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Neurologischen Universitätsklinik Tübingen
Abteilung Neurologie mit Schwerpunkt
neurovaskuläre Erkrankungen

**POCT-DOAC extended – qualitative Testung der Blutgerinnung mit dem Coaguchek® pro
II Point-of-Care Gerinnungstestsystem unter Verwendung des aPTT-Teststreifens bei
Patienten vor und nach Ersteinnahme von direkten oralen Antikoagulanzen**

Inaugural-Dissertation
zur Erlangung des Doktorgrades
der Medizin

der Medizinischen Fakultät
der Eberhard Karls Universität
zu Tübingen

vorgelegt von
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2024

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Tag der disputation: 25.09.2024

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2 Abbreviations

ACT	Activated Clotting Time
aPTT	Activated Partial Thromboplastin Time
CI	Confidence interval
DOAC	Direct Oral Anticoagulation
dTT	Diluted thrombin time
EDTA	Ethylenediaminetetraacetic acid
FEU	Fibrinogen equivalent units
GFR (CDK-EPI)	Glomerular filtration rate (Chronic Kidney Disease Epidemiology Collaboration)
GFR (MDRD)	Glomerular filtration rate (modification of diet in renal disease)
GGT	Gamma-glutamyl transpeptidase
GOT	Glutamat-oxalacetat-transaminase
GPT	Glutamat-pyruvat-transaminase
INR	International Normalized Ration
IQR	Interquartile range
LMWH	Low molecular weight heparin
NSAR	Non-steroidal anti-rheumatic
POCT	Point-Of-Care Testing
PT	Prothrombin time
UPLC-MS/MS	High-Performance Liquid Chromatography/Mass Spectrometry
VKA	Vitamin K Antagonist

3 Introduction

3.1 Direct oral anticoagulation

Since their introduction into the market, direct oral anticoagulants (DOAC) — i.e., the factor-IIa inhibitor, dabigatran etexilate (Pradaxa[®], Boehringer Ingelheim, Germany) and the factor-Xa inhibitors apixaban (Eliquis[®], Bristol-Myers Squibb/Pfizer, USA), edoxaban (Lixiana[®], Daiichi Sankyo, Japan), and rivaroxaban (Xarelto[®] - Bayer, Germany, and Janssen, USA) — have been widely used for the prevention of stroke and systemic thromboembolism resulting as a result of non-valvular atrial fibrillation (Steffel et al., 2021) as well as for the prevention and treatment of venous thromboembolism (Konstantinides et al., 2016).

Dabigatran acts as a reversible thrombin (factor-IIa) inhibitor and thereby prevents the thrombin-induced conversion of fibrinogen to fibrin and the thrombocyte aggregation and thus, the thrombus formation. The factor-Xa inhibitors i.e. apixaban, edoxaban and rivaroxaban inhibit the coagulation cascade one step before, and prevent the conversion of prothrombin to thrombin by reversible inhibition of the activated factor-Xa. Due to the numerous advantages of these pharmaceutical products, dabigatran and rivaroxaban received a marketing authorization valid throughout the European Union in 2008 (European Medicines Agency (EMA), 2008a, European Medicines Agency (EMA), 2008b). Apixaban was approved onto the market in 2011 (European Medicines Agency (EMA), 2011b) whereas edoxaban was approved more recently in 2015 (European Medicines Agency (EMA), 2015). The approval of these drugs took place initially without the availability of an antidote or quick tests for emergencies.

One of the frequent indications for DOAC is for the prevention of venous thromboembolism which has an annual incidence of 75-269 cases per 100 000, with patients 70 years of age or older having an incidence of up to 700 per 100,000 (Konstantinides et al., 2016). In patients who have undergone knee or hip replacement, DOAC is likewise indicated for the prevention of secondary thromboembolism (Rachidi et al., 2013).

The global population is experiencing a surge in medical indications warranting treatment with DOAC. The frequent cardiac arrhythmia, atrial fibrillation, has shown a 3-fold increase in

prevalence over the last 50 years (Schnabel et al., 2015). In 2019 for instance, the global prevalence of atrial fibrillation was 60 million, which was double the number of cases estimated in 1990 (Roth et al., 2020). Atrial fibrillation affects approximately 2% of the German population (Wilke et al., 2013). In Europe, its prevalence is projected to reach 17.9 million by the year 2060, which will be a double of atrial fibrillation prevalence in 2010 (Krijthe et al., 2013). The United States is likewise experiencing an exponential increase in the prevalence of atrial fibrillation. This is projected to reach approximately 12.1 million by 2030 (Colilla et al., 2013) and 16 million by 2050 (Miyasaka et al., 2006).

The general increase in the prevalence of atrial fibrillation is likely due to extended life expectancy, increased cardiovascular risk factors such as obesity, hypertension and diabetes mellitus, and a better survival after cardiovascular events for example myocardial infarction and heart failure, in comparison to previous decades (Heeringa, 2010). In 2017, the GLORIA-AF registry reported that DOAC was prescribed in 52.3% of all patients with atrial fibrillation in Europe and in 47,6% of all patients with atrial fibrillation globally (Huisman et al., 2017). These figures are however, believed to be underestimated (Steinberg et al., 2017). In 2017, an increase of 78.9% in prescription of DOAC since their introduction onto the market in 2010 was reported in the United States alone (Zhu et al., 2018) while in England a surge from 9% in 2015 to 74% in 2019 was recorded (Ho et al., 2020). This implies an increasing tendency in the prescription of DOAC. Furthermore, DOAC have been shown to be a more preferable alternative in handling non-valvular atrial fibrillation compared to vitamin K antagonists (VKA) which are limited by narrow therapeutic interval, the need for frequent monitoring as well as dose adjustments, and increased bleeding complications (Kirchhof et al., 2016).

In addition to cardioembolic ischemic strokes due to atrial fibrillation, a substantial number of cryptogenic ischemic stroke, which accounts for approximately 25% of ischemic stroke, could be attributed to non-valvular atrial fibrillation (Hart et al., 2014).

Although DOAC is prescribed for the secondary prevention of non-valvular cardioembolic stroke, DOAC is nonetheless associated with approximately 2% annual incidence of acute ischemic stroke with an annual stroke risk of 0.9-1.8% (Almutairi et al., 2017, Pfeilschifter et al., 2016, Connolly et al., 2011, Connolly et al., 2009, Patel et al., 2011, Giugliano et al., 2013). The incidence of major

bleeding including intracranial hemorrhage in patients under DOAC therapy is approximately 1.4-3.6% (Connolly et al., 2011, Connolly et al., 2009, Patel et al., 2011, Giugliano et al., 2013). Furthermore, an annual mortality of up to 3.75% has also been reported in association with DOAC (Pirlog et al., 2019).

In spite of the clinical significance of DOAC, it continues to be of neurological concern in patients. In patients undergoing treatment with DOAC, urgent assessment of DOAC plasma concentration is necessary during acute ischemic stroke, to guide decision-making relevant to thrombolysis according to current stroke guidelines. In accordance with recent recommendations, the American Heart Association/American Stroke Association (AHA/ASA) suggests the avoidance of thrombolysis within 48-hours of last DOAC intake in acute ischemic stroke patients (Powers et al., 2019). Based on this recommendation, 12-18% of such patients, who are otherwise potentially eligible for thrombolysis since actual DOAC plasma concentration might not be clinically significant, could be excluded from thrombolysis (Meinel et al., 2021).

In case of intracranial hemorrhage, other major bleedings or emergency surgery, urgent assessment of DOAC plasma concentration is likewise important to guide decision-making relevant to DOAC reversal therapy since unnecessary anticoagulation reversal comes at a considerable financial cost as well as thromboembolic complications: Idarucizumab (Pollack et al., 2017) and andexanet alfa (Connolly et al., 2019) are potent DOAC reversal agents but yet expensive. Prothrombin complex concentrate (Burnett et al., 2016) and andexanet alfa (Gomez-Outes et al., 2021) pose the risk of thromboembolic complications, especially in those 20% of patients in whom DOAC intake is supposed, but no relevant anticoagulant concentration can be detected on emergency admission (Sennesael et al., 2018). With regards to emergency surgery, urgent assessment of DOAC plasma concentration will help guide safe selection of patients for surgical intervention since unnecessary delays due to bleeding concerns might have detrimental consequences (Moster and Bolliger, 2022).

3.2 Definition of clinically relevant DOAC concentrations (treatment thresholds)

Currently, international guidelines recommend plasma DOAC concentrations of 30 or 50 ng/ml as thresholds considered safe for immediate thrombolysis or emergency surgery, or – in case of intracranial and other life-threatening hemorrhage – safe for withholding reversal treatment. It should be noted that although these thresholds have been recommended as clinically relevant by international guidelines, they are yet to be supported by random clinical trials (RCT) and might change with time when supporting RCT data are available (Table 1).

Table 1: Recommended treatment thresholds

	Dabigatran	Rivaroxaban	Apixaban	Edoxaban	VKA
Emergency surgery/invasive surgery permitted when:					
Pradaxa CHMP (EMA), 2011 (European Medicines Agency (EMA), 2011a)	48 ng/mL	–	–	–	–
Eikelboom and Weitz, 2013 (Eikelboom and Weitz, 2013)	50 ng/mL	–	–	–	–
Suggestions GIHP, 2013 (Pernod et al., 2013)	30 ng/mL	30 ng/mL	–	–	–
Recommendation ISTH, 2016 (Levy et al., 2016)	30 ng/mL	30 ng/mL	30 ng/mL	30 ng/mL	–
Thrombolysis during acute ischemic stroke permitted when:					
Steiner et al., 2013(Steiner et al., 2013)	50 ng/mL	100 ng/mL	10 ng/mL	-	INR ≤ 1,7
DGN guidelines 2015 (Neurologie, 2015)	Normal dTT, last intake > 48 h with normal kidney function	Normal AXA, last intake > 48 h with normal kidney function			INR ≤ 1,7
Recommendation ESO-KSU, 2016 (Ahmed et al., 2017)	30 ng/mL	30 ng/mL	30 ng/mL	30 ng/mL	INR ≤ 1,7
Recommendation SFNV and GFHT, 2018 (Touzé et al., 2018)	50 ng/mL	50 ng/mL	50 ng/mL	50 ng/mL	INR ≤ 1,7
Recommendation ESO-KSU, 2018 (Ahmed et al., 2019)	30 ng/mL	30 ng/mL	30 ng/mL	30 ng/mL	INR ≤ 1,7
AHA/ASA guidelines 2019 (Powers et al., 2019)	Normal aPTT/INR, ECT, TT, last intake > 48 h with normal kidney function	Normal INR/aPTT, AXA, last intake > 48 h with normal kidney function			INR ≤ 1,7
ESO guidelines 2021 (Berge et al., 2021)	TT < 60 s	AXA < 0,5 U/mL	AXA < 0,5 U/mL	AXA < 0,5 U/mL	INR ≤ 1,7
DGN guidelines 2021(Neurologie, 2021)	–	–	–	–	–
Antidote during life-threatening bleeding during:					
AHA/ASA guidelines 2015 (Powers et al., 2015)	–	–	–	–	INR > 1,2 to > 1,4
Recommendation ISTH, 2016 (Levy et al., 2016)	50 ng/mL	50 ng/mL	50 ng/mL	50 ng/mL	–
Recommendation ESO-KSU, 2016 (Ahmed et al., 2017)	30 ng/mL	30 ng/mL	30 ng/mL	30 ng/mL	INR > 1,2
Recommendation ESO-KSU, 2018 (Ahmed et al., 2019)	30 ng/mL	30 ng/mL	30 ng/mL	30 ng/mL	INR > 1,2

ESO guidelines 2019 (Christensen et al., 2019)	–	–	–	–	INR > 1,2
DGN guidelines 2021 (Neurologie, 2021)	–	–	–	–	INR > 1,2

CHMP, Committee for Medicinal Products for Human Use; EMEA, European Medicine Agency, KSU, Karolinska Stroke Update Conference; GIHP, Groupe d'Intérêt en hémostase périopératoire; ISTH, International Society on Thrombosis and Haemostasis; SFNV, Société Française Neuro-Vasculaire; GFHT, Groupe Français d'Etudes sur l'Hémostase et la Thrombose; VKA, Vitamin K antagonist

3.3 Anticoagulation testing in emergency situations

Point-of-care testing (POCT) for DOAC although not widely available, are currently part of local clinical guidelines of some institutions. Previous studies that evaluated POCT devices in assessing anticoagulation by VKA found a net time gain of 28 ± 12 minutes compared to routine laboratory-based coagulation tests (Rizos et al., 2009).

It is believed that global laboratory coagulation test such as prothrombin time (PT), activated partial thromboplastin time (aPTT), international normalized ration (INR) and activated clotting time (ACT) do not accurately reflect plasma DOAC concentration and therefore, its use for this purpose is discouraged (Steffel et al., 2021). This happens to be the case even when highly DOAC-sensitive reagents are used.

To guide emergency treatment in patients under DOAC therapy (i.e., can thrombolysis or surgery be initiated? should a reversal agent be administered?) only qualitative assessment is required (i.e., is the DOAC plasma concentration above or below the currently recommended treatment thresholds) (Mbroh and Poli, 2021).

Using the upper limit of the normal range for these parameters as reference values for selecting patients above or below the recommended threshold considered safe for thrombolysis or surgery i.e. recommended treatment threshold of 30 and 50 ng/ml (Ahmed et al., 2019), has been shown to be erroneous (Lippi et al., 2014). By so doing, ineligible patients might be falsely considered for thrombolysis, surgery or DOAC reversal. This has been attributed to an overlap between test results obtained from patients under DOAC therapy and normal results of healthy patients who are not under DOAC therapy (Lippi et al., 2014, Ebner et al., 2017a).

However, by using reagent-specific cut-offs which might be different from the assay's own normal range, global coagulation assays could qualitatively identify plasma concentrations above or below a predefined DOAC concentration (Lippi et al., 2014). These cut-off should be set with safety of the decision-making being the topmost priority. Available data confirm the feasibility of using assay-specific cut-offs to qualitatively identify DOAC plasma concentrations above or below the

recommended treatment thresholds. These cut-offs are set for each DOAC (DOAC-specific), each POCT device (POCT-device-specific) and each POCT assay (POCT-assay-specific). Compared to laboratories which may all use different coagulation assays and test devices, POCT devices and POCT assays are standardized which allows using the cut-offs with the respective device and assay anywhere worldwide. Some global coagulation POCT assays have been found unsuited to detect dabigatran, and data for apixaban has not been promising for any POCT so far (Ebner et al., 2017a).

3.3.1 CoaguChek® POCT

The CoaguChek® (Roche, Switzerland) device is used for measuring global coagulation parameters depending on the type of test strip used. The currently available test strips, aPTT and INR, consist of desiccated reagents which consists of activators, a peptide substrate, and nonreactive components. Upon application of the blood sample on the test strip, the reagent dissolves and the activators start the coagulation process which leads to the formation of the enzyme, thrombin. The built-in meter begins to measure the time. Thrombin cleaves the peptide substrate, generating an electrochemical signal. Depending on the time elapsed when this signal first appears, it is converted via an algorithm into customary coagulation units depending on the test, i.e., INR or seconds for aPTT, and the result is displayed (Roche, 2022). The turnaround time, including blood sampling and measurement is usually between 3-5 minutes.

Apixaban and dabigatran were shown not to be detectable with CoaguChek®-INR with a sufficiently high safety margin (Ebner et al., 2015, Härtig et al., 2020). The CoaguChek®-INR test cards were however shown to detect edoxaban and rivaroxaban plasma concentrations ≤ 30 ng/mL correctly in 44% and 12% of cases respectively when specific INR cut-off of 1.0 and 0.9, respectively, is applied (Härtig et al., 2020). At the same cut-offs, edoxaban and rivaroxaban plasma concentrations > 30 ng/mL could be detected with an accuracy of 96% and 99% respectively (Härtig et al., 2020). Once again, these cut-offs are below the normal range of INR which ranges up to an INR value of 1.2 for the CoaguChek® INR assay (Table 2). Seen from a different perspective, patients under edoxaban (i.e. 44%) and rivaroxaban (i.e. 12%) could be safely treated with thrombolysis, in case of acute ischemic stroke, or safely spared from receiving potentially prothrombotic anticoagulation-reversal therapy, in the case of hemorrhagic stroke, based on results of CoaguChek®-INR (i.e. if $\text{INR} \leq 1.0$ for edoxaban and ≤ 0.9 for rivaroxaban).

On the other hand, > 95% of edoxaban and rivaroxaban patients with CoaguChek®-INR of > 1.0 and > 0.9, respectively, could be timely identified for anticoagulation reversal in case of hemorrhagic stroke or urgent surgery. These patients would however not per se be disqualified from these treatments but may receive delayed therapy based on calibrated DOAC-specific laboratory results. These studies did use blood samples obtained in real-life DOAC-treated patients at 6 different time-points. By assessing DOAC concentration at 6 time-points, these studies aimed to cover a wide range of DOAC plasma concentration including high peak and low trough concentrations.

Table 2: CoaguChek® POCT (Ebner et al., 2015, Härtig et al., 2020)

DOAC	Plasma concentration threshold	Test card (Cut-off)	Sensitivity (95% CI)	Specificity (95% CI)
Edoxaban	30 ng/mL	INR > 1,0	96% (87-99)	44% (30-59)
	50 ng/mL	INR > 1,1	95% (86-99)	86% (74-93)
Rivaroxaban	30 ng/mL	INR > 0,9 [$>1,0$]	99% (92-100) [88% (77-94)]	12% (5-26) [71% (55-84)]
	50 ng/mL	INR > 0,9 [$>1,0$]	100% (93-100) [91% (81-96)]	12% (5-26) [65% (50-78)]
	100 ng/mL	INR > 1,0	96% (85-99)	52% (40-64)

Gray: > 5% false negative POCT-results in favor of higher sensitivity which could pose patients at risk and should only be used in selected cases after careful risk-benefit evaluation.

INR: international normalized ratio; CI: confidence interval

3.3.2 Hemochron® Signature Elite POCT

The use of global coagulation tests with assay-specific and DOAC-specific cut-offs, has also been studied using the Hemochron™ Signature Elite (Werfen, Spain), together with the INR and ACT+ test cartridges. Data was shown to correlate with actual plasma concentrations of the thrombin inhibitor, dabigatran, as well as the factor-Xa inhibitors, edoxaban and rivaroxaban, measured by means of high-performance liquid chromatography/mass spectrometry (UPLC-MS/MS) (Ebner et al., 2017b, Härtig et al., 2021). Similarly to the above-mentioned studies which tested DOAC using the CoaguChek® (Ebner et al., 2015, Härtig et al., 2020), blood samples were obtained in real-life DOAC-treated patients at 6 different time-points.

With sensitivity of > 95%, Hemochron™ Signature Elite (Werfen, Barcelona, Spain) could detect concentrations of dabigatran, edoxaban and rivaroxaban higher than the recommended treatment thresholds of 30 and 50 ng/mL, which permits to identify patients in a pass or fail manner for thrombolysis, anticoagulation reversal therapy or urgent surgery (Table 3).

