC (Apixaban, Edoxaban, Rivaroxaban or Dabigatran) [either for non-valvular atrial fibrillation (NVAF) or venous thromboembolism (VTE)], naïve or shifted from AVK or heparin, were consecutively enrolled in the participating centers at the moment of a routine follow-up visit. The inclusion criteria were: age $\geq$ 18 years, ability to give written informed consent to the blood sampling for the study purpose during a routine visit. Patients already enrolled in clinical trials from phase 1 to 3 were excluded. Most of the patients were enrolled at Cremona hospital (78.9%).

Patients were excluded from the study if: they were treated with other anticoagulants (VKAs within 14 days, low molecular weight heparin within 24 hours, or unfractionated heparin within 12 hours prior to enrollment), had changed the DOAC within 7 days, had spontaneous altered coagulation (PT INR > 1.2, aPTT >1.20 ratio), had known coagulopathies, or presented a lupus anticoagulant positivity and/or high antiphospholipid antibody levels. Use of antiplatelet agents was permitted.

Sample collection and measurements

Blood samples (venous and capillary) were taken during a routine clinical and laboratory control. Most of the samples (90.5%) were collected at trough level (12 hours after Apixaban or Dabigatran and 24 hours after Edoxaban or Rivaroxaban); the remaining samples were collected at pick level (2-4 hours after drug intake).

Baseline characteristics (age, sex, type of drug, clinical indication for anticoagulation, weight, body mass index, kidney and liver function, concomitant medications and diseases) were recorded in a structured database. The REDCap (Research electronic data capture) software was used for data entry. Results of CoaguChek® PRO II tests and of DOAC level measurements were kept blind to the patients and not used to take clinical decisions. Results were recorded in the patient's medical records and transcribed into the REDCap electronic case report form

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(eCRF). Various procedures in clinical data management including eCRF designing, eCRF annotation, database designing, data-entry, data validation, discrepancy management, medical coding, data extraction, and database locking were assessed for quality at regular intervals during the study period. This was an observational study, and the analysis was performed on the total monitored patient sample. Patients were excluded from analysis if they had no exact birth date so that the legal age was questionable, or no exact date of consent.

PT and aPTT tests on CoaguChek® Pro II coagulation testing device were performed using capillary blood obtained through a direct finger puncture using a lancing device. Two POCT instruments for each participating center were provided, completed with controls and strips.

Venous samples for specific DOAC measurements were collected concomitantly with use of POCT, in 0.109M sodium citrate (9:1); plasma aliquots obtained after centrifugation at 2000Xg for 15 min were initially stored at -80°C at the participating centers, and later shipped to the Haemostasis and Thrombosis Center of Cremona Hospital, where the specific drug measurements were performed. DOAC levels expressed as drug concentration-equivalent (ng/mL) were measured with STA-ECA II (Diagnostica Stago, Asnieres-sur-Seine, France) for Dabigatran, and STA-Liquid anti-Xa (Diagnostica Stago) for Apixaban, Edoxaban, and Rivaroxaban. All these tests were calibrated using commercial plasmas from the same supplier and performed on STA-R instrument (Diagnostica Stago).

CoaguChek® Pro II PT and aPTT tests and specific drug measurements were performed according to manufacturers' instructions by thoroughly trained investigators and technicians.

Blinding

CoaguChek® Pro II coagulation testing device operators were blinded of the results of the specific drug measurements as well as technicians performing specific drug measurements were blinded of the CoaguChek® Pro II coagulation testing device results.

Statistical analysis

Categorical variables are presented with absolute and relative frequency; continuous variables with median and range (min-max).

Diagnostic accuracy of CoaguChek® Pro II PT and aPTT tests (reported in seconds) regarding detection of clinically relevant DOAC plasma concentrations was evaluated in terms of sensitivity, specificity, positive and negative predictive values as well as likelihood ratios including respective two-sided 95% confidence intervals (CI). Since data to define relevant DOAC concentrations are currently limited we choose 3 different concentration thresholds for our analysis (30, 50 and 100 ng/mL).[6-10]

Sensitivity is defined as the percentage of samples containing clinically relevant DOAC plasma concentrations that tested positive by the CoaguChek® Pro II PT or aPTT tests and thus correctly identified as patient ineligible for thrombolysis/surgery or requiring reversal therapy. Specificity is defined as the percentage of samples containing no clinically relevant DOAC plasma concentrations that tested negative and were correctly identified by the CoaguChek® Pro II PT or aPTT tests.

Positive predictive value (PPV) is defined as the percentage of samples with clinically relevant DOAC concentrations out of all samples identified as containing clinically relevant DOAC levels by the CoaguChek® Pro II PT or aPTT tests; negative predictive value (NPV) is defined as the percentage of samples with no clinically relevant DOAC plasma concentrations out of all samples identified as containing no clinically relevant drug levels by CoaguChek® Pro II PT or aPTT tests.