Table 3: Hemochron™ Signature Elite POCT (Ebner et al., 2017b, Härtig et al., 2021)

DOAC	Plasma concentration threshold	Test card (Cut-off)	Sensitivity (95% CI)	Specificity (95% CI)
Dabigatran	30 ng/mL	INR > 1,1	99% (92-100)	47% (37-58)
	50 ng/mL	INR > 1,2	98% (88-100)	66% (56-74)
Edoxaban	30 ng/mL	INR > 1,5	99% (91-100)	98% (88-100)
	50 ng/mL	INR > 2,1	97% (88-99)	97% (87-99)
Rivaroxaban	30 ng/mL	ACT+ > 120 s	96% (87-99)	67% (52-80)
	50 ng/mL	ACT+ > 125 s	97% (88-99)	81% (67-90)
	100 ng/mL	ACT+ > 130 s	96% (85-99)	68% (55-78)

ACT: activated clotting time; INR: international normalized ratio; CI: confidence interval; s: seconds

As a guide for emergency physicians, significant plasma concentration of dabigatran, edoxaban and rivaroxaban, i.e. plasma concentrations > 30 ng/mL, could be identified using both, the Hemochron™-INR and the Hemochron™-ACT+ test cartridges. Specificity however was highest with Hemochron™-INR in case of dabigatran and edoxaban and with Hemochron™-ACT+ in case of rivaroxaban (Ebner et al., 2017b, Härtig et al., 2021). The Hemochron™ and the assays i.e. ACT+ and INR test cards, are currently commercially available, approved and widely used for assessing global coagulation e.g. in patients on VKA (Dolscheid-Pommerich et al., 2018).

Hemochron™ is not reliable for assessing apixaban plasma concentration (Ebner et al., 2017b, Härtig et al., 2021). Data indicate weak correlations between Hemochron™-POCT and apixaban concentrations for PT/INR ($r = 0.37$, $p < 0.001$), moderate for ACT+ ($r = 0.48$, $p < 0.001$) and no significant correlation for aPTT or ACT-LR (Ebner et al., 2017b).

Despite a high sensitivity of $> 96\%$ in detecting dabigatran, edoxaban and rivaroxaban plasma concentration above the recommended treatment thresholds of 30 and 50 ng/ml, the specificity for dabigatran (47%) and rivaroxaban (67%) is not very high. HemochronTM nonetheless ensures patients' safety since $> 96\%$ of patients under dabigatran, edoxaban and rivaroxaban with actual DOAC plasma concentration above the recommended treatment thresholds could be correctly identified as such and hence, excluded from thrombolysis in the case of an acute ischemic stroke or urgent surgery, thereby avoiding bleeding complications. In the case of acute hemorrhagic stroke, $> 96\%$ of these patients could be identified for anticoagulation reversal, thereby avoiding hemorrhagic complications. When considered differently, a substantial number of patients the other hand could be identified for thrombolysis or urgent surgery while the remaining could receive late intervention based on results from laboratory coagulation assays. In other words, 47% dabigatran patients and 67% rivaroxaban patients with actual DOAC plasma concentration below the recommended treatment thresholds could be correctly identified as such for thrombolysis or urgent surgery, while the remaining could receive late intervention based on results from laboratory coagulation assays which would have been performed irrespective of POCT. These figures could be argued as significant especially in the case of stroke where urgent intervention is vital in reducing mortality and morbidity. It is worth noticing that the HemochronTM-INR can correctly identify $\geq 97\%$ of patients with actual edoxaban plasma concentration above or below the recommended treatment thresholds as well as identify (Table 3) (Ebner et al., 2017b, Härtig et al., 2021).

3.3.3 Viscoelastic coagulation testing

3.3.3.1 Thromboelastography

The thromboelastography (TEG) analyzer, which makes use of a disposable multi-channel DOAC-specific microfluid cartridge, assesses hemostatic property of blood and its response to antiplatelet therapy by means of resonance-frequency viscoelasticity measurements (Gurbel et al., 2016). A prototype 4-channel cartridge has been developed for DOAC assessment however, these are not commercially available. The direct thrombin inhibitor channel assesses anticoagulation by the thrombin inhibitor, dabigatran, whereas the anti-factor-Xa channel assesses anticoagulation by factor-Xa inhibitors. It has a turn-around time of approximately 10 minutes.

Using the TEG[®]6s (Haemonetics Corporation, Massachusetts, USA) device, data for dabigatran shows a strong correlation with anticoagulation assessment using laboratory-based DOAC-assays, i.e. $r = 0.94$, $p < 0.0001$ (Artang et al., 2019). For apixaban and rivaroxaban, correlations of 0.93 and 0.83, respectively; $p < 0.0001$, were reported. Data for edoxaban were however not provided. In spite of these strong correlations, values of the measured samples with relevance the recommended treatment threshold are less represented in this study. A cut off of 150s for both thrombin inhibitors and factor-Xa inhibitors was associated with 100% sensitivity and $\geq 90\%$ specificity in detecting DOAC plasma concentrations ≥ 50 ng/ml (Artang et al., 2019).

In general, TEG might be appropriate in determining supratherapeutic concentration of DOAC but there seem to be a high uncertainty in discriminating DOAC concentration around the recommended treatment threshold which is relevant in urgent decision-making. TEG is influenced by antiplatelet therapy (Qin et al., 2018) making results obtained from patients under DOAC and concomitant antiplatelet therapy difficult to interpret.

3.3.3.2 Rotational Thromboelastometry (ROTEM[®])

The rotational thromboelastometry (ROTEM[®]) (TEM Innovations GmbH, Munich, Germany) uses a multichannel testcard with standard or modified thromboelastometric assays which allows simultaneous testing to differentiate between thrombin inhibitors and factor-Xa inhibitors (Gorlinger et al., 2016, Schafer et al., 2020). With a turn-around-time of 10 minutes, the DOAC specific ROTEM[®] clotting time assays i.e. low-tissue-factor test (TFTEM) and ecarin-based test (ECATEM) have been shown to correlate strongly with anti-factor-Xa activity ($r = 0.86$; $p < 0.0001$) as well as anti-thrombin activity ($r = 0.72$; $p < 0.0001$) respectively (Schafer et al., 2020). Although not commercially available, these two assays could differentiate between plasma dabigatran and rivaroxaban concentration (Schafer et al., 2020). Data are for apixaban and edoxaban as well as the relevance of ROTEM[®] in identifying DOAC plasma level with respect to recommended treatment thresholds are so far not available. Just as in other thromboelastometric tests, ROTEM[®] might be potentially influenced by antiplatelet therapy.

3.3.4 ClotPro®

The ClotPro® (Enicor GmbH, Munich, Germany) POCT device makes use of six channels for simultaneous coagulation testing (Oberladstatter et al., 2021). It measures clotting time of blood by using an ecarin test clotting time (ECT) for thrombin inhibitors or Russell's viper venom test (RVV) for factor-Xa inhibitors (Fong et al., 2020).

The ClotPro® provides a sensitivity of 100% for the thrombin inhibitor, dabigatran, and a sensitivity of 90-100% for factor-Xa inhibitors, edoxaban and rivaroxaban, in detecting DOAC plasma concentration are actually ≥ 50 ng/ml (Oberladstatter et al., 2021).

For the thrombin inhibitor, dabigatran, plasma concentrations ≥ 50 ng/ml have been shown to be associated with the cut off 180s while factor-Xa inhibitors with plasma concentrations ≥ 50 ng/ml are associated with the cut off 100s (enicor GmbH, 2020).

In spite of the promising data, the ClotPro® is semiautomated and has a long turn-around time partly contributed by sample pipetting which may be cumbersome in the emergency setting. ClotPro® has so far only been evaluated for the 50 ng/ml threshold.

3.3.5 DOASENSE™ DOAC Dipstick

The DOASENSE™ DOAC Dipstick (DOASENSE GmbH, Germany) is the only available POCT method which does not make use of blood. Instead, it assesses anticoagulation by making use of urine due to its ability to detect traces of DOAC in urine (Harenberg et al., 2013, Harenberg et al., 2019) since approximately 54–85% of DOAC is excreted in urine due to their hydrophilic nature and the small molecular weight of DOAC (Gomez-Outes et al., 2021). The DOASENSE™ DOAC Dipstick has two test pads for the detection of the presence of the thrombin inhibitor dabigatran and the factor Xa inhibitors. Two additional test pads indicate abnormal urine color and creatinine (Harenberg et al., 2019). The DOASENSE™ DOAC Dipstick is currently commercially available and has been recommended in some guidelines for detecting DOAC (Tan et al., 2023).

At > 30 ng/ml threshold, a sensitivity of 100% has been reported for thrombin inhibitors (Margetic et al., 2022, Merrelaar et al., 2022, Ord et al., 2022). For factor-Xa inhibitors, sensitivities ranging from 90%-100% have been reported in different studies (Margetic et al., 2022, Merrelaar et al.,

2022, Papageorgiou et al., 2023, Ord et al., 2022). At a threshold > 50 ng/ml, 92% sensitivity was recorded for DOAC-naïve patients 4 to 6 hours after first DOAC intake (Tan et al., 2023).

In spite of these data, the DOASENSE™ DOAC Dipstick requires urine to be obtained from patients which in the case of reduced consciousness or aphasia, could lengthen the turn-around time since urine would have to be obtained by means of urine catheter insertion, and moreover, under sterile conditions. Interpretation of changes in color of the urine pads are most often dependent on the observer although a low interobserver variability has been reported (Harenberg et al., 2021). Nonetheless, specific DOASENSE readers are available to assist interpretation of color changes. It is also unknown how long it takes after drug intake before a positive urine test could be detected. Since urine concentration is not the same as plasma concentration of DOAC, the relevance of a mere positive test result to the recommended treatment threshold is arguable.

4 Aims of the project

The aim of this project was to study the diagnostic accuracy of the CoaguChek[®] (Roche, Rotkreuz, Switzerland) used together with the aPTT test strips (Roche, Rotkreuz, Switzerland) to qualitatively identify patients with plasma DOAC concentrations above the currently recommended treatment relevant thresholds (i.e., DOAC concentrations that would allow for immediate reversal treatment in case of intracranial or other life-threatening hemorrhage or surgery, or that would justify withholding thrombolysis if necessary) for all four currently Food and Drug Administration- and European Medicines Agency-approved DOAC, i.e. the thrombin inhibitor dabigatran, and the factor-Xa inhibitors apixaban, edoxaban and rivaroxaban, in real-life patient samples.

5 Materials and Methods

5.1 Study design

Single-center, prospective diagnostic study with blinded outcome assessment. The study was conducted at the Department of Neurology & Stroke of the Tübingen University Hospital, a tertiary care facility. We planned to enroll a total of 80 patients, 20 patients for each of the 4 DOAC, who were scheduled to receive DOAC for secondary thromboembolic prevention. Study was registered on ClinicalTrials.gov with the unique identifier NCT04679298. The abstract and preliminary results for this study was recently presented at the World Stroke Conference in October 2023 (Sage Journals, 2023).

5.2 Standard protocol approval

An approval was obtained from an independent review board of the Ethics Committee of the Tübingen University (protocol no. 703/2020BO1) before all study-related activities began. The final version of the ethics proposal was submitted on 16.11.2020 and approved on 23.11.2020.

5.3 Eligibility criteria

5.3.1 Inclusion criteria

Patients were considered for enrollment into the study if they were ≥ 18 years, had an indication for the initiation of DOAC therapy and could provide a written informed consent. A written informed consent was obtained from all patients prior to enrollment.

5.3.2 Exclusion criteria

Patients were excluded from enrollment on the basis of treatment with VKA ≤ 14 days, heparinoids (e.g. fondaparinux) ≤ 72 hours, unfractionated heparin ≤ 12 hours and LMWH ≤ 24 hours prior to first DOAC intake. Patients on prior DOAC treatment were excluded if last DOAC intake was $\leq$

72 hours before enrollment. Furthermore, patients were excluded if they had a known coagulopathy, abnormal coagulation parameters at baseline defined by INR > 1.2/Quick < 70% or aPTT > 40 s.

5.4 Data and Sample collection

5.4.1 Data collection

Data collected as part of the baseline characteristics of enrolled patients included age, sex, height, weight, baseline serum creatinine, GFR (MDRD), GFR (CDK-EPI), GFR (Cockcroft-Gault), liver function tests as well as cardiovascular risk factors including heart insufficiency, arterial hypertension, hypercholesterinemia, diabetes mellitus, nicotine abuse, coronary artery disease, previous myocardial infarction, peripheral artery disease, previous hemorrhage, abnormal INR, current therapy with NSAR or antiplatelet and alcohol consumption. Based on these baseline characteristics, the CHA₂DS₂-VASc (Lip et al., 2010) and HAS-BLED (Pisters et al., 2010) scores were calculated. CHA₂DS₂-VASc was scored according to the following criteria: 1 point for age 65-74 or 2 points for age ≥ 75, 2 points for stroke or transient ischemic attack (TIA), and 1 point each for heart insufficiency, arterial hypertension, diabetes mellitus as well as coronary artery disease. HAS-BLED was scored according to the following criteria: 1 point each for age > 65, serum creatinine >2.3mg/dl, GGT/GOT/GPT >3x the normal range or bilirubin >2x the normal range, arterial hypertension, previous hemorrhage, abnormal INR, current therapy with NSAR or antiplatelet therapy, alcohol consumption as well as stroke or TIA.

5.4.2 Sample collection

Blood samples were collected either directly by means of venipuncture or through a venous catheter at six defined time points, i.e., at baseline before DOAC intake, 0.5, 1, 2, 8 hours after DOAC intake and directly before the next DOAC intake i.e., at 12 hours for dabigatran and apixaban or 24 hours for edoxaban and rivaroxaban. At baseline, blood was collected into one EDTA blood sampling tube for assessing full blood count and one heparin blood sampling tube for assessing inflammatory markers, protein/albumin as well as liver and kidney function tests at the Tübingen University Hospital central laboratory. During each blood sampling, 0.5–1 ml blood was collected

directly into a non-heparinized syringe (Injekt, B.Braun, Melsungen, Germany) before transferring onto aPTT test strips which has been inserted into the pre-programed CoaguChek® POCT device within 5 seconds. At the same time, 2–3 ml blood was collected into 3 citrated blood sampling tubes (S-Monovette Citrate 3.2%, Sarstedt, Nümbrecht, Germany). One of the tubes containing citrated whole blood was transported to the Tübingen University Hospital central laboratory, where coagulation parameters were assessed (see 5.4.3 laboratory assessment). The remaining two citrated whole blood were centrifuged at 1500 revolutions per minute for 10 minutes to yield citrated plasma which were pipetted into at least 0.5 ml aliquots and stored at -80°C at our center. One citrated plasma per timepoint of blood collection for each patient was transported on dry ice to the Institute for Laboratory and Transfusion Medicine at the Heart and Diabetes Center of Ruhr University (Bad Oeynhausen, Germany) for UPLC-MS/MS.

5.5 Coagulation assessment

5.5.1 Point-of-care testing

Point-of-care testing was performed with the CoaguChek® Pro II device. It is required to switch on the device, followed by selection “patient test” and then “aPTT”. The insertion of a valid aPTT test strip is then required. The insertion of the aPTT test strip was done in such a way so as to avoid contact with the blood sampling surface of the test strip. A period of 5-10 seconds was allowed for test strip to warm up after which the device indicates end of warming up. After this, the whole blood sample, usually 8µl, is applied within a maximum of 3 minutes. Upon application of the blood sample, time is allowed, usually 2-3 minutes, until aPTT results are shown on the device.

5.5.2 Laboratory assessment

Laboratory-based tests were conducted at the Tübingen University Hospital central laboratory. One standard tube of citrated blood was sent to the central laboratory for assessing DOAC-specific coagulation. Dabigatran was measured using Innovance direct thrombin inhibitor on a Sysmex CS-5100 (both Siemens Healthineers, Erlangen, Germany). Calibrated anti-Xa activity was assessed using the Innovance Heparin assay on a Sysmex CS-5100 (both Siemens Healthineers, Erlangen, Germany).

Laboratory-based global coagulation parameters assessed included INR measured using Dade Innovin® (Siemens Healthineers, Erlangen, Germany) and aPTT measured using Dade® Actin FS® (Siemens Healthineers, Erlangen, Germany). Other laboratory-based coagulation tests performed included fibrinogen (Clauss) and D-dimers. All laboratory coagulation testing was performed according to manufacturers' instructions by well trained technicians.

At baseline, the following tests were performed using blood samples collected into standard EDTA tubes: creatinine, urea, total protein, albumin, C-reactive protein, procalcitonin, cholinesterase, glutamat-pyruvat-transaminase/alanin-aminotransferase and glutamate-oxalacetat-transaminase/aspartat-aminotransferase.

5.5.3 Ultra-performance liquid chromatography - tandem mass spectrometry

UPLC-MS/MS was performed at the Institute for Laboratory and Transfusion Medicine, Heart and Diabetes Center, Ruhr University Bochum, Bad Oeynhausen, Germany. Test was performed using citrated plasma from blood sample collected simultaneously during POCT.

Blood samples in citrated tubes were centrifuged at 1500 revolutions per minute for 10 minutes shortly after blood sampling. Citrated plasma samples of at least 1.5 ml were collected into Eppendorf tubes and stored in -80°C until transport for UPLC-MS/MS. In case citrated plasma samples were stored at -20°C, UPLC-MS/MS was performed within 2 weeks of sample collection. Details of UPLC-MS/MS has been previously described (Kuhn et al., 2018).

5.6 Blinding

All laboratory tests as well as UPLC-MS/MS tests were conducted and interpreted by independent technicians blinded to POCT results, which was conducted by an investigator who was likewise blinded to all laboratory and UPLC-MS/MS tests.

5.7 Statistics

The statistics for this study was designed in accordance to recommendations from the institute for clinical epidemiology and applied biometry of the Eberhard Karls University of Tübingen. Statistics was performed using the SPSS 29 (IBM, Armonk, NY, USA) software.

Spearman's rho correlation coefficient for non-parametric correlations was used to estimate the strength of correlation between CoaguChek®-aPTT values and UPLC-MS/MS values as well as all other correlation analyses. Correlation strength was graded as proposed by Evans: <0.20 very weak, 0.20–0.39 weak, 0.40–0.59 moderate, 0.60–0.79 strong, and >0.80 very strong (Evans, 1996).

Fisher's exact test was calculated to determine differences in the proportions of baseline CoaguChek®-aPTT results between the study groups and to examine the association between CoaguChek®-aPTT results and DOAC concentrations dichotomized to concentrations above and below the currently recommended treatment thresholds of 30 and 50 ng/ml.

The diagnostic accuracy of the CoaguChek®-aPTT test stripes was assessed based on sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and likelihood ratio of different CoaguChek®-aPTT cut-off values to qualitatively discriminate DOAC plasma concentrations above from below the treatment thresholds of 30 and 50ng/ml. The free online VassarStats Clinical Calculator 1 was used for calculating the diagnostic sensitivity and specificity, negative predictive values and likelihood ratios with 95% confidence intervals according to the efficient-score method as described by Newcombe (Newcombe, 1998).

Sensitivity was defined as the percentage of samples containing clinically relevant plasma DOAC concentrations as detected by UPLC-MS/MS, that were correctly identified as such by high CoaguChek®-aPTT values. These group of patients theoretically belong to a those eligible for anticoagulation reversal therapy in the case of hemorrhagic stroke or severe bleeding, or might be excluded from immediate thrombolysis or surgery. Specificity on the other hand was defined as the percentage of samples containing no clinically relevant plasma DOAC concentrations as detected by UPLC-MS/MS, that were correctly identified as such by low CoaguChek®-aPTT values. These group of patients theoretically belong to a those eligible for immediate thrombolysis

or surgery, or might be excluded from anticoagulation reversal therapy in the case of hemorrhagic stroke or severe bleeding.

PPV was defined as the ratio of samples containing clinically relevant plasma DOAC concentrations as detected by high CoaguChek[®]-aPTT values to all samples containing clinically relevant plasma DOAC concentrations as detected by UPLC-MS/MS expressed as percentage. NPV on the other hand was defined as the ratio of samples containing no clinically relevant plasma DOAC concentrations as detected by low CoaguChek[®]-aPTT values to all samples containing no clinically relevant plasma DOAC concentrations as detected by UPLC-MS/MS expressed as percentage.

Likelihood ratio (sensitivity/1-specificity) indicates how much more likely a CoaguChek[®]-aPTT result is found in samples containing ≥ 30 or ≥ 50 ng/ml compared to samples containing < 30 or < 50 ng/ml of DOAC.

For the sake of safety, an ideal CoaguChek[®]-aPTT cut-off was pre-defined as the highest possible value that yielded a sensitivity of $> 95\%$, i.e., a misprediction percentage ($1 - \text{specificity}$) $< 5\%$, in order to avoid false positives. All DOAC concentrations are reported as median and interquartile range (IQR). In further analysis, a less conservative approach was implemented, i.e. sensitivity of 90% , to see to which extent the specificity for CoaguChek[®]-aPTT could be improved. This was done for the purpose of identifying a possible balance between sensitivity and specificity.

6 Results

6.1 Baseline characteristics

In total, 80 patients were enrolled in the study, 20 patients for each DOAC. First patient was enrolled on 05.01.2021, and last patient on 28.09.2022. A total of 459 out of the planned 480 samples, corresponding to 96%, were successfully collected and analyzed; dabigatran $n = 115$, apixaban $n = 113$, edoxaban $n = 116$ and rivaroxaban $n = 115$. Samples which were not collected were due to several reasons including early discharge ($n = 12$), study drop-out (4), futility in blood sampling ($n=2$) and erroneous administration of heparin during the study ($n = 3$).

34 women, i.e. 42.5%, were enrolled. The median age was 78 years (IQR 63—86) with the youngest being 19 and the oldest being 99 years. The average body mass index (BMI) was 26.5 ± 5.0 ; dabigatran $n = 28.1 \pm 4.6$, apixaban $n = 25.5 \pm 4.1$, edoxaban $n = 26.3 \pm 5.6$ and rivaroxaban $n = 26.0 \pm 5.3$. The median baseline creatinine was 0.1 mg/dl (IQR 0.7—1.1) which corresponded to a median GFR (Cockcroft Gault) of 74.3 ml/min/1.73 m² (IQR 53.1—102.3). The lowest creatinine across the population was 0.5 mg/dl while the highest was 2.4 mg/dl corresponding to GFR (Cockcroft Gault) of 271.5 ml/min/1.73 m² and 24.2 ml/min/1.73 m² respectively. A median urea value of 33.5 mg/dl (IQR 27.0—42.8) was recorded; lowest was 14 mg/dl while highest was 85 mg/dl. Median D-dimer was 1.2 µg/ml FEU (IQR 0.5—1.9), lowest being 0.19 µg/ml FEU and the highest 22 µg/ml FEU.

Abnormal liver function, defined as GGT/GOT/GPT > 3x normal value or bilirubin > twice the normal value, was observed in only five patients, 2 under apixaban treatment and 3 under edoxaban treatment. The most predominant cardiovascular risk factor of this study population was hypertension, followed by hypercholesterinemia with the entire population being generally pre-obese. The frequency of other cardiovascular risk factor were coronary heart disease, nicotine abuse and diabetes mellitus in descending order. It was observed that majority of the patients, i.e. 63.7%, received DOAC due to cardioembolic causes of stroke with a corresponding median CHA₂DS₂-VASc score of 5 points. 85% of all patients had a history of stroke and in most cases were being treated for a recent stroke at the time of study enrollment. The median HAS-BLED score was 2 points. Data for the baseline characteristics are summarized in table 4.

Table 4: Baseline characteristics of study population

	Dabigatran (N=20)	Apixaban (N=20)	Edoxaban (N=20)	Rivaroxaban (N=20)	All (N=80)
Age [years]	72 (63—88)	76 (56—80.5)	84 (78—90)	78 (60—87)	78 (63—86)
No. of samples collected	116	115	113	115	459
Female (N)	6 (30%)	8 (40%)	9 (45%)	11 (55%)	34 (42.5%)
BMI, kg/m ²	28.1 ± 4.6	25.5 ± 4.1	26.3 ± 5.6	26.0 ± 5.3	26.5 ± 5.0
Risk factors					
Abnormal liver function*	3 (15%)	0	2 (10%)	0	5 (6.3%)
Baseline creatinine, mg/dL	0.9 (0.7—1)	0.9 (0.8—1.2)	0.9 (0.7—1.3)	0.9 (0.6—1)	0.9 (0.7—1.1)
GFR [mL/min] (MDRD)	87.3 ± 31.3	74.6 ± 21.4	71.7 ± 27.1	85.7 ± 24.3	79.8 ± 26.7
GFR [mL/min] (CDKEPI)	76.1 ± 18.0	73.1 ± 19.0	64.7 ± 20.3	77.9 ± 15.1	72.9 ± 18.6
GFR [mL/min] (Cockcroft-Gault)	89.5 (61.8—106.8)	78.4 (56.2—106.1)	55.3 (39.9—73.5)	78.8 (60.3—111.8)	74.3 (53.1—102.3)
CrCl < 30 ml/min	1 (5%)	0	1 (5%)	0	2 (2.5%)
CrCl < 50 ml/min	3 (15%)	4 (20%)	8 (40%)	3 (15%)	18 (22.5%)
Congestive heart failure	2 (10%)	1 (5%)	0	2 (10%)	5 (6.3%)
Arterial hypertension	18 (90%)	15 (75%)	16 (80%)	12 (60%)	61 (76.3%)
Hypercholesterinemia	8 (40%)	6 (30%)	7 (35%)	5 (25%)	26 (32.5%)
Diabetes mellitus	0	1 (5%)	5 (25%)	3 (15%)	9 (11.3%)
Nicotine abuse	7 (35%)	5 (25%)	1 (5%)	5 (25%)	18 (22.5%)
Coronary heart disease	7 (35%)	5 (25%)	8 (40%)	2 (10%)	22 (27.5%)

History of myocardial infarction	3 (15%)	1 (5%)	4 (20%)	2 (10%)	10 (12.5%)
History of PAD	2 (10%)	1 (5%)	1 (5%)	2 (10%)	6 (7.5%)
Previous oral anticoagulation	4 (20%)	6 (30%)	5 (25%)	8 (40%)	13 (16.3%)
Therapy with antiplatelets	4 (20%)	4 (20%)	6 (30%)	8 (40%)	22 (27.5%)
Alcohol abuse	1 (5%)	1 (5%)	0	1 (5%)	3 (3.8%)
History of stroke	17 (85%)	18 (90%)	16 (80%)	17 (85%)	68 (85%)
CHA ₂ DS ₂ -VASc Score	5 (3—6)	4,5 (4—5)	6 (3,3—7)	4 (3—6)	5 (3—6)
HAS-BLED Score	2 (1—3)	2 (1,3—2)	2 (1,3—3)	2 (2—3)	2 (1—3)
Indication for anticoagulation therapy					
Atrial fibrillation	9 (45%)	17 (85%)	11 (55%)	14 (70%)	51 (63.7%)
Patent foramen ovale	10 (50%)	1 (5%)	6 (30%)	5 (25%)	22 (27.5%)
Others**	1 (5%)	2 (10%)	3 (15%)	1 (5%)	7 (8.8%)

BMI, body mass index; CHE, choline esterase; CrCl, creatinine clearance; GOT, Glutamic-Oxaloacetic Transaminase; GGT, gamma-glutamyl transferase GPT, glutamic-pyruvic transaminase; PAD, peripheral artery disease

*GGT/GOT/GPT > 3x normal value or bilirubin > 2x normal value

** others, Embolic stroke of undetermined source, pulmonary embolism, deep venous thrombosis, cerebral venous sinus thrombosis, paraneoplastic syndrome

Ideally, a baseline plasma DOAC level of 0 ng/dl detected by UPLC-MS/MS was expected. In 9 patients however, traces of DOAC, i.e. 0.9–9.8 ng/ml (apixaban, n = 5; dabigatran, n = 1), up to significant DOAC concentration, i.e. 34.3–75.4 ng/ml (edoxaban, n = 1; rivaroxaban, n = 2), was observed at baseline. These patients were enrolled at least 3 days after last known DOAC intake and were all patients who had at time of study enrollment, received a DOAC different from that previously administered prior to study enrollment. Only one of these nine patients had a renal

impairment with creatinine clearance of 31.5 ml/min and a corresponding baseline plasma DOAC level of only 0.9 ng/ml. This patient belonged to the apixaban group. Of the nine patients, four had a creatinine clearance of 50–89 ml/min (apixaban, n = 2; edoxaban, n = 1; rivaroxaban n = 1), and the remaining four had a creatinine clearance of > 100 ml/min (apixaban, n = 2; dabigatran, n = 1; rivaroxaban n = 1). All the nine patients did not have any history or present manifestation of hepatic dysregulation. The age of patients with significant plasma DOAC concentration ranged between 59–92 years.

6.2 Descriptive statistics of coagulation parameters

6.2.1 CoaguChek®-aPTT

The mean CoaguChek®-aPTT and corresponding SD recorded for all samples was 35s ± 10. The lowest value of 19s was recorded for all four DOAC. The highest value of 69s was recorded across all samples. This was seen in a patient the edoxaban (Table 5).

Table 5: Descriptive data of coagulation parameters

	Dabigatran		Apixaban		Edoxaban		Rivaroxaban		All	
	Mean ± SD or Median (IQR)	Min.– Max.	Mean ± SD or Median (IQR)	Min.– Max.	Mean ± SD or Median (IQR)	Min.– Max.	Mean ± SD or Median (IQR)	Min.– Max.	Mean ± SD or Median (IQR)	Min.– Max.
CoaguChek®- aPTT, s	29 ± 5	19–43	32 ± 8	19– 61.2	37 ± 10	19–69.4	40 ± 11	19–65	35 ± 10	19–69
UPLC-MS/MS, ng/ml	12 (0–58)	0–234	39 (4–71)	0– 267	61 (3– 191)	0–1349	113 (24– 185)	0–444	55 (2–115)	0–1349
Lab-based calibrated DOAC, ng/ml	19 (19–54)	19–203	40 (10– 69)	8– 235	70 (19– 184)	19–513	101 (22– 182)	10–420	43 (19–110)	8–513
Anti-Xa activity, IU/ml			0.2 (0.1– 0.4)	0.1– 1.6	0.3 (0.1– 1.2)	0.1–1.6	0.8 (0.10– 1.4)	0.1–1.6	0.3 (0.1– 1.0)	0.1–1.6
Lab-based aPTT, s	25 (23–29)	19–48	24 (22– 26)	19– 31	25 (23– 30)	18–57	27 (24– 30)	18–47	25 (23–29)	2–57
INR	1.1 (1–1.1)	0.9–1.3	1.1 (1.1– 1.2)	0.9– 1.6	1.1 (1– 1.3)	1–3.1	1.2 (1.1– 1.3)	0.9–4.1	1.1 (1.0– 1.2)	0.9–4.1

INR, international normalized ration; PTT partial thromboplastin time; UPLC-MS/MS, Ultra-performance liquid chromatography – tandem mass spectrometry

6.2.2 High-performance liquid chromatography/mass spectrometry (UPLC-MS/MS)

The median UPLC-MS/MS recorded for all samples was 55 ng/ml (IQR 2—115). A trough level of 0 ng/ml was recorded for all DOAC, mostly at baseline and in some cases up to 2 hours after DOAC intake. The highest peak concentration of 1349 ng/ml was recorded in the edoxaban group (Table 5).

6.2.3 Laboratory-based calibrated DOAC plasma level

The mean laboratory-based calibrated DOAC plasma concentration varied across all DOAC groups. DOAC plasma concentration as low as 8 ng/ml was recorded in the apixaban group and as high as 513 ng/ml was recorded in the edoxaban group with a median of 43 ng/ml (IQR 19–110) across all samples (Table 5).

6.2.4 Anti-Xa-Activity

The median anti-Xa-activity for across samples was 0.3 IU/ml (0.1–1.0) which was mainly influenced by the relatively higher anti-Xa-activity of rivaroxaban, i.e. 0.75 IU/ml (IQR 0.10—1.08), in comparison to the other DOAC. The lowest value of 0.1 IU/ml and highest value of 1.62 IU/ml were recorded for all factor Xa inhibitors corresponding to trough and peak concentration respectively (Table 5).

6.2.5 Laboratory-based aPTT

The median aPTT did not vary among all the individual DOAC. The individual median as well as the cumulative median for all DOAC were below the laboratory-specific normal range of 40s for the reagent used, i.e. Dade[®] Actin[®] FS (Siemens Healthineers, Erlangen, Germany). The highest aPTT value of 48s, which lies outside the normal range was found in the dabigatran group.

6.2.6 INR

The median INR did not vary across the different DOAC. The highest INR of 4.1 was recorded in the rivaroxaban group after 24 hours of DOAC intake.

6.3 Correlation analysis

The Spearman's rho correlation analyses was used for the non-parametric data at 95% confidence interval.

6.3.1 Correlation between CoaguChek®-aPTT and UPLC-MS/MS

Dabigatran and apixaban showed moderate correlations between CoaguChek®-aPTT and UPLC-MS/MS, i.e. 0.49 and 0.50 respectively each, while edoxaban and rivaroxaban showed very strong and strong correlations i.e. 0.84 and 0.79 respectively, all at $p < 0.001$ (Figure 1a-1d).

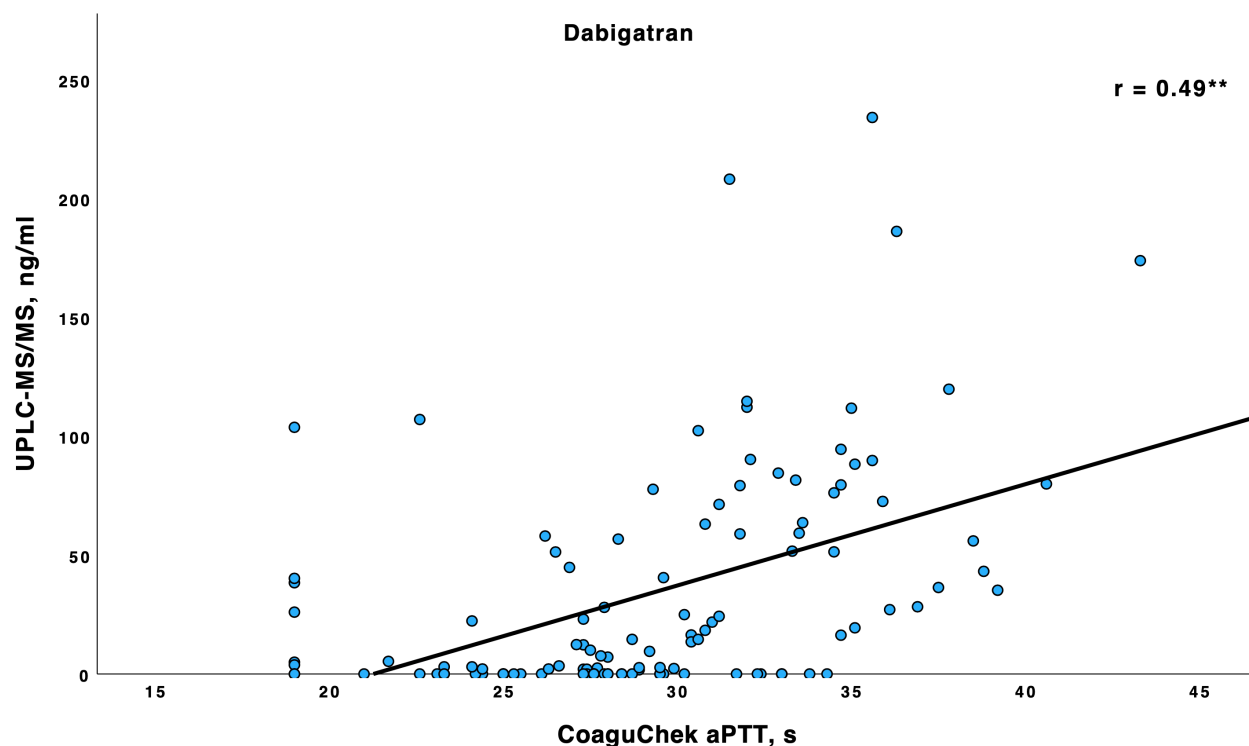


Figure 1a: Correlation between CoaguChek®-aPTT and UPLC-MS/MS for dabigatran

**significant at the 0.01 level

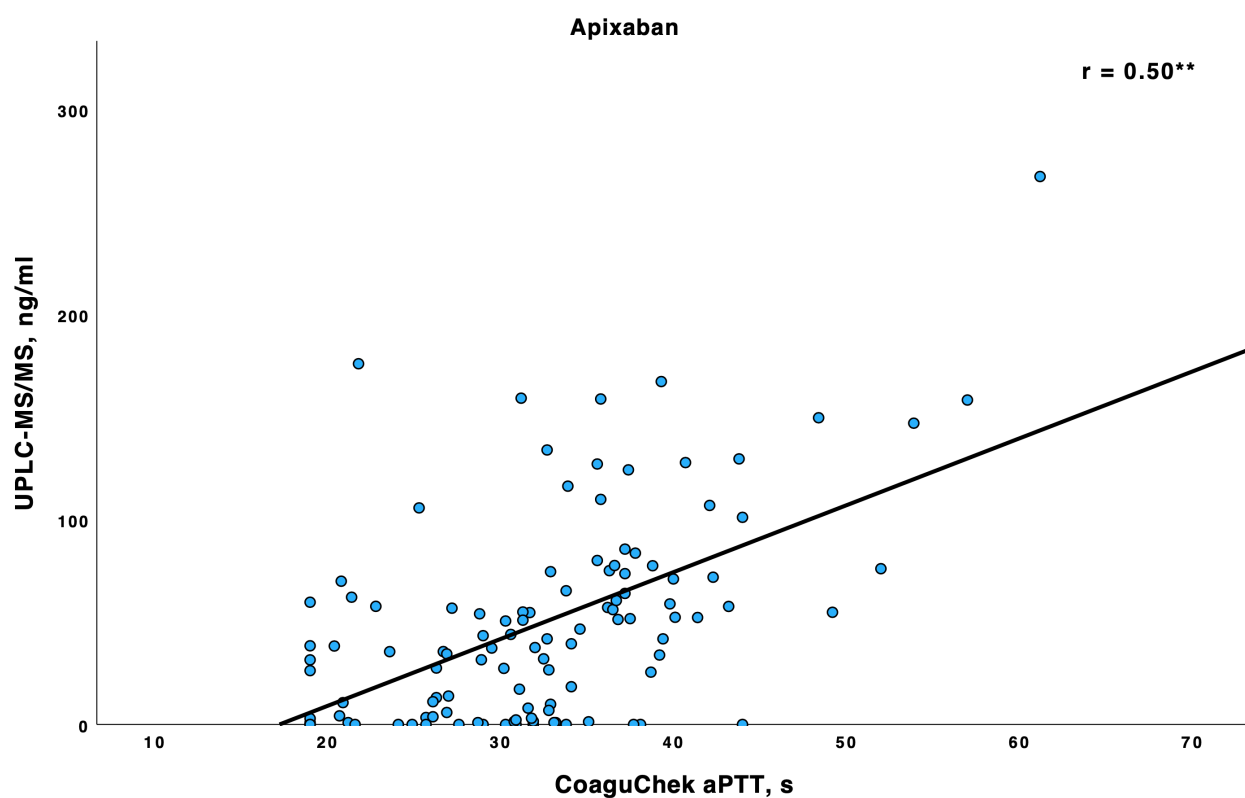


Figure 1b: Correlation between CoaguChek®-aPTT and UPLC-MS/MS for apixaban
**significant at the 0.01 level