Receiver operating characteristic (ROC) curves were drawn and the area under the ROC curves (AUROCs) were calculated for CoaguChek® Pro II PT and aPTT tests at the three different thresholds of DOAC plasma concentrations (>30, >50 and > 100 ng/mL) (Figures 1-3); AUROCs were considered as a measure of overall test performance and are given with the two-sided 95% CI. Using the ROC curves, for each test/DOAC threshold, as the ideal cut-off point was selected the one that yielded a sensitivity of at least 95% (misprediction percentage < 5%, considered sufficiently safe for clinical application) associated to the highest possible specificity, to avoid false-negative results but simultaneously to identify the largest number of patients eligible for emergency treatment such as thrombolysis or emergency surgery.

This study was performed in accordance with the STARD (Standards for Reporting Diagnostic Accuracy) guidelines for studies on diagnostic tests.[11]

All statistical analyses were performed using GraphPad Prism for MacOS version 9.3.1 (GraphPad Software, San Diego, CA, USA) and online MedCalc version 20.027 Ltd (MedCalc Software, Ostend, Belgium).

RESULTS

The characteristics of patient population and the DOAC treatments are described in Table 1. The median age of patients was 74 years (range 21-94) and 59.2% were males. Apixaban was used in most of the subjects (n=222, 43.4%), Edoxaban and Rivaroxaban in 23.4% (n=120) and 21.7% (n=111) of cases, respectively, while few enrolled patients were treated with Dabigatran (n=59, 11.5%). This drug distribution reflects the current real-life situation of DOAC prescription in Italy.

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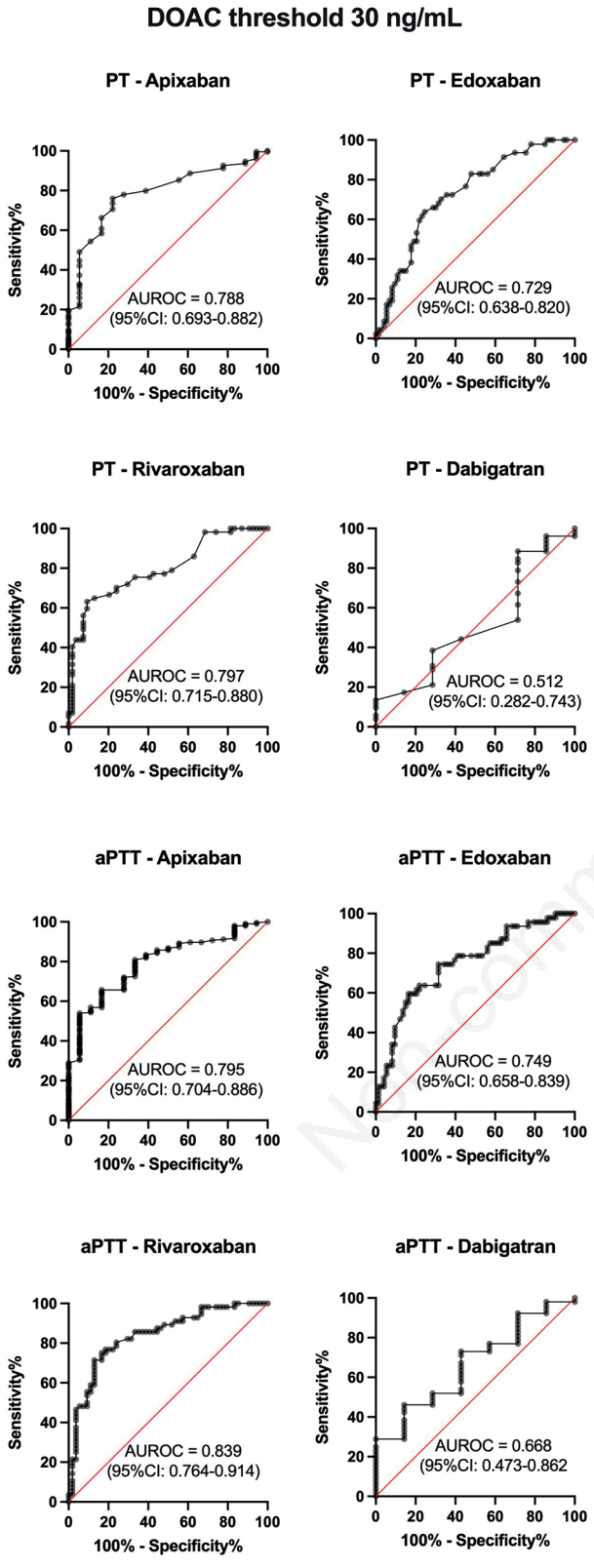

Figure 1. Receiver operating characteristic (ROC) curves for CoaguChek®Pro II PT and aPTT tests when testing for detection of samples containing 30 ng/mL DOAC concentrations.

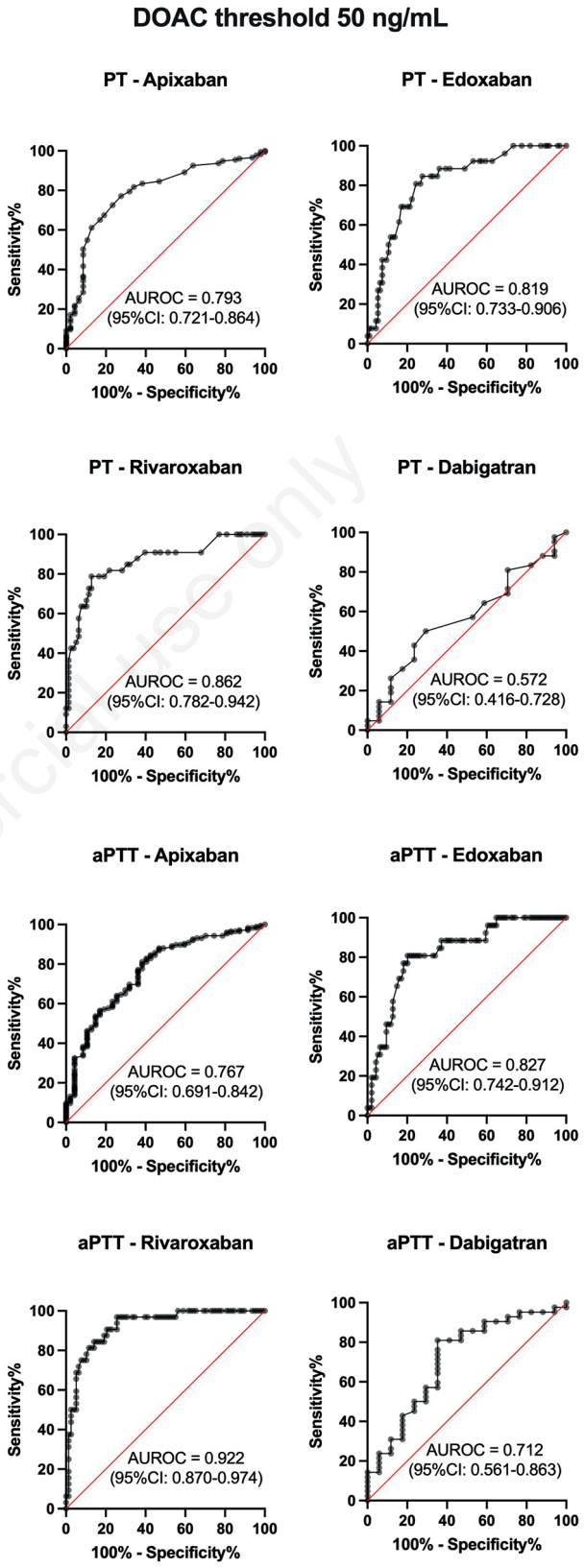

Figure 2. Receiver operating characteristic (ROC) curves for CoaguChek®Pro II PT and aPTT tests when testing for detection of samples containing 50 ng/mL DOAC concentrations.

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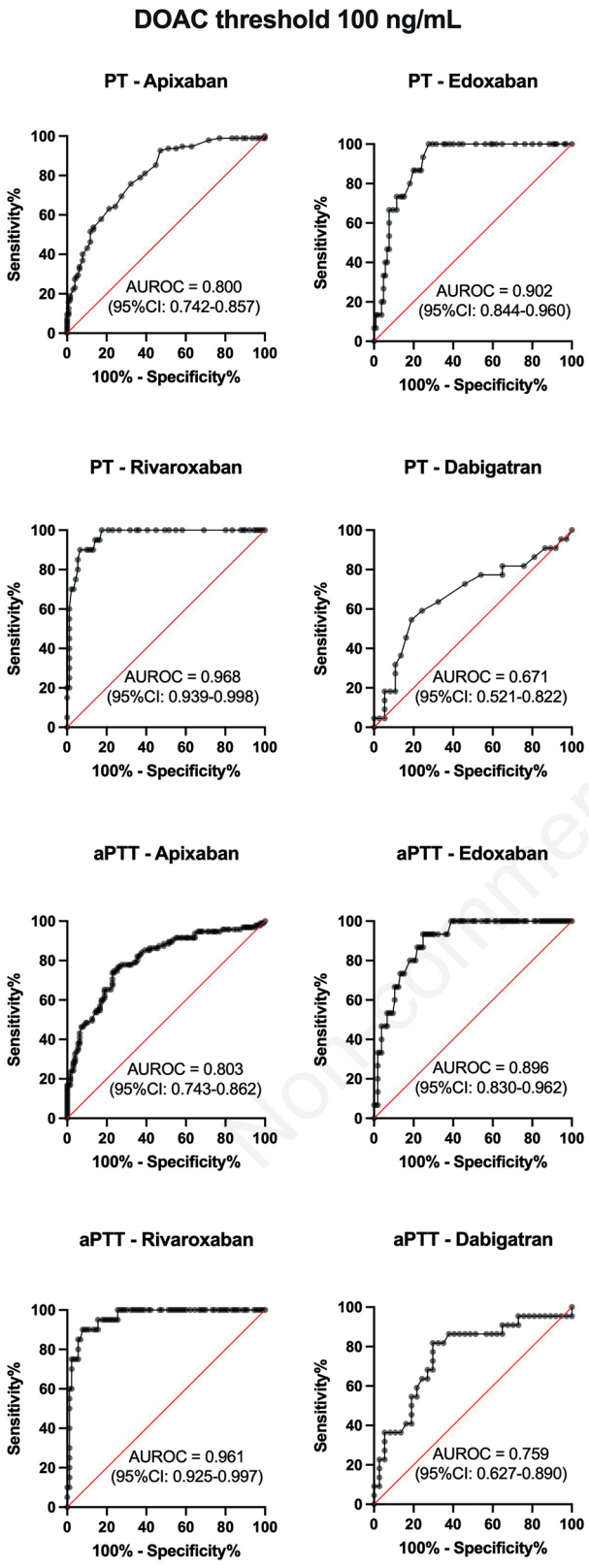