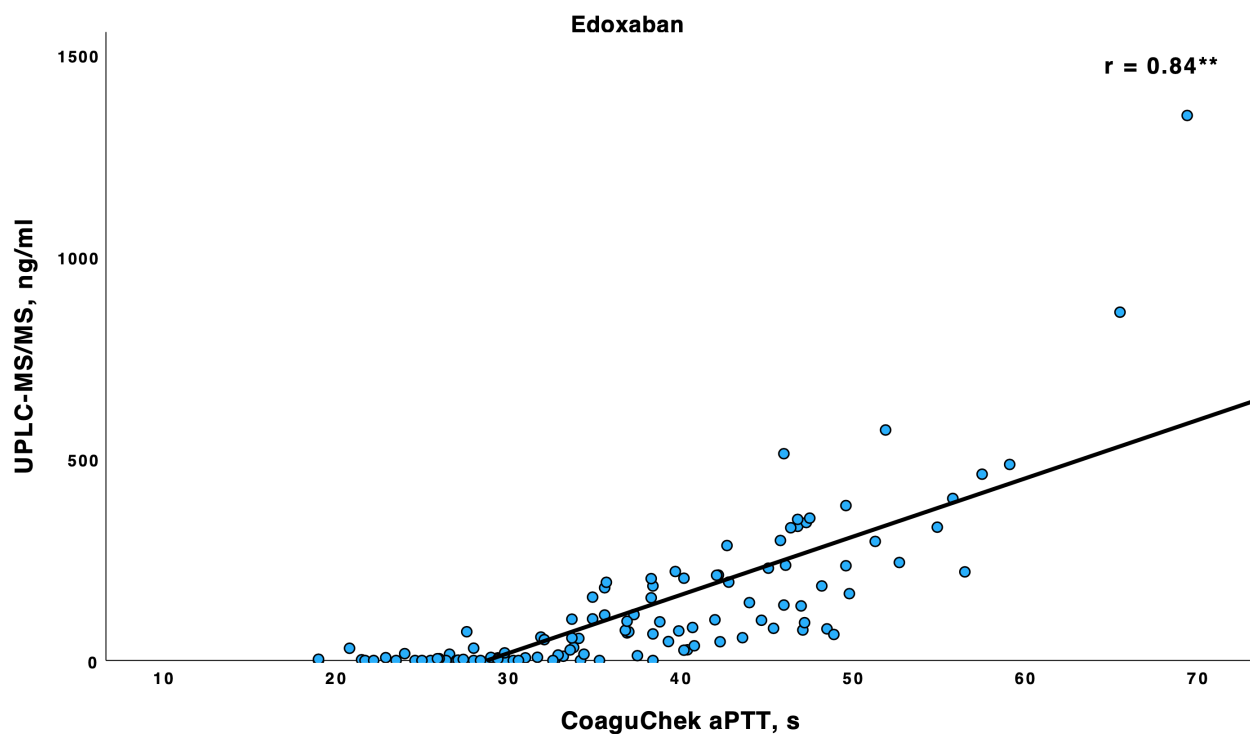


Figure 1c: Correlation between CoaguChek®-aPTT and UPLC-MS/MS for edoxaban

**significant at the 0.01 level

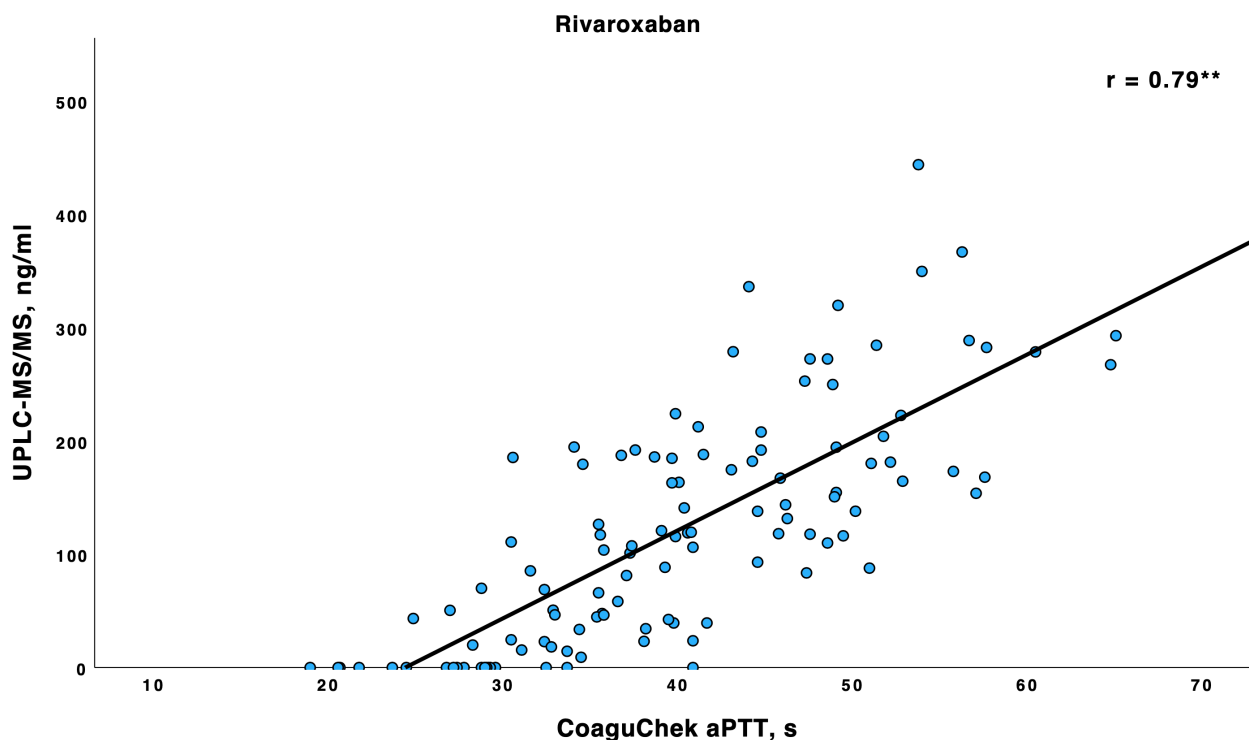


Figure 1d: Correlation between CoaguChek®-aPTT and UPLC-MS/MS for rivaroxaban

**significant at the 0.01 level

6.3.2 Correlation between laboratory-based calibrated DOAC assay and UPLC-MS/MS

All DOAC showed a very strong correlation between laboratory-based calibrated DOAC plasma level and UPLC-MS/MS. Spearman's rho correlation coefficients of 0.88, 0.95, 0.98 and 0.99 were recorded for dabigatran, apixaban, edoxaban and rivaroxaban respectively, all at significant at $p < 0.001$ (Figure 2a-d).

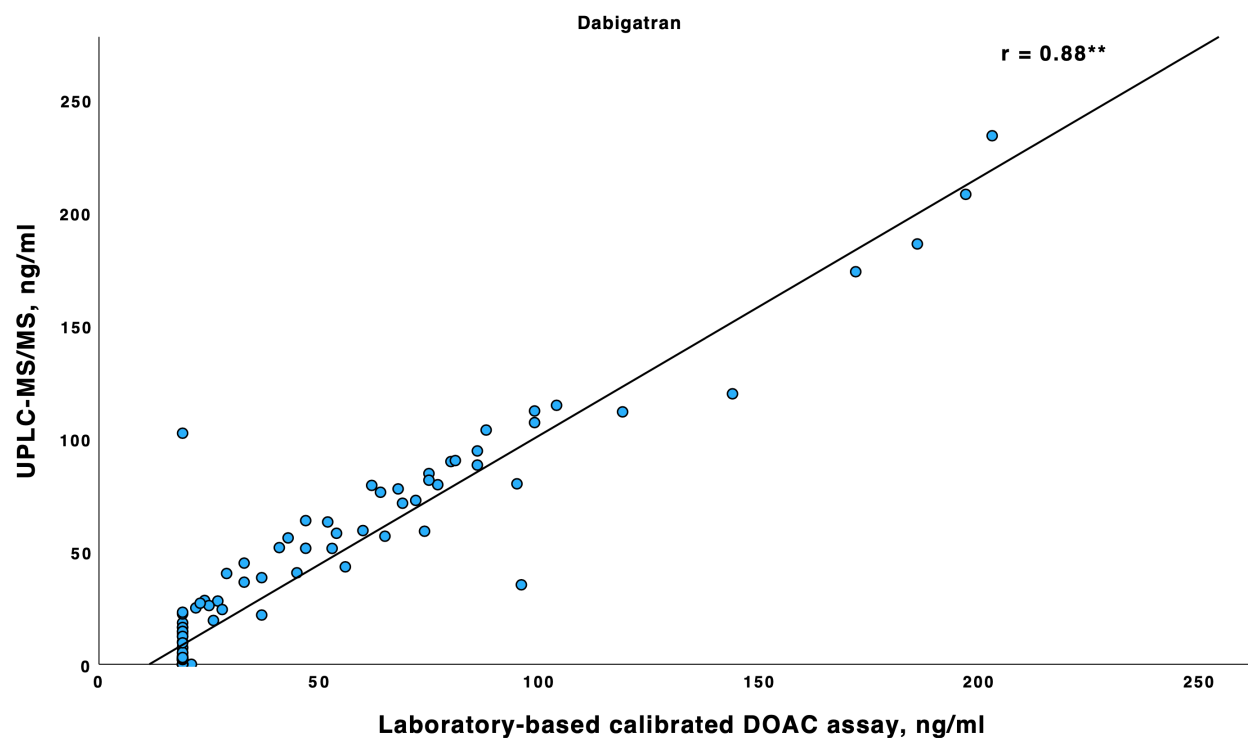


Figure 2a: Correlation between laboratory-based calibrated DOAC assay and UPLC-MS/MS for dabigatran

****significant at the 0.01 level**

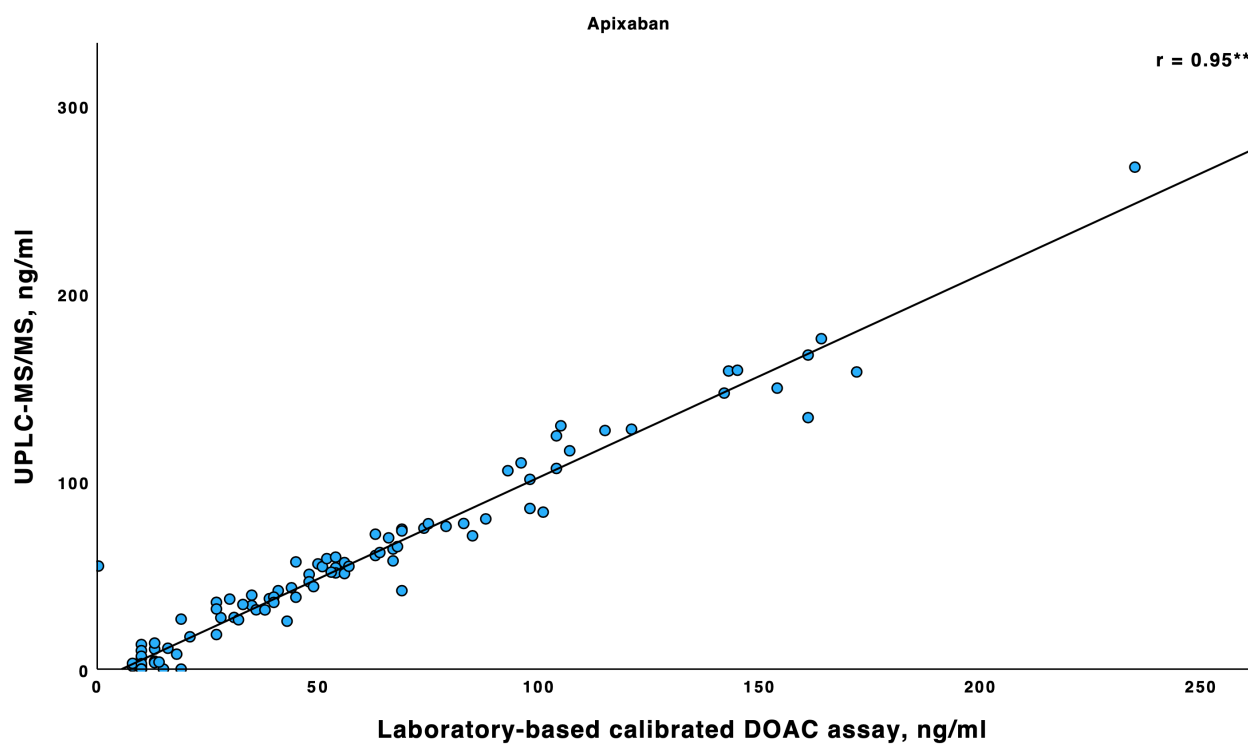


Figure 2b: Correlation between laboratory-based calibrated DOAC assay and UPLC-MS/MS for apixaban

**significant at the 0.01 level

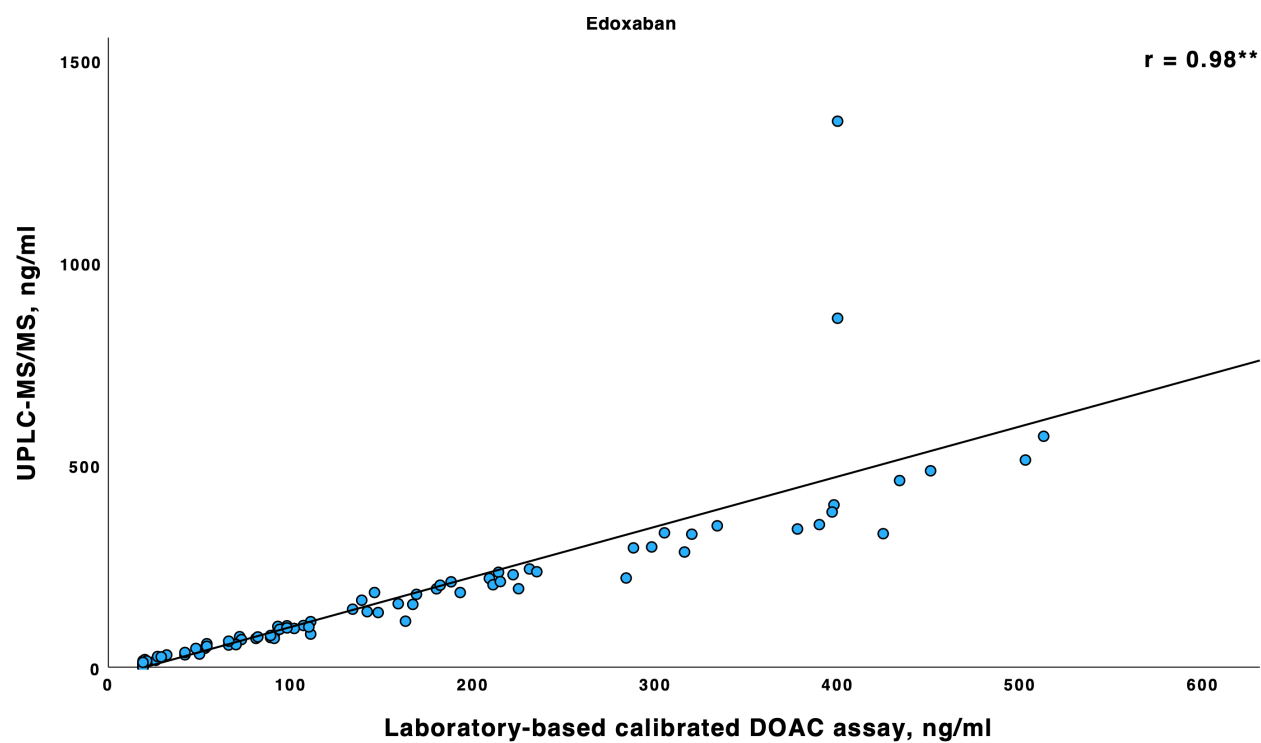


Figure 2c: Correlation between laboratory-based calibrated DOAC assay and UPLC-MS/MS for edoxaban

**significant at the 0.01 level

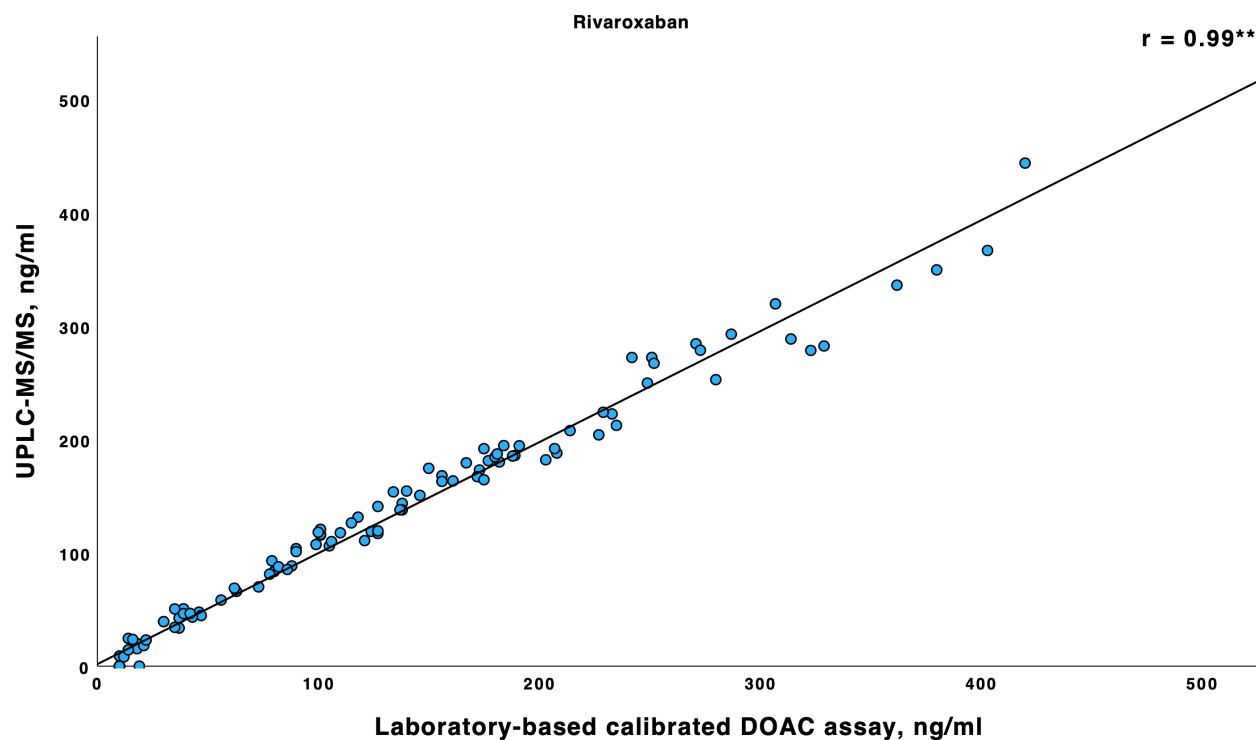


Figure 2d: Correlation between laboratory-based calibrated DOAC assay and UPLC-MS/MS for rivaroxaban

**significant at the 0.01 level

6.3.3 Correlation between anti-Xa-activity and UPLC-MS/MS

All factor-Xa inhibitors, i.e. apixaban, edoxaban and rivaroxaban, showed a very strong correlation with UPLC-MS/MS (Figure 3).

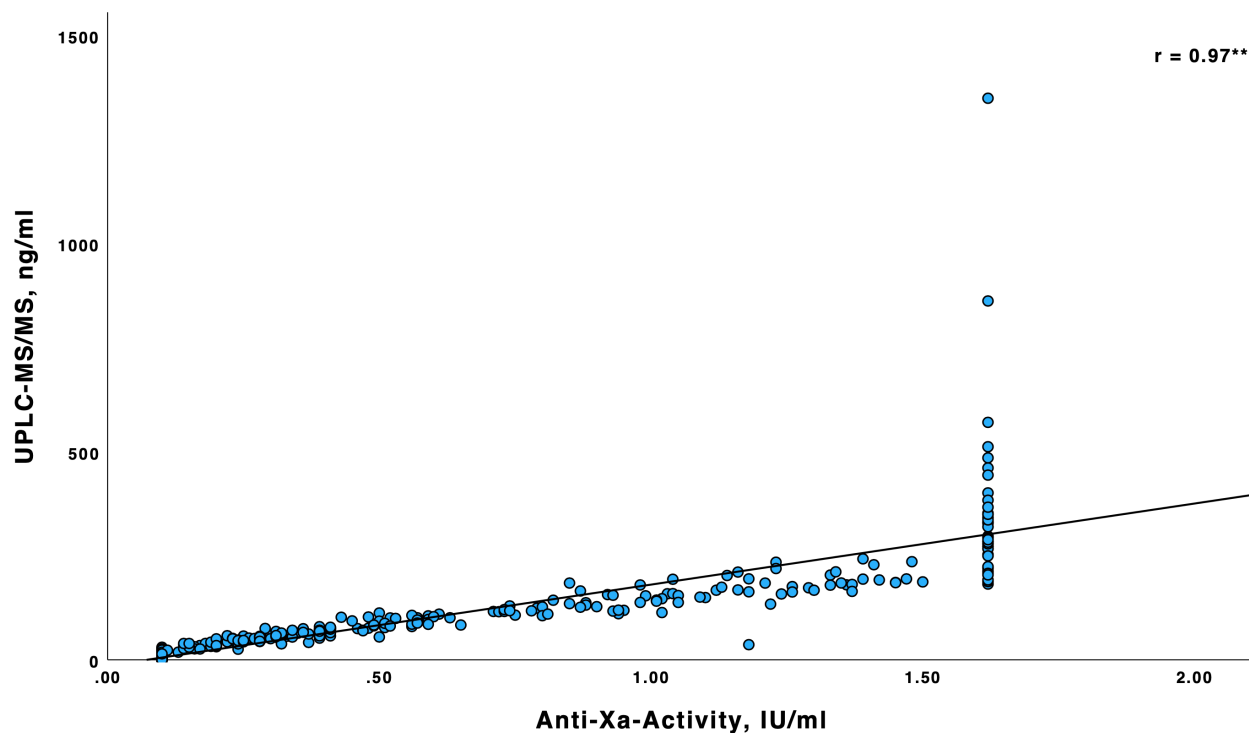


Figure 3: Correlation between anti-Xa activity and UPLC-MS/MS for all factor-Xa inhibitors
 **significant at the 0.01 level

6.3.4 Correlation between laboratory-based aPTT and UPLC-MS/MS

There was a strong correlation between laboratory-based aPTT and UPLC-MS/MS for dabigatran, i.e. 0.67 (Figure 4a). For factor-Xa inhibitors, a weak correlation was recorded for apixaban, i.e. 0.22 (Figure 4b). This correlation was however very strong for edoxaban and strong for rivaroxaban, i.e. 0.83 and 0.73 respectively, all at $p < 0.001$ (Figure 4c-d).

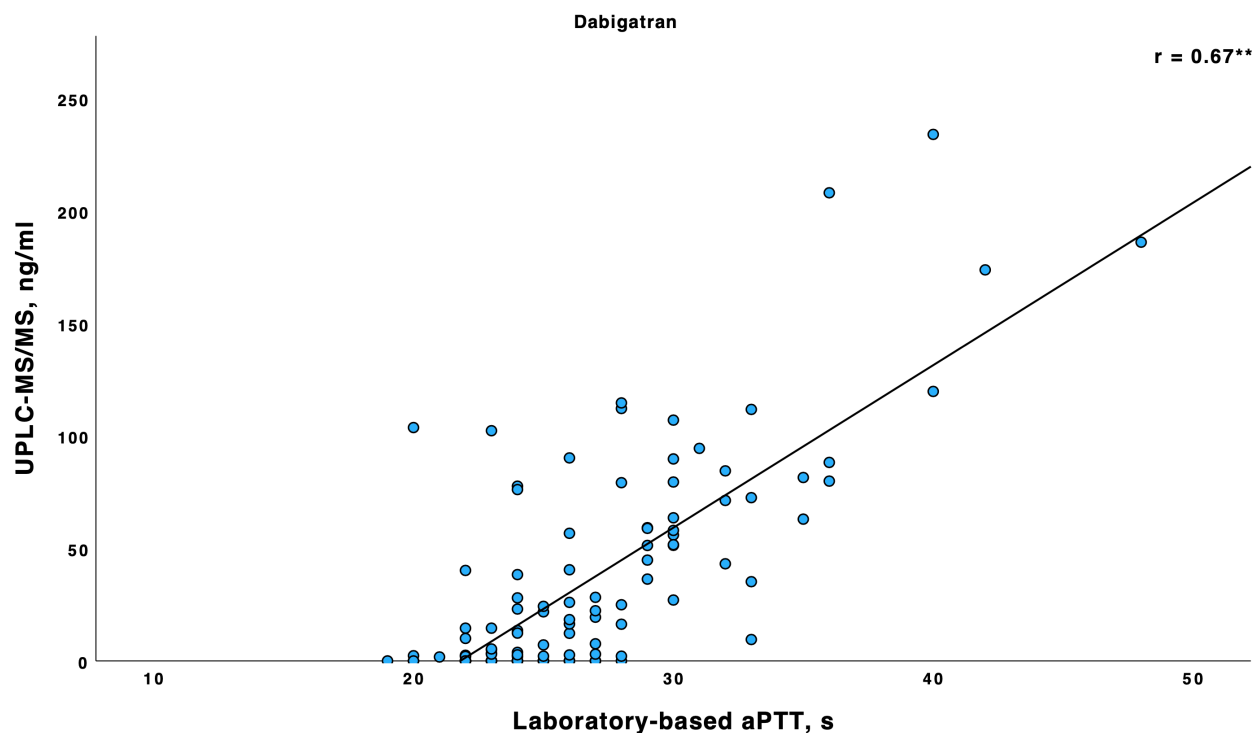


Figure 4a: Correlation between laboratory-based aPTT and UPLC-MS/MS for dabigatran
**significant at the 0.01 level

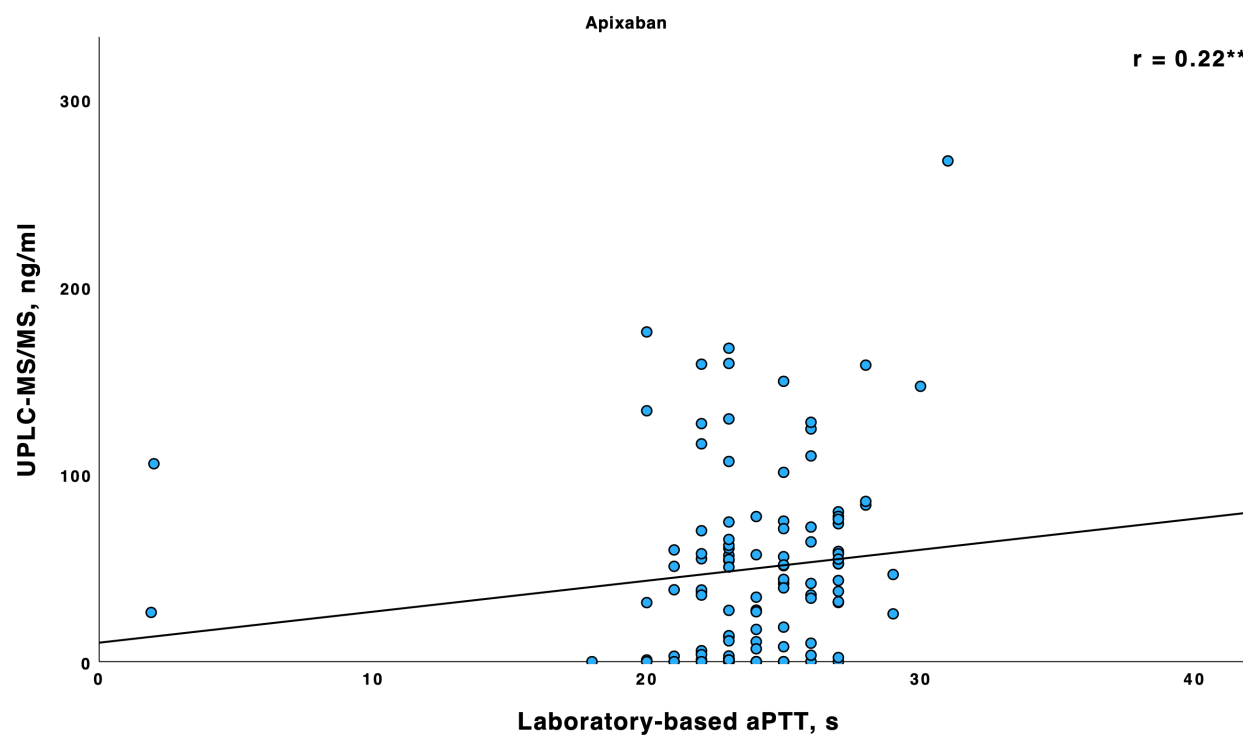


Figure 4b: Correlation between laboratory-based aPTT and UPLC-MS/MS for apixaban
**significant at the 0.01 level

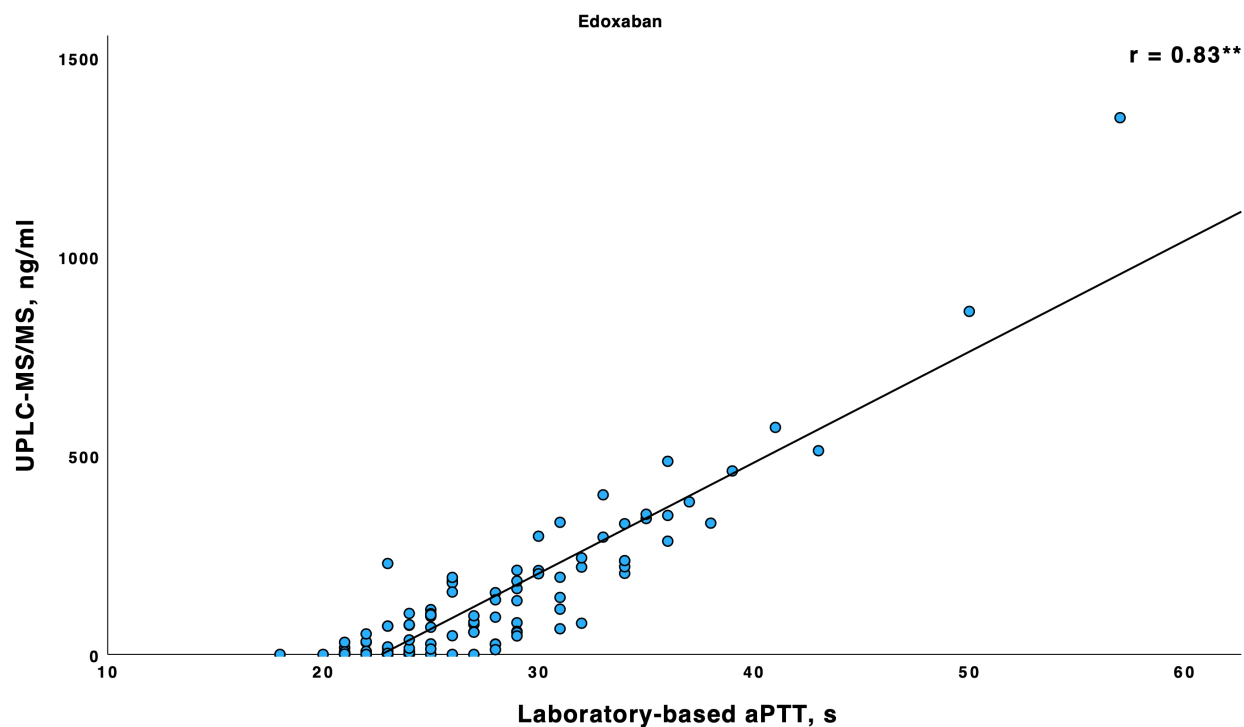


Figure 4c: Correlation between laboratory-based aPTT and UPLC-MS/MS for edoxaban
**significant at the 0.01 level

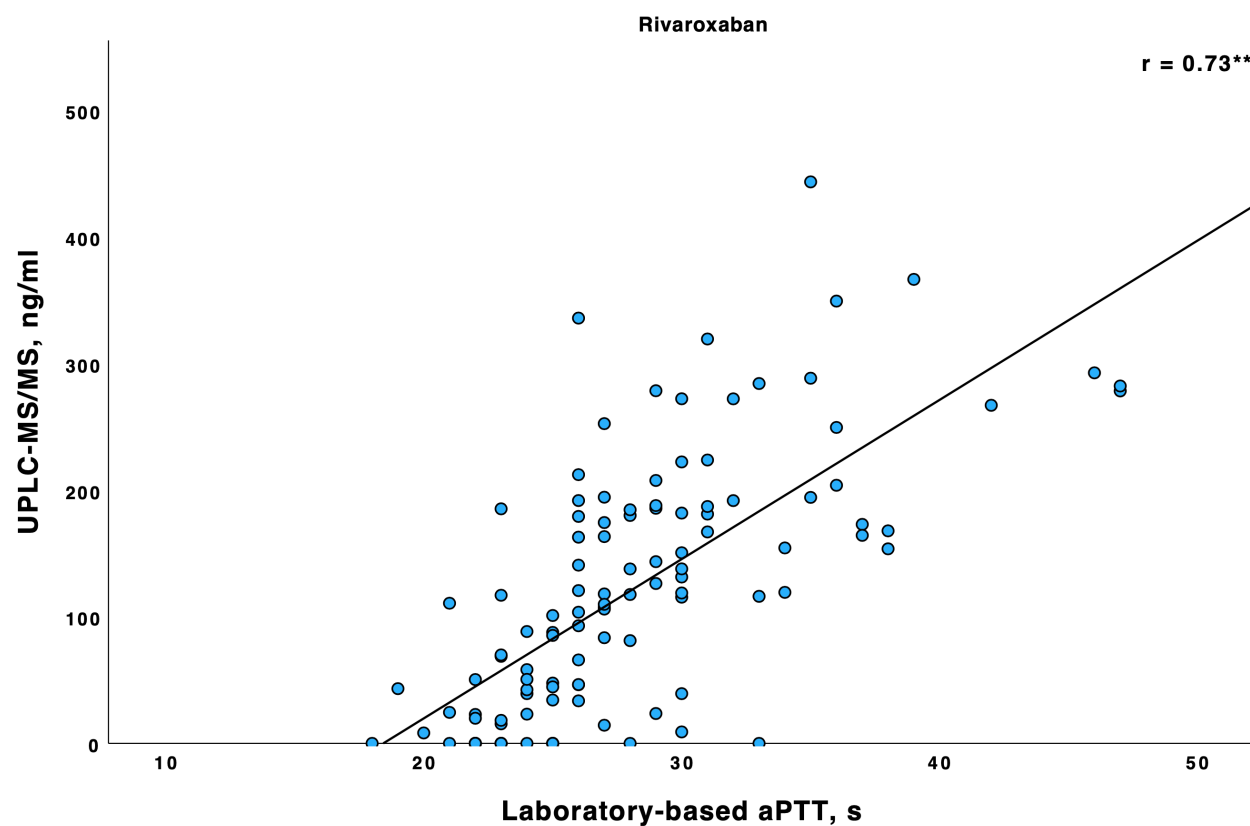


Figure 4d: Correlation between laboratory-based aPTT and UPLC-MS/MS for rivaroxaban

**significant at the 0.01 level

6.3.5 Correlation between CoaguChek®-aPTT and laboratory-based aPTT

The correlation between CoaguChek®-aPTT and laboratory-based aPTT was moderate for dabigatran and apixaban, i.e. 0.62 and 0.64 respectively, but very strong for edoxaban and rivaroxaban, i.e. 0.86 and 0.87 respectively, all at $p < 0.001$ (Figure 5a-d).