Figure 3. Receiver operating characteristic (ROC) curves for CoaguChek®Pro II PT and aPTT tests when testing or detection of samples containing 100 ng/mL DOAC concentrations.

Low doses of DOACs were used in about half of the patients [41.9% Apixaban (2.5 mg/BID); 55.0% Edoxaban (30 mg); 37.8% Rivaroxaban (10 or 15 mg); 42.3% Dabigatran (110 mg/BID)]. Approximately half patients were treated for NVAF and less than half for a previous VTE event (50.4% and 47.7%, respectively), whereas only 1.9% of cases were treated for both VTE and NVAF. Concerning the associated treatments, no significant differences between groups treated with the different DOACs were found regarding antiplatelet, antiarrhythmic, antidiabetic, antihypertensive, thyroid dysfunction and lipid lowering drugs; no patients were using antiviral or antiepileptic drugs. The only significant difference in co-medications was a higher prevalence (p=0.030) of gastroprotective use in patients treated with Dabigatran and Edoxaban (42.4 and 47.5%, respectively) vs those treated with Apixaban and Rivaroxaban (34.2 and 30.6%, respectively).

Edoxaban and Rivaroxaban median levels (25 and 31 ng/mL, respectively) resulted lower than that of Apixaban and Dabigatran (90 and 83 ng/mL, respectively); this probably because, differently for Apixaban and Dabigatran, Edoxaban and Rivaroxaban are taken once daily and blood sampling was drawn in the morning, at the trough level, in most patients.

Using the ROC analysis, we calculated optimized "ideal" cut-off values for CoaguChek® Pro II PT and aPTT tests (Table 2) which provide the > 95% sensitivity and the highest specificity for different threshold DOAC concentrations: 30 ng/mL (Fig. 1), 50 ng/mL (Fig. 2) and 100 ng/mL (Fig. 3). Based on AUROC values,[12] both CoaguChek® Pro II PT and aPTT tests resulted from moderately (0.7<AUROC≤0.9) to highly informative

Table 1. Characteristics of the 512 enrolled patients

Males, n (%)	303 (59.2)
Age, median (min-max), y	74 (21-94)
BMI, median (min-max), kg/m^2	26.9 (17.0-46.1)
eGRF, median (min-max), mL/min/1.73m^2	71.4 (18.7-150.5)
ALT, median (min-max), U/L	19.5 (4.0-85.0)
AST, median (min-max), U/L	21.0 (9.0-72.0)
DOAC treatment, n (%)	
Apixaban	222 (43.4)
Edoxaban	120 (23.4)
Rivaroxaban	111 (21.7)
Dabigatran	59 (11.5)
Indication for treatment, n (%)	
VTE	244 (47.7)
NVAF	258 (50.4)
VTE+NVAF	10 (1.9)

eGRF, Glomerular Filtration Rate (estimated by the Chronic Kidney Disease-Epidemiology Collaboration equation); NVAF, non-valvular atrial fibrillation; VTE, venous thromboembolism.

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(0.9<AUROC<1.0) for Edoxaban and Rivaroxaban for all threshold DOAC concentrations. AUROC values were lower for Apixaban compared to those obtained for Edoxaban and Rivaroxaban, while both CoaguChek® Pro II PT and aPTT tests seem not to be informative for Dabigatran at all DOAC threshold concentrations.

Table 2 reports the CoaguChek® Pro II PT and aPTT diagnostic test evaluation regarding detection of DOAC concentrations at 30, 50 and 100 ng/mL. Regarding Apixaban, for both CoaguChek® Pro II PT and aPTT tests, a sensitivity > 95% corresponded to low specificity values at all Apixaban thresholds, that reached the highest values at the 100 ng/mL Apixaban threshold (28.3 and 21.3% for CoaguChek® Pro II PT and aPTT tests, respectively); the NPVs were acceptable only at the 100 ng/mL Apixaban threshold (94.7% and 87.1% for CoaguChek® Pro II PT and aPTT tests, respectively). Conversely, for Edoxaban and Rivaroxaban both for CoaguChek® Pro II PT and aPTT tests a sensitivity > 95% corresponded to satisfying specificity values, especially at the 50 and 100 ng/mL threshold DOAC concentrations; the NPVs resulted in the range 90-100% both for CoaguChek® Pro II PT and aPTT tests at all DOAC thresholds. Finally, probably due to the low number of enrolled patients, CoaguChek® Pro II PT and aPTT tests did not seem to be useful to identify patients with Dabigatran levels above the pre-defined threshold Dabigatran concentrations; indeed, the specificity values were very low thus generating a large number of false positive cases; only at 100 ng/mL Dabigatran threshold the NPV was acceptable for CoaguChek® Pro II aPTT test. Finally, only for Edoxaban and Rivaroxaban the negative likelihood ratio values resulted closer to zero, indicating a markedly low probability of having DOAC concentrations above the pre-defined thresholds, if the CoaguChek® Pro II PT or aPTT tests resulted negative.