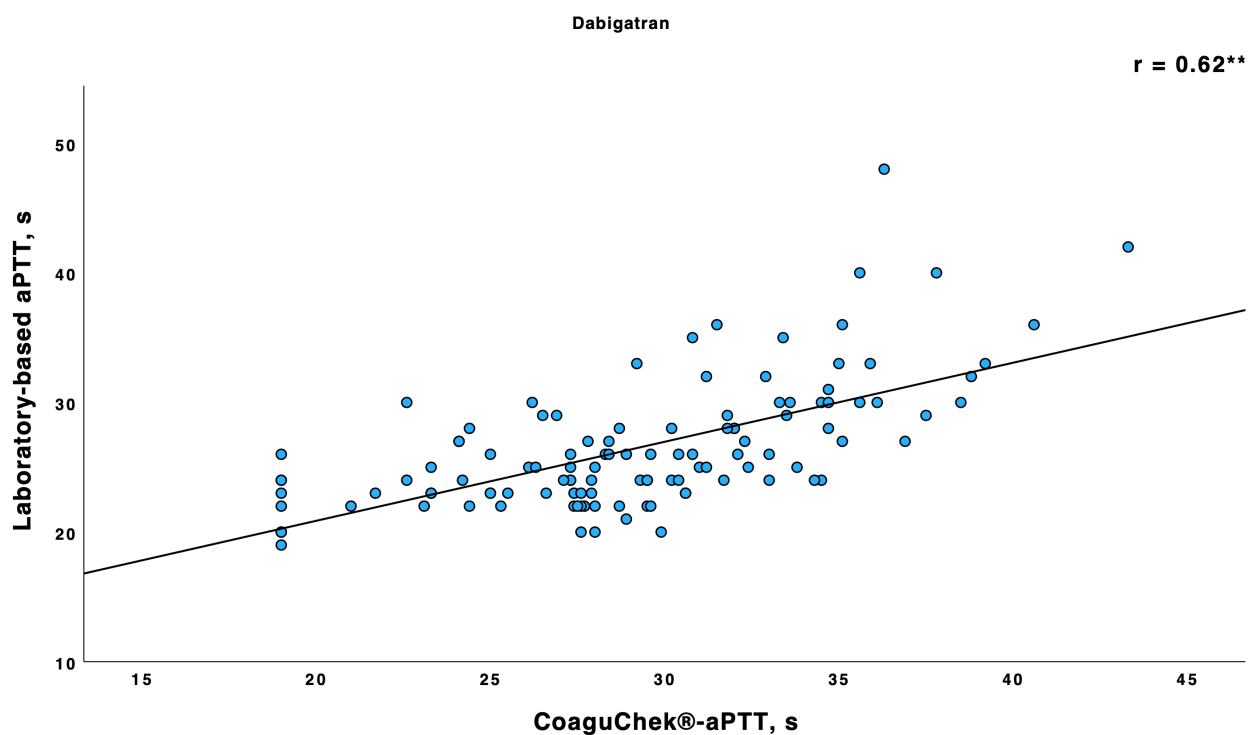


Figure 5a: Correlation between lab-based aPTT and CoaguChek®-aPTT for dabigatran

**significant at the 0.01 level

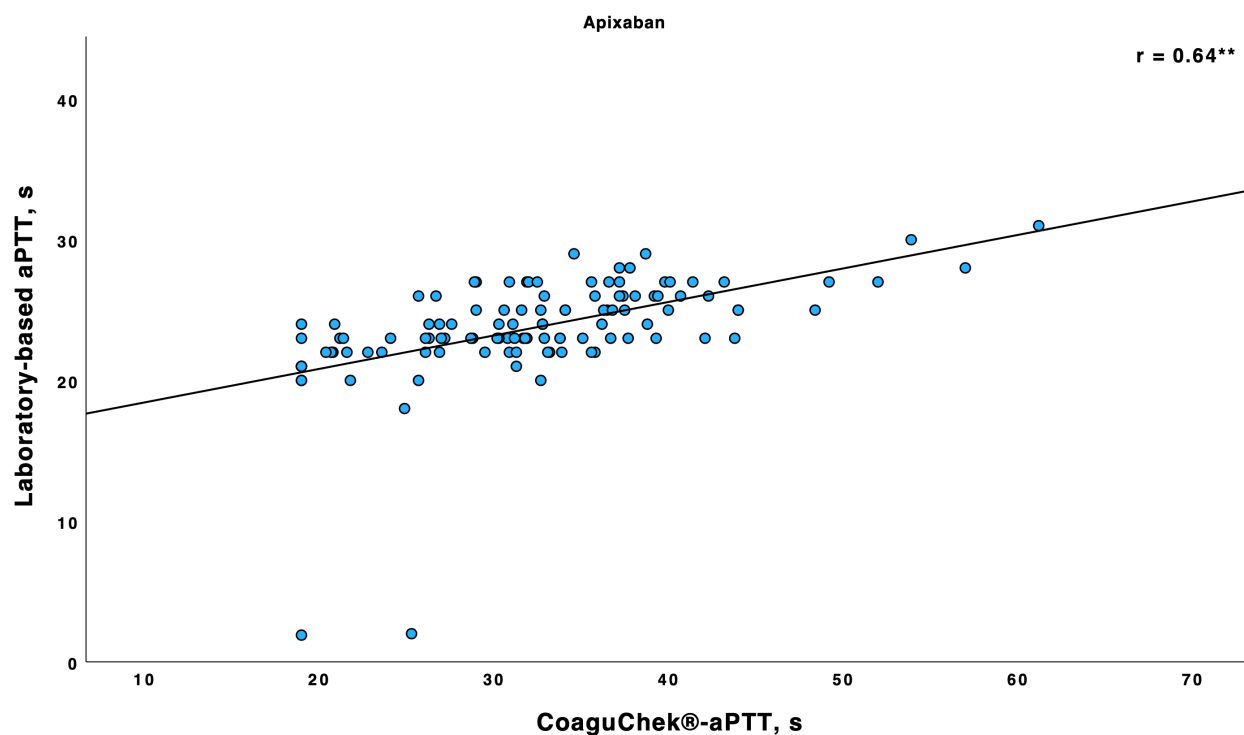


Figure 5b: Correlation between lab-based aPTT and CoaguChek®-aPTT for apixaban
**significant at the 0.01 level

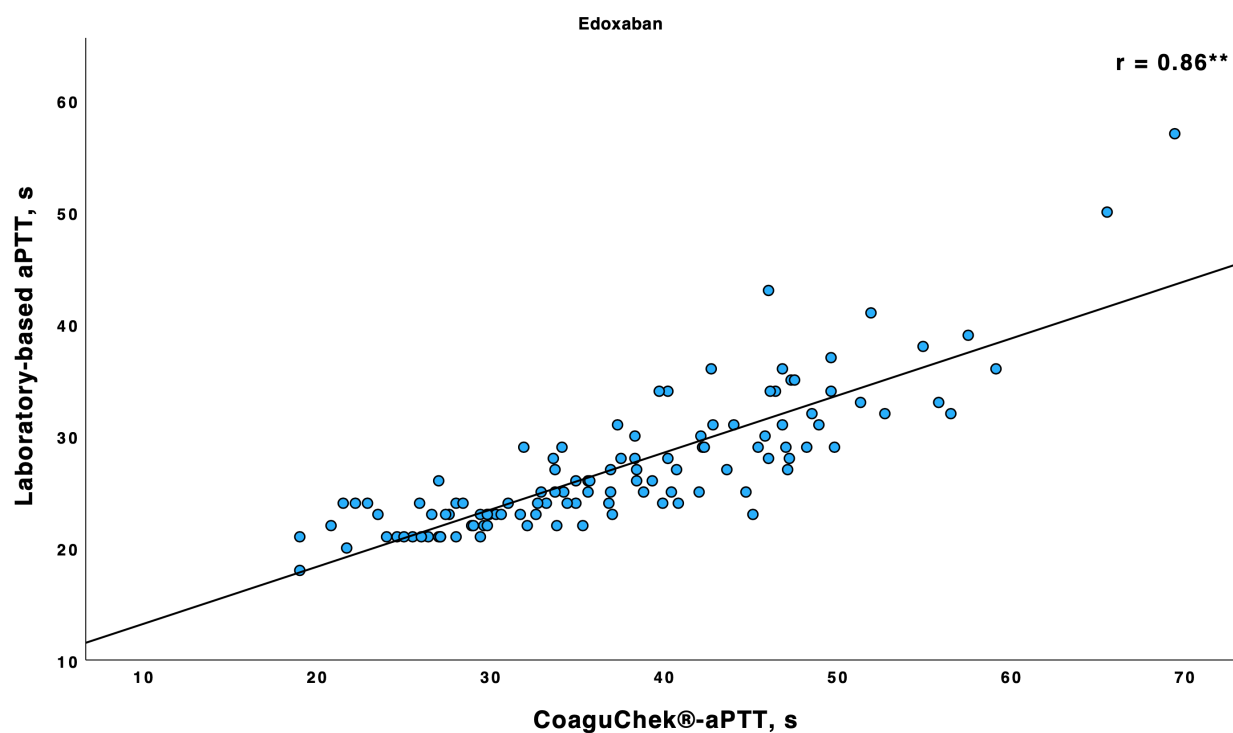


Figure 5c: Correlation between lab-based aPTT and CoaguChek®-aPTT for edoxaban
**significant at the 0.01 level

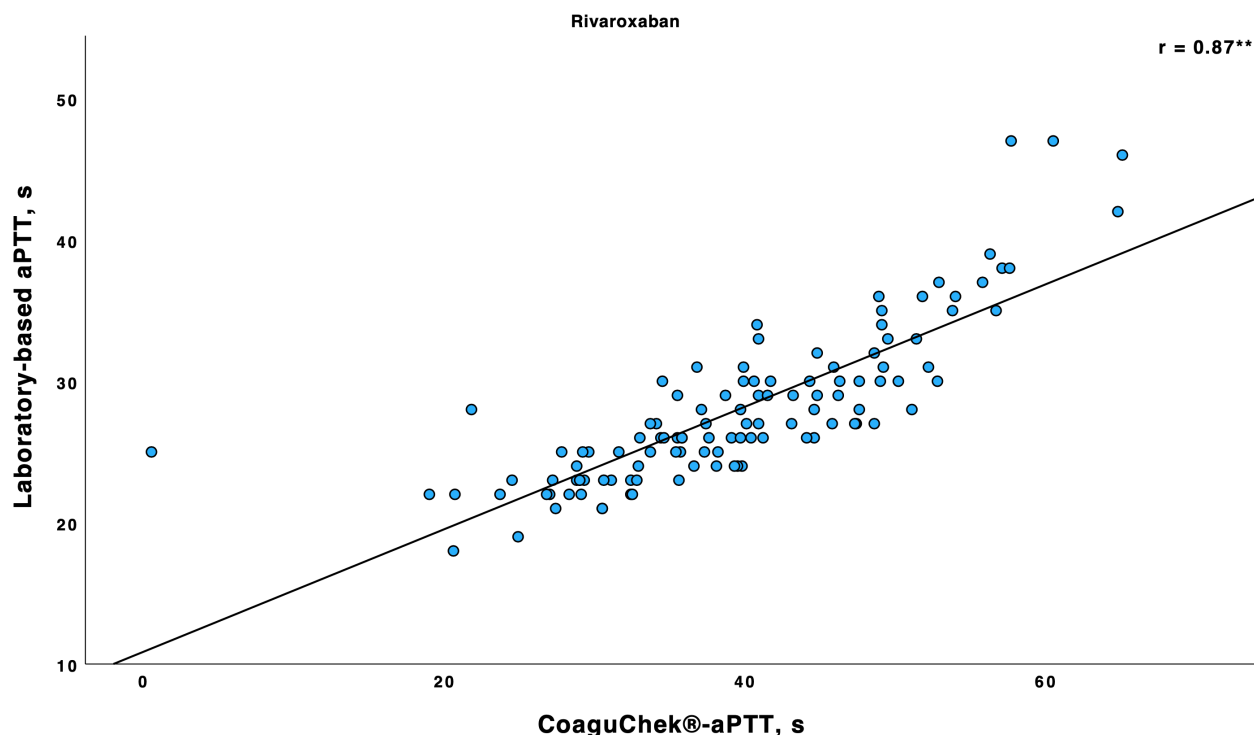


Figure 5d: Correlation between lab-based aPTT and CoaguChek®-aPTT for rivaroxaban

****significant at the 0.01 level**

6.4 Diagnostic accuracy of CoaguChek®-aPTT at ≥ 30 ng/ml threshold

Samples with actual plasma concentration ≥ 30 ng/ml detected by means of UPLC-MS/MS were detected as such by CoaguChek®-aPTT in 38/107 (35.5%), 65/104 (62.5%), 66/78 (84.6%) and 82/93 (88.2%) samples for dabigatran, apixaban, edoxaban and rivaroxaban respectively. Percentage values represent the definition for sensitivity (see section 5.8). On the other hand, 5/8 (62.5%), 6/9 (66.7%), 35/38 (92.1%) and 18/22 (81.8%) samples were correctly identified as samples < 30 ng/ml for dabigatran, apixaban, edoxaban and rivaroxaban respectively, representing specificity of CoaguChek®-aPTT (See section 5.8).

To avoid false positives, the ideal cut-off was pre-defined as the highest possible CoaguChek® aPTT value that yielded a sensitivity of $> 95\%$ and thus a misprediction percentage $< 5\%$.

For dabigatran, no cut-off could yield a sensitivity of at least 95%. For apixaban, a sensitivity and specificity of 95.6% (95%-CI 89.0–98.9) and 13.3% (95%-CI 5.5–25.2) respectively were recorded when using a cut-off of 20s, all $p < 0.00$. At cut-offs of 32s and 30s, edoxaban and rivaroxaban yielded a sensitivities of 95.7% (95%-CI 89.1–98.9) and 95.3% (95%-CI 89.5–98.5) respectively, with a corresponding specificity of 74.5% (95%-CI 60.9–85.4) and 62.1% (95%-CI 43.9–78.2) respectively, $p < 0.00$ (Table 6).

Table 6: Diagnostic parameters of CoaguChek®-aPTT for all DOAC with corresponding cut-offs using 30 and 50 ng/ml thresholds using a more and less conservative definition for sensitivity at > 95% and > 90% respectively.

		(Ideal) Cut off for CoaguChek®-aPTT (s)	Sensitivity (%)		Specificity (%)		PPV (%)		NPV (%)		LR	
				p		p		p		p	N	p
Dabigatran	30 ng/ml	20	92.7 (82.1–98.1)	0.00	6.8 (2.5–14.0)	0.02	35.5 (26.9–44.8)	0.00	62.5 (29.0–89.0)	0.00	0.01	0.91
		26	90.2 (78.8–96.9)	0.00	31.1 (21.3–42.1)	0.00	42.0 (32.1–52.6)	0.00	85.2 (68.9–95.1)	0.00	7.41	0.01
	50 ng/ml	22	97.1 (87.7–99.8)	0.00	11.1 (5.5–19.1)	0.00	31.4 (23.1–40.7)	0.00	90.0 (62.8–99.4)	0.00	2.42	0.12
		26	91.2 (78.7–97.7)	0.00	32.1 (22.6–42.7)	0.00	36.0 (26.4–46.5)	0.00	89.7 (75.3–97.3)	0.00	7.92	0.00
Apixaban	30 ng/ml	20	95.6 (89.0–98.9)	0.00	13.3 (5.5–25.2)	0.01	62.5 (53.0–71.4)	0.00	66.7 (34.5–90.5)	0.00	2.87	0.09
		22	91.1 (81.0–97.2)	0.00	22.1 (13.3–32.8)	0.00	78.9 (57.6–92.9)	0.00	43.6 (33.9–53.7)	0.00	2.90	0.09
	50 ng/ml	21	96.1 (88.4–99.3)	0.00	19.4 (10.9–30.3)	0.00	49.5 (39.7–59.3)	0.00	85.7 (62.1–97.5)	0.00	6.86	0.01
		25	90.2 (80.1–96.4)	0.00	25.8 (16.0–37.5)	0.00	50.0 (39.9–60.1)	0.00	76.2 (55.6–90.7)	0.00	4.99	0.03
Edoxaban	30 ng/ml	32	95.7 (89.1–98.9)	0.00	74.5 (60.9–85.4)	0.00	84.6 (75.5–91.4)	0.00	92.1 (80.8–98.0)	0.00	68.65	0.00
		34	90.0 (81.3–95.5)	0.00	85.1 (73.2–93.3)	0.00	89.9 (81.3–95.5)	0.00	85.1 (73.2–93.3)	0.00	71.75	0.00
	50 ng/ml	34	95.2 (88.1–98.8)	0.00	79.2 (67.1–88.6)	0.00	84.5 (74.9–91.6)	0.00	93.3 (83.6–98.3)	0.00	76.68	0.00
		35	90.5 (81.6–96.1)	0.00	84.9 (73.7–92.8)	0.00	87.7 (78.3–94.2)	0.00	88.2 (77.6–95.1)	0.00	74.51	0.00
Rivaroxaban	30 ng/ml	30	95.3 (89.5–98.5)	0.00	62.1 (43.9–78.2)	0.00	88.2 (80.6–93.7)	0.00	81.8 (62.7–94.0)	0.00	41.41	0.00
		33	90.7 (83.4–95.6)	0.00	79.3 (62.5–91.2)	0.00	92.9 (86.1–97.1)	0.00	74.2 (57.3–87.3)	0.00	51.25	0.00
	50 ng/ml	31	95.0 (88.2–98.3)	0.00	51.3 (35.9–66.5)	0.00	79.1 (70.0–86.6)	0.00	83.3 (65.4–94.5)	0.00	32.43	0.00
		34	90.0 (81.3–95.0)	0.00	69.2 (53.8–82.2)	0.00	85.0 (76.1–91.7)	0.00	77.1 (61.6–88.8)	0.00	42.04	0.00

LR: likelihood ratio; NPV: negative prediction value; PPV: positive prediction value. Significant at the 0.01 level.

Receiver operator characteristic (ROC) curves are shown in figure 6. The highest area under the ROC curve (AUROC) was recorded for edoxaban with 0.93 (95%-CI 0.89–0.98), followed by rivaroxaban with 0.91 (95%-CI 0.85–0.97). Dabigatran and apixaban recorded an AUROC of 0.78 (95%-CI 0.68–0.88) and 0.74 (95%-CI 0.65–0.83) respectively, all at $p < 0.000$ (Table 7).

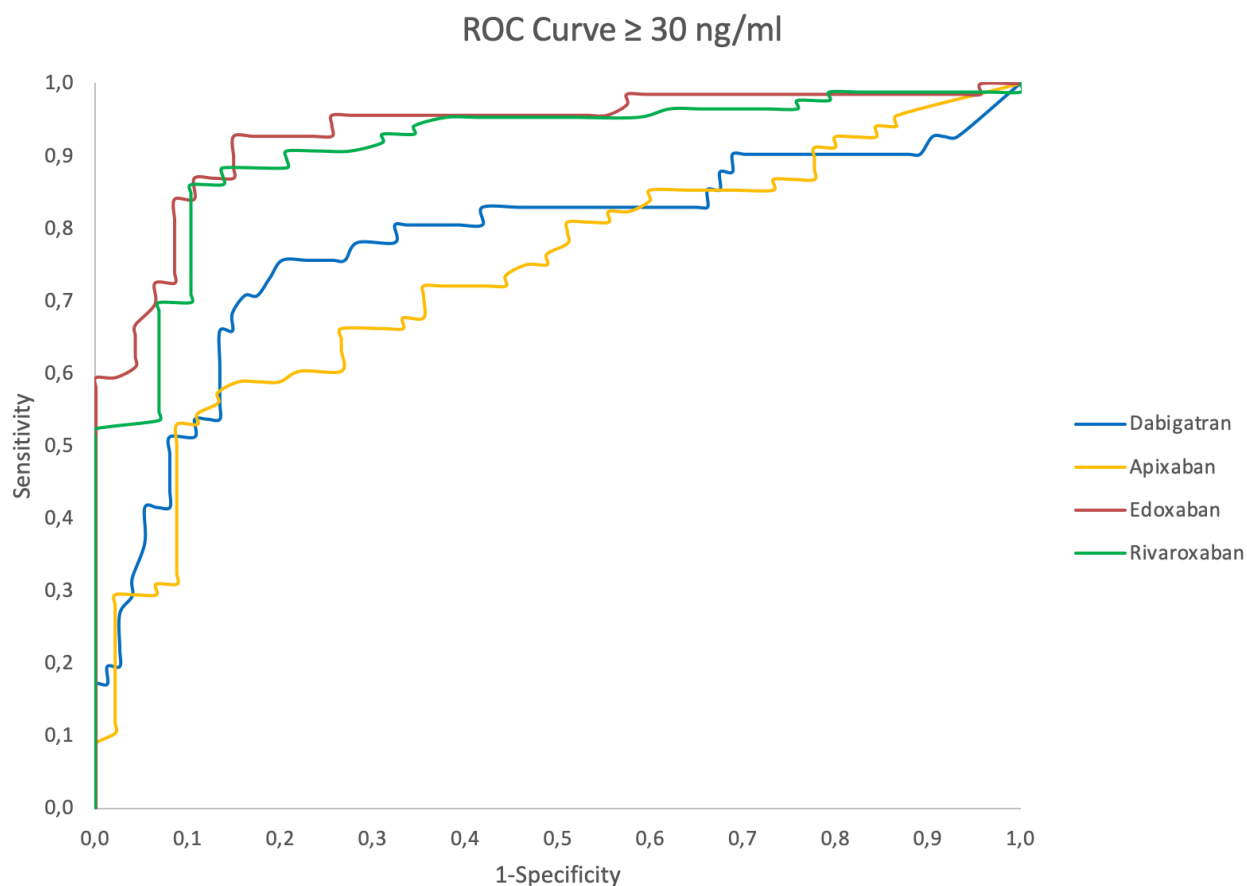


Figure 6: Receiver operator characteristics curve at ≥ 30 ng/ml for all DOAC

Table 7 Area under the receiver operator characteristics curve for all DOAC

DOAC	Threshold	AUROC	Two-tailed p-Value
Dabigatran	≥ 30 ng/ml	0.78 (0.68–0.88)	0.00
	≥ 50 ng/ml	0.8 (0.70–0.89)	0.00
Apixaban	≥ 30 ng/ml	0.74 (0.65–0.83)	0.00
	≥ 50 ng/ml	0.79 (0.71–0.88)	0.00
Edoxaban	≥ 30 ng/ml	0.93 (0.89–0.98)	0.00
	≥ 50 ng/ml	0.94 (0.90–0.98)	0.00
Rivaroxaban	≥ 30 ng/ml	0.91 (0.85–0.97)	0.00
	≥ 50 ng/ml	0.89 (0.83–0.95)	0.00

AUROC: area under the receiver operator characteristics curve. Significant at the 0.01 level.

6.5 Diagnostic accuracy of CoaguChek[®]-aPTT at ≥ 50 ng/ml threshold

Samples ≥ 50 ng/ml were correctly detected by CoaguChek[®]-PTT in 33/105 (31.4%), 60/71 (84.5%), 66/78 (84.6%) and 72/91 (79.1%) samples for dabigatran, apixaban, edoxaban and rivaroxaban respectively. On the other hand, 9/10 (90.0%), 12/14 (85.7%), 42/45 (79.2%) and 20/24 (83.3%) samples were correctly identified as samples < 50 ng/ml for dabigatran, apixaban, edoxaban and rivaroxaban respectively.

Using a cut-off of 22s, the dabigatran group showed a sensitivity of 97.1% (95%-CI 87.7–99.8) and a low specificity of 11.1% (95%-CI 5.5–19.1), $p < 0.00$. For apixaban, although a sensitivity of $> 95\%$ was attainable using a cut-off of 21s, a corresponding low specificity of 19.4% (95%-CI 10.9–30.3) was recorded, $p < 0.00$. At a cut-off of 34s and 31s, edoxaban and rivaroxaban yielded a sensitivity of 95.2% (95%-CI 88.1–98.8) and 94.7% (95%-CI 88.2–98.3) respectively, with a corresponding specificity of 79.2% (95%-CI 67.1–88.6) and 51.3% (95%-CI 35.9–66.5) respectively, $p < 0.00$ (Table 6).

ROC curve for ≥ 50 ng/ml threshold are shown in figure 7. Dabigatran and apixaban recorded an AUROC of 0.80 (95%-CI 0.70–0.89) and 0.79 (95%-CI 0.71–0.88) respectively, all at $p < 0.000$ (Table 7). The highest area under the receiver operator characteristics curve (AUROC) was recorded for edoxaban with 0.94 (95%-CI 0.90–0.98), followed by rivaroxaban with 0.89 (95%-CI 0.83–0.85).

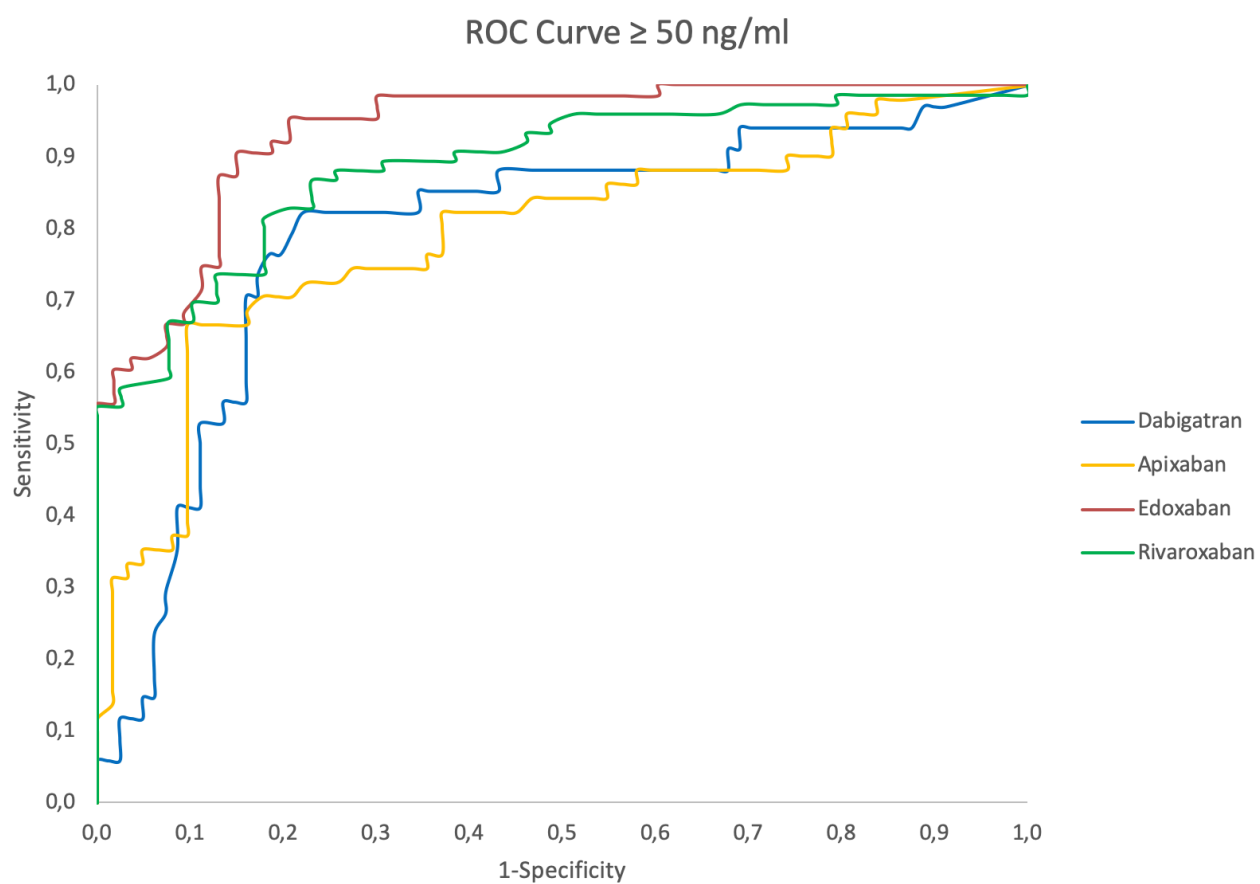


Figure 7: Receiver operator characteristics curve at ≥ 50 ng/ml for all DOAC

7 Discussion

This study assessed the diagnostic accuracy of the CoaguChek[®] used together with the aPTT test strips to qualitatively guide urgent decision-making with regards to thrombolysis, surgery or anticoagulation reversal in patients under DOAC. Based on the results of previous studies, CoaguChek[®] used together with the then available test strips, i.e. INR, was shown not to be suitable to safely exclude clinically relevant plasma concentration of dabigatran and apixaban prior to thrombolysis, or guide reversal therapy in stroke patients (Ebner et al., 2015, Härtig et al., 2020). This current study therefore sought to extend the capability of the CoaguChek[®] with the now available test strips, i.e. aPTT, to safely exclude clinically relevant plasma concentration of all four currently Food and Drug Administration- and European Medicines Agency-approved DOAC.

Similar to the previous CoaguChek[®] studies (Ebner et al., 2015, Härtig et al., 2020), a low correlation between the CoaguChek[®]-aPTT was found for the thrombin inhibitor, i.e. dabigatran and the factor Xa inhibitor, apixaban. A high correlation was detected for the factor Xa inhibitors, edoxaban and rivaroxaban. The CoaguChek[®]-aPTT could further on detect plasma concentrations > 30 and 50 ng/ml in edoxaban and rivaroxaban with a sensitivity of > 95% and corresponding specificity of > 51%. Using a less conservative limit for sensitivity, i.e. 90%, the study could show an improvement in specificity of > 69%, hence, making room for balance in both sensitivity and specificity.

7.1 Diagnostic capability of CoaguChek[®]-aPTT

Analyses showed a very strong correlation between CoaguChek[®]-aPTT and UPLC-MS/MS for edoxaban ($r = 0.84$) (Figure 1c) and strong correlation for rivaroxaban ($r = 0.79$) (Figure 1d). These data were comparable to that obtained with the CoaguChek[®]-INR for edoxaban ($r = 0.73$) (Härtig et al., 2020) and rivaroxaban ($r = 0.82$) (Ebner et al., 2015). Similarly to CoaguChek[®]-INR, the CoaguChek[®]-aPTT was shown to be not suitable for the qualitative assessment of dabigatran and apixaban plasma concentration prior to thrombolysis, or guide reversal therapy in stroke patients. Although correlational analyses depicted a superiority of the CoaguChek[®]-aPTT, i.e. dabigatran ($r = 0.49$; $p < 0.001$) (Figure 1a) and apixaban ($r = 0.50$; $p < 0.001$) (Figure 1b), compared to the

CoaguChek[®]-INR dabigatran ($r = 0.04$; $p < 0.001$) and apixaban ($r = 0.32$; $p < 0.001$) (Ebner et al., 2015), this effect is still not significant. For dabigatran, just as for all DOAC, its sensitivity to aPTT might rather be assay-specific (Cuker et al., 2014). None of the currently studied global coagulation POCT systems has been shown to qualitatively assess plasma concentration of apixaban (Ebner et al., 2015, Ebner et al., 2017b, Ebner et al., 2017a). Since CoaguChek[®]-aPTT might be reliable for qualitative assessment of edoxaban and rivaroxaban with regards to urgent decision-making, prior knowledge of the DOAC administered is therefore essential.

7.1.1 Qualitative anticoagulation assessment using 30 ng/ml threshold

As a primary requirement, this study sought to establish cut-offs whereby safety was the main priority. For this reason, a more conservative definition for sensitivity, i.e. $\geq 95\%$, was strived for in identifying DOAC concentration ≥ 30 and 50 ng/ml. In this case, $\geq 95\%$ of patients with actual DOAC plasma concentration ≥ 30 and 50 ng/ml would be correctly identified as such and excluded from thrombolysis in the case of acute ischemic stroke, or be included for anticoagulation reversal in the case of hemorrhagic stroke or urgent surgery. By using the idea of qualitative assessment of DOAC in the emergency setting (Mbroh and Poli, 2021), a cut-off of 20s yielded a sensitivity of 95.6% for apixaban and 92.7% for dabigatran with a corresponding specificity of 13.3% and 6.8%, hence limiting its diagnostic capability (Table 6). It is worth noticing that for dabigatran, a sensitivity of 95% was not even attainable. A cut-off of 32s and 30s yielded a sensitivity of 95.7% and 95.3% with a corresponding specificity of 74.5% and 62.1% for edoxaban and rivaroxaban ≥ 30 ng/ml respectively (Table 6). Data showed a high AUROC for edoxaban 0.93 and rivaroxaban 0.91 (Table 7). In comparison, the CoaguChek[®]-INR only yielded a specificity of 44.0% and 11.9% for edoxaban and rivaroxaban respectively, at sensitivity $\geq 95\%$ with a corresponding AUROC of 0.92 for edoxaban and 0.90 for rivaroxaban (Ebner et al., 2015, Härtig et al., 2020). In spite of CoaguChek[®]-aPTT being better in identifying samples < 30 ng/ml than CoaguChek[®]-INR, the general diagnostic performance of both CoaguChek[®]-aPTT and CoaguChek[®]-INR is fairly similar.

In further analysis, a less conservative approach was implemented, i.e. sensitivity of 90%, to see to which extent the specificity for CoaguChek[®]-aPTT could be improved. This approach could not improve the general diagnostic capability of CoaguChek[®]-aPTT for apixaban and dabigatran. Using a cut off of 34s for edoxaban, a sensitivity of 89.9% (81.3–95.5) yielded corresponding

specificity of 85.1% (73.2–93.3). A cut-off of 33s for rivaroxaban likewise showed a good balance for sensitivity and specificity, i.e. 90.7% (83.4–95.6) and 79.3% (62.5–91.2) respectively (Table 6).

7.1.2 Qualitative anticoagulation assessment using 50 ng/ml threshold

Using 50 ng/ml as threshold, CoaguChek®-aPTT does not provide any evidence of the possibility of extending the diagnostic use of CoaguChek® to include apixaban and dabigatran. Using a conservative definition, i.e. 95%, as well as a less conservative definition, i.e. 90%, for sensitivity, data from this study did not show any improvement in the diagnostic capability of CoaguChek®-aPTT for both edoxaban and rivaroxaban (Table 6). AUROC for edoxaban and rivaroxaban were 0.94 (95%-CI 0.90–0.98) and 0.89 (95%-CI 0.83–0.95) respectively using CoaguChek®-aPTT (Table 7) which is similar to that reported for CoaguChek®-INR (Ebner et al., 2015, Härtig et al., 2020).

A threshold of ≥ 50 ng/ml did not show much variation in the cut-offs for attaining a sensitivity $\geq 95\%$ compared to the cut-offs for ≥ 30 ng/ml threshold. This shows that the thresholds 30 and 50 ng/ml are rather low and close to each other (Härtig et al., 2020). The choice of threshold in clinical practice could therefore be individualized and the risk-to-benefit ratio should always be considered.

Based on current data, the Hemochron™ shows superiority over the CoaguChek® in general by showing a higher sensitivity and specificity at both 30 and 50 ng/ml thresholds for dabigatran, edoxaban and rivaroxaban (Table 2).

7.2 Diagnostic capability of other coagulation parameters

7.2.1 Global coagulation parameters

DOAC seemed to influence global coagulation parameters such as INR and CoaguChek®-aPTT values. Different DOAC seemed to show different extent of influence on INR and CoaguChek®-aPTT values (Table 5). On the contrary, the median laboratory-based aPTT was fairly similar among all DOAC (Table 5). The difference in response between CoaguChek®-aPTT and laboratory-based aPTT and the absence of a perfect correlation between CoaguChek®-aPTT and

laboratory-based aPTT (Figure 5a-d) is due to different assays, which measure the same parameter, responding differently to the same DOAC (Douxflis et al., 2014). This reagent-dependency was also evident in the correlational analysis between laboratory-based aPTT and UPLC-MS/MS (Figure 4a-d) compared that between CoaguChek®-aPTT and UPLC-MS/MS (Figure 5a-d). In some cases, reagents of laboratory-based global coagulation parameters are optimized to avoid the influence of DOAC or the influence of DOAC on laboratory-based global coagulation parameters are dependent on the sensitivity of the reagent to DOAC (Dunois, 2021). The lack of clear response of laboratory-based aPTT to different DOAC in this study could also be due to the optimization of the reagents in order to avoid the influence of DOAC or due to a lower sensitivity to DOAC.

7.2.2 Specific coagulation parameters

The specific laboratory-based coagulation parameter, anti-Xa-activity, and calibrated DOAC-specific assays are already adjusted to detect DOAC. As expected, factor Xa inhibitors showed an effect on anti-Xa-activity. Rivaroxaban however showed the highest response to anti-Xa-activity in comparison to apixaban and edoxaban (Table 5). Similarly, all four DOAC influenced the values of calibrated DOAC-specific assays to different extents.

Laboratory-based calibrated DOAC assays correlated very strongly with UPLC-MS/MS for all factor Xa inhibitors (Figure 2a-d) while anti-Xa-activity also correlated very strongly with UPLC-MS/MS for both the thrombin inhibitor, dabigatran, and all factor Xa inhibitors (Figure 3). This continues to show that in the absence of the possibility of exact measurement of plasma concentration of DOAC by means of UPLC-MS/MS, and also in the absence of urgency, the specific coagulation parameters, i.e. anti-Xa-activity for factor Xa inhibitors and DOAC-specific laboratory-based calibrated DOAC assays continue to be the most reliable alternative.

After DOAC intake, a trough level of 0 ng/dl could be recorded even up to 2 hours which indicates the need for careful interpretation of plasma DOAC concentration irrespective of the test used. A history of the last DOAC intake is therefore essential in interpreting results of plasma DOAC concentration.

7.3 Strengths and limitations of study

In this study, blood samples of patients in a non-emergency setting were collected in which measurements were done under physiologic conditions making it more ideal than other studies which used spiked plasma (Van Ryn et al., 2012). As a standard of measuring DOAC plasma concentration, UPLC-MS/MS, which is the most sensitive and considered the gold standard for assessing DOAC plasma concentration, was used. This, as opposed to other qualitative POCT studies for DOAC, provides more reliable correlational analyses (Groene et al., 2021, Oberladstatter et al., 2021, Ord et al., 2022, Margetic et al., 2022, Schafer et al., 2021, Artang et al., 2019). Analogous to several other previous studies (Ebner et al., 2017b, Härtig et al., 2021, Ebner et al., 2015, Härtig et al., 2020, Artang et al., 2019), this study assessed POCT for DOAC with respect to the two currently recommended treatment thresholds of 30 and 50 ng/ml instead of only 30 ng/ml (Margetic et al., 2022) or 50 ng/ml (Oberladstatter et al., 2021). It is worth mentioning that in spite the absence of data for 30 ng/ml, the latter is yet still in approved in Europe for assessing DOAC. Although the currently recommend treatment thresholds are yet to be validated in prospective clinical studies, data from this study could be used for recalculation of DOAC specific ideal cut-offs to match any treatment thresholds in the future. In spite of the low specificity, CoaguChek®-aPTT ensures high safety in guiding emergency decision-making. Meaning, $\geq 95\%$ of patients with actual DOAC plasma concentration ≥ 30 and 50 ng/ml would be correctly identified as such and excluded from thrombolysis in the case of acute ischemic stroke, or be included for anticoagulation reversal in the case of hemorrhagic stroke or urgent surgery.

As a limitation, this study is based on a single-center experience and future trials could benefit from a multi-center study, ideally spontaneous POCT measurement in the emergency setting. Similar to most available POCT for DOAC, CoaguChek® requires knowledge of the type of DOAC taken by the patient for qualitative assessment of anticoagulation by DOAC.