DISCUSSION

DOACs are administered at fixed doses, without routine frequent laboratory monitoring. However, DOAC

Table 2. CoaguChek® Pro II PT and aPTT diagnostic test evaluation regarding detection of DOAC concentration at 30, 50 and 100 ng/mL (95% confidence interval in brackets)

Test	Threshold (ng/mL)	(Ideal) Cut-off (sec)	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)	Negative LR	Positive LR
				Apixaban				
PT	>30	11.5	96.1 (92.4-98.3)	5.6 (0.1-27.3)	11.1 (1.6-48.6)	92.0 (91.1-92.8)	0.7 (0.1-5.3)	1.0 (0.9-1.1)
	>50	11.3	98.3 (95.1-99.6)	2.1 (0.0-11.3)	25.0 (3.4-75.8)	78.9 (78.1-79.7)	0.8 (0.1-7.6)	1.0 (0.9-1.1)
	>100	12.1	97.9 (92.6-99.7)	28.3 (20.7-37.0)	94.7 (81.6-98.6)	50.5 (47.7-53.4)	0.1 (0.0-0.3)	1.4 (1.2-1.5)
aPTT	>30	28.8	95.1 (91.2-97.6)	16.7 (3.6-41.4)	23.1 (8.3-49.8)	92.8 (91.3-94.1)	0.3 (0.1-1.0)	1.1 (0.9-1-4)
	>50	30.0	95.4 (91.2-98.0)	19.1 (9.1-33.3)	52.9 (31.5-73.4)	81.5 (79.2-83.5)	0.2 (0.1-0.6)	1.2 (1.1-1.4)
	>100	31.5	97.8 (89.6-98.8)	21.3 (14.5-29.4)	87.1 (71.0-94.9)	47.6 (45.2-50.1)	0.2 (0.1-0.6)	1.2 (1.1 (1.3)
				Edoxaban				
PT	>30	11.9	97.9 (88.7-99.9)	21.9 (13.1-33.1)	94.1 (68.7-99.1)	44.7 (41.5-47.9)	0.1 (0.0-0.7)	1.2 (1.1-1.4)
	>50	12.2	96.1 (80.4-99.9)	30.8 (21.7-41.2)	96.7 (80.6-99.5)	27.8 (24.8-31.0)	0.1 (0.0-0.9)	1.4 (1.2-1.6)
	>100	13.8	100 (78.2-100)	72.4 (62.8-80.7)	100	34.1 (27.5-41.3)	0	3.6 (2.7-4.9)
aPTT	>30	31.2	95.7 (85.5-99.5)	23.3 (14.2-34.7)	89.5 (67.3-97.2)	44.5 (41.1-48.0)	0.2 (0.1-0.7)	1.2 (1.1-1.4)
	>50	33.4	96.1 (80.4-99.9)	39.4 (29.4-50.0)	97.4 (84.2-99.6)	30.5 (26.8-34.4)	0.1 (0.0-0.7)	1.6 (1.3-1.9)
	>100	37.3	100 (78.2-100)	60.9 (50.9-70.3)	100	26.8 (22-4-31.7)	0	2.6 (2.0-3.2)
				Rivaroxaban				
PT	>30	12.2	98.2 (90.6-99.7)	31.5 (19.5-45.6)	94.4 (70.1-99.2)	60.2 (55.7-64.3)	0.1 (0.0-0.4)	1.4 (1.2-1.7)
	>50	12.2	100 (89.4-100)	23.1 (14.3-34.0)	100	35.5 (32.7-38.3)	0	1.3 (1.1-1-5)
	>100	13.9	95.0 (75.1-99.9)	85.7 (76.8-92.2)	98.7 (92.0-99.8)	59.4 (46.7-70.9)	0.1 (0.0-0.4)	6.6 (4.0-11.1)
aPTT	>30	32.6	96.4 (87.7-99.6)	33.3 (21.1-47.5)	90.0 (68.7-97.4)	60.0 (55.2-64.6)	0.1 (0.0-0.4)	1.4 (1.2-1.8)
	>50	38.7	96.9 (83.8-99.9)	74.4 (63.2-83.6)	98.3 (89.3-99.7)	60.8 (51.4-69.5)	0.0 (0.0-0.3)	3.8 (2.6-5.5)
	>100	42.6	95.0 (75.1-99.9)	84.4 (75.3 (91.2)	98.7 (91.8-99.8)	57.6 (45.3-68.9)	0.1 (0.0-0.4)	6.1 (3.7-10.0)
				Dabigatran				
PT	>30	14.5	95.2 (83.8-99.4)	5.9 (0.1-28.7)	33.3 (4.6-83.8)	71.4 (68.6-74.1)	0.8 (0.1-8.3)	1.0 (0.9-1.2)
	>50	11.5	95.2 (83.8-99.4)	5.9 (0.1-28.7)	33.3 (4.6-83.8)	71.4 (68.6-74.1)	0.8 (0.1-8.3)	1.0 (0.9-1.2)
	>100	11.5	95.4 (77.1-99.9)	5.4 (0.7-18.2)	66.7 (16.2-95.4)	37.5 (34.7-40.3)	0.8 (0.1-8.7)	1.0 (0.9-1.1)
aPTT	>30	30.2	96.1 (86.8-99.5)	14.3 (0.3-57.9)	33.3 (4.9-82.8)	89.3 (86.0-91.9)	0.3 (0.0-2.6)	1.1 (0.8-1.5)
	>50	32.8	95.2 (83.8-99.4)	23.5 (6.8-49.9)	66.7 (28.7-90.8)	75.5 (70.1-80.2)	0.2 (0.0-1.0)	1.2 (0.9-1.6)
	>100	34.1	95.4 (77.1-99.9)	27.0 (13.8-44.1)	90.9 (57.8-98.6)	43.7 (38.5-49.1)	0.2 (0.0-1.2)	1.3 (1.0-1.6)