8 Summary

There is a global surge in the prescription of DOAC, i.e. the thrombin (factor-IIa) inhibitor, dabigatran and the factor-Xa inhibitors apixaban, edoxaban, and rivaroxaban, for the prevention of stroke and systemic thromboembolism resulting from non-valvular atrial fibrillation, prevention and treatment of venous thromboembolism, as well as prevention of secondary thromboembolism (Steffel et al., 2021, Konstantinides et al., 2016, Rachidi et al., 2013, Huisman et al., 2017). DOAC has gradually replaced VKA over the past ten to fifteen years in the management of non-valvular atrial fibrillation since VKA are limited by narrow therapeutic interval, need for frequent monitoring as well as dose adjustments, and increased bleeding complications (Kirchhof et al., 2016). In spite of the primary indication for DOAC which also includes secondary stroke prevention, DOAC are associated with approximately 2% annual incidence of acute ischemic stroke with an annual stroke risk of 0.9-1.8% (Almutairi et al., 2017, Pfeilschifter et al., 2016, Connolly et al., 2011, Connolly et al., 2009, Patel et al., 2011, Giugliano et al., 2013), 1.4-3.6% incidence of major bleeding including intracranial bleeding (Connolly et al., 2011, Connolly et al., 2009, Patel et al., 2011, Giugliano et al., 2013) and an annual mortality of up to 3.75% (Pirlog et al., 2019). This means several patients under DOAC continue to present with acute neurological and surgical emergencies where urgent decision-making with regards to thrombolysis or DOAC reversal is imminent.

POCT for DOAC is believed to facilitate urgent decision-making since this has been shown to provide a net time gain of 28 ± 12 minutes compared to routine laboratory-based coagulation tests (Rizos et al., 2009). In view of this, several studies have researched into the extent to which POCT devices can assess anticoagulation of DOAC. Majority of these studies are based on the principle of qualitative detection of DOAC plasma concentration above or below the currently recommended treatment thresholds of 30 and 50 ng/ml. The CoaguChek® together with the INR test strips provides > 95% sensitivity in detecting plasma concentration of edoxaban and rivaroxaban which are actually above 30 and 50 ng/ml (Ebner et al., 2015, Härtig et al., 2020). The Hemochron™ together with the INR and ACT+ provides > 95% sensitivity in detecting plasma concentration of dabigatran, edoxaban and rivaroxaban which are actually above 30 and 50 ng/ml (Ebner et al., 2017b, Härtig et al., 2021). The ClotPro® provides a sensitivity of 100% for the thrombin inhibitor, dabigatran, and a sensitivity of 90-100% for the factor-Xa inhibitors edoxaban, and rivaroxaban in

detecting DOAC plasma concentration which are actually above 50 ng/ml (Oberladstatter et al., 2021). The DOASENSE™ DOAC Dipstick which unlike the above mentioned POCT tests the presence or absence of DOAC in urine as an indirect determination of presence of DOAC in plasma, provides > 95% sensitivity in detecting positive test results (Ord et al., 2022, Margetic et al., 2022, Papageorgiou et al., 2023).

In this study, we assessed the diagnostic accuracy of the CoaguChek® used together with the aPTT test strips to qualitatively identify patients with plasma DOAC concentrations above the currently recommended treatment relevant thresholds (Mbroh and Poli, 2021) (i.e., DOAC concentrations that would allow for immediate reversal treatment in case of intracranial or other life-threatening hemorrhage or surgery, or that would justify withholding thrombolysis if necessary) for all four currently Food and Drug Administration- and European Medicines Agency-approved DOAC, i.e. apixaban, dabigatran, edoxaban and rivaroxaban, in real-life patient samples. Analogous to the previous CoaguChek® study which used INR test strips (Ebner et al., 2015, Härtig et al., 2020), patients ≥ 18 years who had an indication for the initiation of DOAC therapy were enrolled. Patients with known coagulopathy, abnormal coagulation parameters at baseline which could also be as a result a recent treatment with VKA ≤ 14 days, heparinoids ≤ 72 hours, unfractionated heparin ≤ 12 hours and low molecular weight heparin (LMWH) ≤ 24 hours prior to first DOAC intake were excluded. Blood samples were collected at six defined time points, i.e., at baseline before DOAC intake, 0.5, 1, 2, 8 hours after DOAC intake and directly before the next DOAC intake i.e., at 12 hours for dabigatran and apixaban or 24 hours for edoxaban and rivaroxaban. As a standard for measuring the actual DOAC concentration, high-performance liquid chromatography/mass spectrometry (UPLC-MS/MS) was performed.

A total of 80 patients were enrolled in the study, 20 patients for each DOAC. A total of 459 out of the planned 480 samples, corresponding to 96%, were successfully collected and analyzed. The number of samples collected for each of the four DOAC was fairly equal. DOAC groups did not show much variation in baseline characteristics which included demographics and cardiovascular risk factors. Coagulation parameters including laboratory-based aPTT, CoaguChek®-aPTT and INR did not show much variation across all four DOAC. Laboratory-based calibrated DOAC, anti-Xa activity and UPLC-MS/MS were elevated in the rivaroxaban group. To address our primary aim, data showed that correlation between CoaguChek®-aPTT and UPLC-MS/MS was weak for

dabigatran and apixaban, but strong – very strong for edoxaban and rivaroxaban. These results therefore restrict diagnostic capability of CoaguChek®-aPTT to edoxaban and rivaroxaban. and does not extend the diagnostic capability of CoaguChek® to all four all four currently Food and Drug Administration- and European Medicines Agency-approved DOAC. For the sake of patient safety, we strived for a sensitivity of $\geq 95\%$ in identifying samples with actual plasma concentration ≥ 30 and 50 ng/ml. Using the CoaguChek®-aPTT, cut offs of 32s and 30s for edoxaban and rivaroxaban respectively, met the conservative definition for sensitivity, i.e. $\geq 95\%$ in detecting plasma concentration ≥ 30 . For plasma concentration ≥ 50 ng/ml, cut offs of 34s and 31s for edoxaban and rivaroxaban respectively provided sensitivity of $\geq 95\%$. These cut offs can be considered safe since $\geq 95\%$ of patients with actual edoxaban and rivaroxaban plasma concentrations ≥ 30 and 50 ng/ml, could be excluded from thrombolysis and could be spared the bleeding complications associated with thrombolysis. Patients under treatment with edoxaban and rivaroxaban with CoaguChek®-aPTT below the suggested cut off (Table 6) could be therefore spared the administration of DOAC reversal agents.

At a sensitivity of $\geq 95\%$, CoaguChek®-aPTT shows a better balance for specificity and therefore shows a considerable preference compared to the CoaguChek®-INR with respect to edoxaban and rivaroxaban.

The very strong correlation between laboratory-based calibrated DOAC assays and UPLC-MS/MS for all DOAC, as well as the very strong correlation between anti-Xa Activity and UPLC-MS/MS for all factor-Xa inhibitors shown in this study continues to support the fact that laboratory-based calibrated DOAC assays and anti-Xa-activity are by far the most and practically reliable in assessing DOAC plasma concentration. These tests are however still challenged by the long turn-around time and its scarcity in most laboratories, limiting its applicability in urgent DOAC assessment (Dolscheid-Pommerich et al., 2018, Rizos et al., 2010). As a compromise for this time-factor, these laboratory tests could supplement and address the lower specificities of CoaguChek®-aPTT, hence allowing emergency physicians make initial clinical decisions with regards to thrombolysis, anticoagulation reversal and urgent surgery, which are considered safe and not detrimental in patient management. An algorithm for safe implementation of CoaguChek®-aPTT for edoxaban and rivaroxaban is shown in Figure 8. Validation through prospective, multi-centric studies, ideally in emergency setting, are warranted.

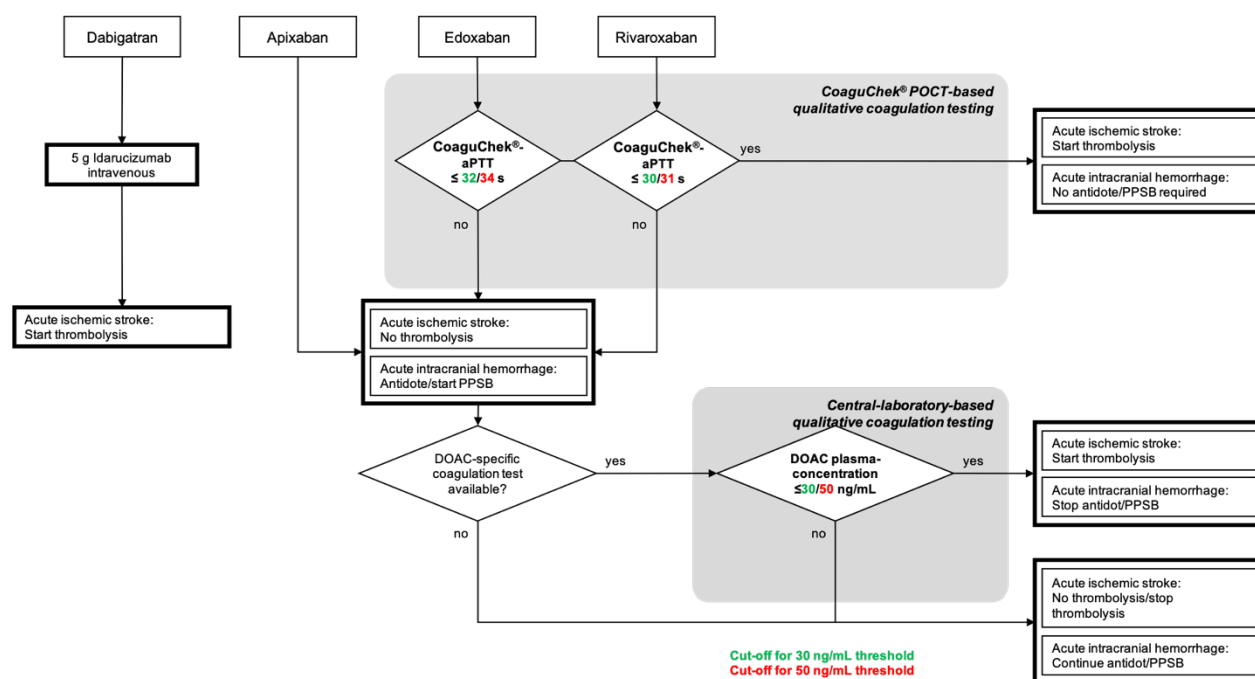


Figure 8: Proposed algorithm for using the CoaguChek®-aPTT (CC-aPTT) point-of-care testing system for qualitative anticoagulation assessment of edoxaban and rivaroxaban to guide urgent decision-making with respect to thrombolysis, anticoagulation reversal and intracranial hemorrhage (ICH).

Deutsche Zusammenfassung

Weltweit werden direkte orale Antikoagulanzen (DOAK; der Thrombinhemmer Dabigatran und die Faktor-Xa-Hemmer Apixaban, Edoxaban und Rivaroxaban) zur Vorbeugung von Schlaganfällen und systemischen Thromboembolien infolge nicht-valvulärem Vorhofflimmerns sowie zur Vorbeugung und Behandlung venöser Thromboembolien zunehmend häufiger verschrieben (Steffel et al., 2021, Konstantinides et al., 2016, Rachidi et al., 2013, Huisman et al., 2017). DOAK haben Vitamin-K-Antagonisten (VKA) in diesen Indikationen im letzten Jahrzehnt aufgrund gleicher Effektivität bei besserer Sicherheit (v.a. weniger Hirnblutungen) und höherem Einnahmekomfort (kein Therapiemonitoring erforderlich) graduell ersetzt (Kirchhof et al., 2016). Trotz DOAK Einnahme liegt das jährlichen Schlaganfallrisiko bei 0,9–1,8 % (Almutairi et al., 2017, Pfeilschifter et al., 2016, Connolly et al., 2011, Connolly et al., 2009, Patel et al., 2011, Giugliano et al., 2013) und für schwere Blutungen bei 1,4–3,6 % (Connolly et al., 2011, Connolly et al., 2009, Patel et al., 2011, Giugliano et al., 2013). Konsekutiv steigt die Inzidenz DOAK assoziierter neurologischer und chirurgischer Notfälle, bei denen eine dringende Entscheidung zum Beispiel hinsichtlich einer Thrombolyse oder einer DOAK-Antagonisierung erforderlich wird.

Bei VKA assoziierten Notfällen beschleunigt eine bettseitige Point-of-care-Gerinnungstestung (POCT) der International Normalized Ratio (INR) die Entscheidungsfindung um ca. 30 Minuten (Rizos et al., 2009). POCT für DOAK wurden erst rezent in Studien untersucht. Diese verfolgten das Prinzip der qualitativen Beurteilung, d.h. können Blutproben von Patienten mit therapierelevanter Antikoagulation sicher identifiziert werden. Je nach Leitlinie und Indikation (d.h. Thrombolyse oder Notfalloperation bzw. Antidotgabe) werden hierfür die DOAK-Plasmakonzentrationen unterhalb bzw. oberhalb 30 und 50 ng/ml empfohlen.

Mit dem CoaguChek® und den INR-Teststreifen gelang die qualitative Beurteilung für Edoxaban und Rivaroxaban (Ebner et al., 2015, Härtig et al., 2020). Mit dem Hemochron™ Signature Elite gelang dies zusätzlich auch für Dabigatran (Ebner et al., 2017b, Härtig et al., 2021). Der ClotPro® wurde zwar für alle vier DOAK zugelassen, allerdings nur der 50 ng/ml Plasmakonzentrationsschwellenwert untersucht (Oberladstatter et al., 2021). Auch das DOASENSE™ DOAC Dipsticks ist für alle DOAK geeignet, jedoch wird nicht die DOAK-Konzentration im Plasma sondern – mit fraglicher Relevanz für die klinische Einschätzung der

Gerinnungssituation – im Urin getestet (Ord et al., 2022, Margetic et al., 2022, Papageorgiou et al., 2023).

In dieser Promotionsarbeit wurde nun die diagnostische Genauigkeit des CoaguChek® zusammen mit den neu kommerziell verfügbaren aPTT-Teststreifen untersucht (Mbroh and Poli, 2021) therapierelevante DOAK-Konzentrationen in Patientenblutproben zu erkennen. Analog zur vorherigen CoaguChek®-Studie, bei der INR-Teststreifen verwendet wurden (Ebner et al., 2015, Härtig et al., 2020), wurden Patienten ≥ 18 Jahre, bei denen eine Indikation zur Einleitung einer DOAK-Therapie bestand, eingeschlossen. Ausgeschlossen wurden Patienten mit bekannter Koagulopathie, abnormalen Gerinnungsparametern zu Studienbeginn und zuvor mit anderen Antikoagulanzen behandelte Patienten. Blutproben wurden zu sechs definierten Zeitpunkten entnommen, i.e. bei Baseline vor der DOAK-Einnahme, $\frac{1}{2}$, 1, 2, 8 Stunden nach und direkt vor der nächsten DOAK-Einnahme, i.e. nach 12 Stunden bei Dabigatran und Apixaban bzw. 24 Stunden bei Edoxaban und Rivaroxaban. Die tatsächliche DOAK-Plasmakonzentration wurde mit Ultra-Performance-Flüssigchromatographie-Tandem-Massenspektrometrie (UPLC-MS/MS) bestimmt.

80 Patienten wurden in die Studie eingeschlossen, 20 pro DOAK. Insgesamt wurden 459 (96%) der geplanten 480 Proben erfolgreich gesammelt und analysiert. Die vier DOAK-Gruppen zeigten keine relevanten Unterschiede in Demografie und kardiovaskulären Risikofaktoren. Die Korrelation zwischen CoaguChek®-aPTT und den mittels UPLC-MS/MS waren nur für Edoxaban und Rivaroxaban ausreichend, nicht aber für Dabigatran und Apixaban. Die zur Patientensicherheit prädefinierte Sensitivität $\geq 95\%$ zur Identifizierung von Blutproben mit tatsächlichen Plasmakonzentrationen ≥ 30 ng/ml wurde mit den CoaguChek®-aPTT Cut-offs von 32 s bzw. 30 s für Edoxaban bzw. Rivaroxaban erreicht. Cut-offs von 34 s bzw. 31 s ergaben eine Sensitivität von $\geq 95\%$ für Edoxaban bzw. Rivaroxaban zur Identifizierung von Blutproben mit tatsächlichen Plasmakonzentrationen ≥ 50 ng/ml.

Mit einer Sensitivität von $\geq 95\%$ weist CoaguChek®-aPTT ein besseres Balance hinsichtlich der Spezifität auf (75% bzw. 62% für Edoxaban bzw. Rivaroxaban bei Plasmakonzentrationen ≥ 30 ng/ml und 79% bzw. 51% bei Plasmakonzentrationen ≥ 50 ng/ml) und zeigt daher eine deutliche Bevorzugung gegenüber CoaguChek®-INR (44% bzw. 12% für Edoxaban bzw. Rivaroxaban bei Plasmakonzentrationen ≥ 30 ng/ml und 86% bzw. 12% bei Plasmakonzentrationen ≥ 50 ng/ml).

Labor-basierte kalibrierte DOAK-Assays waren erwartungsgemäß in der Beurteilung der DOAK-vermittelten Antikoagulation am zuverlässigsten. Diese Tests sind jedoch aufgrund der längeren Bearbeitungszeit für die DOAK-Notfalltestung weniger geeignet (Dolscheid-Pommerich et al., 2018, Rizos et al., 2010). Für den DOAK-Notfall schlagen wir daher einen sequenziellen Algorithmus vor welcher im Falle Edoxaban und Rivaroxaban sowohl die CoaguChek®-aPTT als auch labor-basierte Testung beinhaltet (Abbildung 8). Eine Validierung dieses Algorithmus durch prospektive, multizentrische Studien, idealerweise in Notfallsituationen, ist gerechtfertigt.

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10 Declaration of personal contribution to the dissertation

This study was carried out in the Department of Neurology under the supervision of Prof. Dr. Sven Poli, deputy medical director of the Department of Neurology, neurovascular diseases and head of the study group. The study was designed by Prof. Dr. Sven Poli.

Introduction to the entire study process was carried out by Prof. Dr. Sven Poli and Mr. Mark Divkovic. Patient information and patient enrolment was carried out by me with the support of other medical doctors of the study group: Prof. Dr. Sven Poli, Dr. Khoulood Poli, Ms. Alexandra Gómez Expósito, Mr. Kamaldeen Adeyemi and Dr. Johannes Tünnerhoff. Samples were taken by me with the support of Mr. Mark Divkovic.

The laboratory tests were carried out in the central laboratory of the University Hospital, Tübingen. Ultra-performance liquid chromatography – tandem mass spectrometry (UPLC-MS/MS) was carried out at the Institute for Labor and Transfusion Medicine, Heart and Diabetes Center, Ruhr University Bochum, Bad Oeynhausen, Germany under the supervision of PD Dr. med. Dr. rer. nat. Ingvild Birschmann.

The statistical analysis was carried out independently by me after consultation with Dr. Gunnar Blumenstock, Institute for Biometrics, University of Tübingen.

I carried out data research in the form of digital patient documents from the clinic's internal management system SAP, ICCA and LAURIS as well as data collection and data analysis independently.

I herewith declare that I wrote the manuscript independently and that I did not use any sources other than those specified by me. Parts that are direct quotes or paraphrases are identified as such.

Joshua Kobina Mbroh

Tübingen, den 13.05.2024