LR, Likelihood ratio; NPV, Negative predictive value; PPV, Positive predictive value.

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measurement may be useful in some clinical conditions such as emergencies.[2,13,14] Acute clinical scenarios, including major bleeding, need for emergency surgery, or decision making for DOAC reversal, would utilize a one-time measurement to determine if a DOAC is present. In these situations, DOAC testing should consist of simple sample processing, rapid turnaround times, and considerable sensitivity to be useful. Specific coagulation tests including diluted thrombin time, ecarin chromogenic assay, and drug-specific anti-Xa assays are available, but, unfortunately, their applicability is still limited in the setting of urgent/emergent conditions; furthermore, these tests are not implemented in many laboratories. In most cases, the only coagulation tests always available are PT and aPTT, for this reason several authors have argued these unspecific global tests might suffice to rule out relevant DOAC concentrations.[7,15,16] However, both PT and aPTT are performed in the central laboratory and therefore results are inevitably delayed by sample transportation and subsequent sample handling, which under ideal conditions takes approximately 35 minutes.[17] Moreover, standard PT and aPTT are not adequate when used to monitor DOACs due to significant variability in methodology and sensitivity of reagents used.[13,14,18-20]

Therefore, a coagulation test that can be processed shortly and at the patient's bed by a POCT, provides a binary yes or no answer for the presence of DOAC anticoagulant effect, exhibits improved reliability and sensitivity over standard coagulation tests, with a favorable cost-effectiveness would be extremely useful in critical conditions and/or in emergency situations.

Our study aimed to evaluate the diagnostic accuracy of the CoaguChek® Pro II® coagulation testing device, a POCT coagulometer, to qualitative rule out relevant concentrations of Apixaban, Dabigatran, Edoxaban and Rivaroxaban in real-life patients. Data on what constitutes a relevant DOAC concentration are scarce, and concentrations that leads to clinically significant coagulation impairment have not been established in prospective clinical trials. Different thresholds have been proposed for each DOAC, which differed between thrombolysis for acute stroke and emergency surgery.[6-10] Based on these recommendations, we investigated 3 different concentration thresholds (30, 50 and 100 ng/mL) that, at present time, may be considered relevant for a safe urgent/elective surgery and may permit thrombolysis.

Using the ROC curves, for each test/DOAC threshold, we selected as ideal cut-off point the one that yielded a sensitivity of at least 95% (false-negative results <5%, considered sufficiently safe for clinical application) associated to the highest possible specificity, to avoid false-negative results but also to identify the largest number of patients eligible for emergency treatment such as thrombolysis or emergency surgery.

For Edoxaban and Rivaroxaban we found that a sensitivity higher than 95% was associated to an acceptable/good specificity for both CoaguChek® Pro II® PT and aPTT tests at all selected threshold concentrations; overall, negative predictive values resulted between 90-100%. This means that CoaguChek® Pro II PT and aPTT tests could identify patients with Edoxaban and Rivaroxaban concentrations above the pre-defined thresholds with lower number of false-positive cases, thus allowing a higher number of patients eligible for thrombolysis or emergency surgery. Surprisingly, our study showed that for Edoxaban- and Rivaroxaban-treated patients similar results were obtained either with PT or aPTT CoaguChek® Pro II coagulation testing device. Results of DOAC-specific tests, when available, are commonly slower to be obtained in comparison with POCT; POCT and DOAC-specific coagulation tests can therefore be used sequentially to optimize speed and patient management: if POCT yield results above pre-specified cutoff values, emergency procedure may not be initiated, without the need of further coagulation testing. Calibrated DOAC-specific tests are only required if the results of POCT are below pre-specified cutoff values. Such an approach has been recently proposed.[21]

Conversely, CoaguChek® Pro II PT and aPTT tests could identify patients with Apixaban concentrations above the pre-defined thresholds, but only at the expense of a high number of false-positive cases, thus excluding from thrombolysis/surgery a too large number of patients. Finally, CoaguChek® Pro II PT and aPTT tests did not seem to be useful to identify Dabigatran concentrations higher than the pre-defined thresholds; acceptable results were only obtained for CoaguChek® Pro II aPTT test at the threshold of 100 ng/mL Dabigatran concentration.

Our results agree with those of previously published data. Indeed, all previous papers evaluating a similar POCT, found that the results of PT/INR obtained with this device can accurately rule out relevant concentrations of Edoxaban[22] or Rivaroxaban[23-25] but not of Apixaban[23,25] or Dabigatran.[23] Our study confirms that the three factor Xa inhibitors, Edoxaban, Rivaroxaban and Apixaban, differed in their effects on different POCT devices.[23,25,26] Other studies have recorded less-pronounced effects of Apixaban on PT test performed in laboratory.[27,28] As expected, CoaguChek® Pro II PT test resulted not accurate to identify samples with no relevant Dabigatran concentrations; surprisingly, this was the case also for CoaguChek® Pro II aPTT test.

Prompt availability and instantaneous results make CoaguChek® Pro II coagulation testing device adoption an attractive option in emergency situations, since it can be used at patient's bedside and without loss of time between sampling, testing and results. Indeed, the lag time between blood sampling and laboratory test execution

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should particularly be considered, because the DOAC plasma concentration increases rapidly after intake until reaching a peak after 2 to 4 hours.

Our study has strengths and limitations. Strengths include the large sample size and the acquisition of all samples from real-life patients. The use of real-life patients instead of spiked plasma has increased the inter-sample variability, thus DOAC plasma concentrations around 30-50-100 ng/mL thresholds are well represented in the dataset. Furthermore, collecting samples during treatment, mostly at trough level, provided a high number of samples containing minimal or very low DOAC concentrations and therefore this study is suitable to assess the effects of low DOAC concentrations on CoaguChek® Pro II PT and aPTT tests. For the first time, we focused on the diagnostic accuracy of a widely available POCT, the CoaguChek® Pro II coagulation testing device, to identify patients with clinically relevant DOAC concentrations investigating all 4 currently approved DOACs. To our knowledge, Edoxaban has never been tested with this specific device.

There are also some limitations. First, since there is no single CoaguChek® Pro II PT and aPTT test cut-off value that can be used for all DOACs, knowledge of the patient's medication history is necessary. Second, despite the overall large number of participants, there were a limited number of samples in the Dabigatran cohort. Third, despite the potential use of POCT in the emergency department, we did not include any patients in the emergency setting. The non-emergency setting was chosen to ensure fast patient recruitment, feasibility of POCT measurements and to analyze a wide spectrum of low DOAC plasma concentrations. Furthermore, the investigated concentration thresholds, albeit based on current literature, were established retrospectively and warrant prospective clinical evaluation. Finally, these results are not transferable to other laboratory-based assays and other PT/aPTT -based POCT devices than the CoaguChek® Pro II coagulation testing device; since generalizability of our results is limited, validation of our data is warranted, ideally including clinical outcome-oriented endpoints.

CONCLUSIONS

Our results suggest that CoaguChek® Pro II coagulation testing device can be used to qualitatively identify relevant concentrations of Edoxaban or Rivaroxaban, but not of Apixaban or Dabigatran. It allows the rapid identification of a relevant fraction of patients who are ineligible for emergency procedures, without needing to await the results of much slower laboratory-based specific coagulation tests. Anti-Xa assays most accurately detect low Edoxaban and Rivaroxaban levels and should be used in these situations but, if the specific tests are not available, the CoaguChek® Pro II coagulation testing device may be a fast and reliable alternative for guiding emergency decision/treatment in patients on Edoxaban or Rivaroxaban therapy. However, as the suggested cut-offs were determined retrospectively, further evaluation in a prospective clinical trial, ideally in emergency situations, is warranted to investigate the clinical safety of this approach.

REFERENCES

1. Douxfils J, Gosselin RC. Laboratory Assessment of Direct Oral Anticoagulants. Semin Thromb Hemost 2017;43:277-90.
2. Douxfils J, Adcock D, Bates SM, et al. 2021 Update of the International Council for Standardization in Haematology Recommendations for Laboratory Measurement of Direct Oral Anticoagulants. Thromb Haemost 2021;121:1008-20.
3. Iapichino GE, Bianchi P, Ranucci M, Baryshnikova E. Point-of-Care Coagulation Tests Monitoring of Direct Oral Anticoagulants and Their Reversal Therapy: State of the Art. Semin Thromb Hemost 2017;43:423-32.
4. Wool GD. Benefits and Pitfalls of Point-of-Care Coagulation Testing for Anticoagulation Management: An ACLPS Critical Review. Am J Clin Pathol 2019;151:1-17.
5. Favaloro EJ, Lippi G. Laboratory testing in the era of direct or non-vitamin k antagonist oral anticoagulants: a practical guide to measuring their activity and avoiding diagnostic errors. Semin Thromb Hemost 2015;41:208-27.
6. Pernod G, Albaladejo P, Godier A, et al. Management of major bleeding complications and emergency surgery in patients on long-term treatment with direct oral anticoagulants, thrombin or factor-Xa inhibitors: proposals of the working group on perioperative haemostasis (GIHP) - March 2013. Arch Cardiovasc Dis 2013;106:382-93.
7. Steiner T, Bohm M, Dichgans M, et al. Recommendations for the emergency management of complications associated with the new direct oral anticoagulants (DOACs), apixaban, dabigatran and rivaroxaban. Clin Res Cardiol 2013;102:399-412.
8. Levy JH, Ageno W, Chan NC, et al. When and how to use antidotes for the reversal of direct oral anticoagulants: guidance from the SSC of the ISTH. J Thromb Haemost 2016;14:623-7.
9. Ebner M, Birschmann I, Peter A, et al. Limitations of Specific Coagulation Tests for Direct Oral Anticoagulants: A Critical Analysis. J Am Heart Assoc 2018;7:e009807.
10. Touze E, Gruel Y, Gouin-Thibault I, et al. Intravenous thrombolysis for acute ischaemic stroke in patients on direct oral anticoagulants. Eur J Neurol 2018;25:747-e52.
11. Bossuyt PM, Reitsma JB, Bruns DE, et al. STARD 2015: An Updated List of Essential Items for Reporting Diagnostic Accuracy Studies. Clin Chem 2015;61:1446-52.
12. Swets JA. Measuring the accuracy of diagnostic systems. Science 1988;240:1285-93.
13. Tripodi A, Ageno W, Ciaccio M, et al. Position Paper on laboratory testing for patients on direct oral anticoagulants. A Consensus Document from the SISET, FCSA, SIBioC and SIPMeL. Blood Transfus 2018;16:462-70.
14. Dunois C. Laboratory Monitoring of Direct Oral Anticoagulants (DOACs). Biomedicines 2021;9.

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15. Siegal DM, Crowther MA. Acute management of bleeding in patients on novel oral anticoagulants. Eur Heart J 2013;34:489-98b.
16. Tran H, Joseph J, Young L, et al. New oral anticoagulants: a practical guide on prescription, laboratory testing and peri-procedural/bleeding management. Australasian Society of Thrombosis and Haemostasis. Intern Med J 2014;44:525-36.
17. Seiffge DJ, Traenka C, Polymeris A, et al. Feasibility of rapid measurement of Rivaroxaban plasma levels in patients with acute stroke. J Thromb Thrombolysis 2017;43:112-6.
18. Douxfils J, Ageno W, Samama CM, et al. Laboratory testing in patients treated with direct oral anticoagulants: a practical guide for clinicians. J Thromb Haemost 2018;16:209-19.
19. Gosselin RC, Adcock DM, Bates SM, et al. International Council for Standardization in Haematology (ICSH) Recommendations for Laboratory Measurement of Direct Oral Anticoagulants. Thromb Haemost 2018;118:437-50.
20. Gosselin RC, Adcock DM, Douxfils J. An update on laboratory assessment for direct oral anticoagulants (DOACs). Int J Lab Hematol 2019;41:33-9.
21. Kepplinger J, Prakapenia A, Barlinn K, et al. Standardized use of novel oral anticoagulants plasma level thresholds in a new thrombolysis decision making protocol. J Thromb Thrombolysis 2016;41:293-300.
22. Härtig F, Birschmann I, Peter A, et al. Point-of-care testing for emergency assessment of coagulation in patients treated with direct oral anticoagulants including edoxaban. Neurol Res Pract 2021;3:9.
23. Ebner M, Peter A, Spencer C, et al. Point-of-Care Testing of Coagulation in Patients Treated With Non-Vitamin K Antagonist Oral Anticoagulants. Stroke 46:2741-7.
24. Fontana P, Alberio L, Angelillo-Scherrer A, et al. Impact of rivaroxaban on point-of-care assays. Thromb Res 2017;153:65-70.
25. Padayattil Jose S, Banzato A, Carraro P, et al. Point of Care Testing (POCT) to assess drug concentration in patients treated with non-vitamin K antagonist oral anticoagulants (NOACs). Thromb Res 2018;163:100-4.
26. Ebner M, Birschmann I, Peter A, et al. Point-of-care testing for emergency assessment of coagulation in patients treated with direct oral anticoagulants. Crit Care 2017;21:32.
27. Cuker A, Siegal DM, Crowther MA, Garcia DA. Laboratory measurement of the anticoagulant activity of the non-vitamin K oral anticoagulants. J Am Coll Cardiol 2014;64:1128-39.
28. Douxfils J, Tamigniau A, Chatelain B, et al. Measurement of non-VKA oral anticoagulants versus classic ones: the appropriate use of hemostasis assays. Thromb J 2014;12:24.

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