Edoxaban: Impact on routine and specific coagulation assays

A practical laboratory guide

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Summary

Assessment of plasma concentration/effect of edoxaban may be useful in some situations. Also, clinicians need to know how routine coagulation assays are influenced. It was our aim to determine coagulation tests useful for the assessment of edoxaban's pharmacodynamics and provide recommendations for the interpretation of haemostasis diagnostic tests. Edoxaban was spiked at concentrations ranging from 0 to 1,000 ng/ml in platelet-poor plasma which covers the on-therapy range (from ± 25 ng/ml at C_{trough} to ± 170 ng/ml at C_{max}). aPTT, PT, dRVVT, chromogenic anti-Xa assays, TGA and a large panel of haemostasis diagnostic tests were performed using several reagents. A concentration-dependent prolongation of aPTT, PT and dRVVT was observed. The effect was dependent on the reagents. FXa chromogenic assays showed high sensitivity and a linear correlation depending on the methodology. TGA may be useful to assess the pharmacodynamics of edoxaban but its turnaround time and the lack of standardisation are limitations. Edoxaban impairs the assessment of lupus anticoagulant, protein S (clotting method), APC-R, antithrombin (FXa-based assay) and measurement of clotting factor activity. Immunological assays and assays acting below the FXa are not influenced by edoxaban. In conclusion, some PT reagents could be used to estimate edoxaban activity. Chromogenic anti-Xa assays are required to assess the plasma concentration. TGA may be useful but requires standardisation. In case of thrombophilia or in the exploration of a haemorrhagic event, immunological assays should be recommended, when applicable. Standardisation of the time between the last intake and the sampling is mandatory to provide a proper assessment of the result.

Keywords

Edoxaban, drug measurement, coagulation tests, guidelines, factor Xa inhibitors

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Introduction

Edoxaban, a direct factor-Xa inhibitor, has received its market authorisation, under the brand-name of Savaysa®, on the 8th of January 2015 in the United States (US) for the prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation (NVAF) as well as for the treatment of deep-vein thrombosis (DVT) and pulmonary embolism (PE). The product was already available in Japan since April 2011 for the prevention of thromboembolic events in patients with major orthopaedic surgery under the brand name of Lixiana®. The Ministry of Health in Japan has extended in September 2014 its indication to the prevention of stroke in NVAF patients and in the treatment of DVT and PE. The marketing approvals relied on the broad development program that evaluated the efficacy and safety of edoxaban versus standard of care in these different indications (1–3).

The dose regimens in the HOKUSAI and in the ENGAGE AF-TIMI 48 have been cautiously chosen based on patient's characteristics and/or concomitant medications (i.e. doses were halved in case of concomitant treatment with potent P-gp inhibitors, in case of creatinine clearance (CrCl) of 30 to 50 ml/minute [min] or in case of a body weight of 60 kg or less) (1, 2). Interestingly, the investigators of the ENGAGE-AF TIMI 48 study proposed two dose regimens, a high- (60 mg once daily [QD]) and a low- (30 mg QD) one. After the randomisation, if the patients had one or more of the above-mentioned conditions, the doses were halved. This reveals that even if the majority of the patients are likely to receive a "normal" dose regimen, some dose adaptation can be proposed based on the solely clinical characteristics (4). However, in the ENGAGE AF-TIMI 48 trial patients with CrCl < 30 ml/min were excluded while the position of the Food and Drug Administration (FDA) was to allow the use of edoxaban in patients with CrCl ≥ 15 ml/min (5); a clinical feature for which no

Table 1: Overview of the different assays performed in this study.

Coagulation assay	Reagent	Manufacturer	Method	Coagulation Analyser (Manufacturer)
Prothrombin Time	TriniCLOT® PT Excel S®	Tcoag, Bray, Ireland	Chronometric	STA-R Evolution® (Diagnostica Stago)
	TriniCLOT® PT Excel®			
	TriniCLOT® PT HTF®			
	STA®-Neoplastine® R	Diagnostica Stago, Asnieres, France		
	STA®-Neoplastine® CI+			
	Dade® Innovin®	Siemens Healthcare Diagnostics, Deerfield, IL, USA		
	RecombiPlasTin 2G®	Instrumentation Laboratory, Lexington, KY, USA		
Activated Partial Thromboplastin Time	STA®-C. K. Prest®	Diagnostica Stago, Asnieres, France	Chronometric	STA-R Evolution® (Diagnostica Stago)
	STA®-Cephascreen®			
	STA®-PTT Automate			
	Actin® FS	Siemens Healthcare Diagnostics, Deerfield, IL, USA		
	SynthASil®	Instrumentation Laboratory, Lexington, KY, USA		
Thrombin Time	STA®-Thrombin	Diagnostica Stago, Asnieres, France	Chronometric	STA-R Evolution® (Diagnostica Stago)
Ecarin Clotting Time	Ecarin 5IU/ml	Diagnostica Stago, Asnieres, France	Chronometric	STA-R Evolution® (Diagnostica Stago)
Reptilase Time	STA®-Reptilase	Diagnostica Stago, Asnieres, France	Chronometric	STA-R Evolution® (Diagnostica Stago)
Lupus Anticoagulant	STA®-Staclot® DRVV Screen	Diagnostica Stago, Asnieres, France	Chronometric	STA-R Evolution® (Diagnostica Stago)
	STA®-Staclot® DRVV Confirm			
Protein C	HemosIL® Protein C	Instrumentation Laboratory, Lexington, KY, USA	Chromogenic	ACL-TOP® (Instrumentation Laboratory)
Protein S	STA®-Staclot® Protein S	Diagnostica Stago, Asnieres, France	Chronometric	STA-R Evolution® (Diagnostica Stago)
Free Protein S Antigen	HemosIL® Free Protein S	Instrumentation Laboratory, Lexington, KY, USA	Immunoturbidimetric	ACL-TOP® (Instrumentation Laboratory)
Activated Protein C Resistance	Pefakit® APC-R Factor V Leiden	Pentapharm, Aesch, Switzerland	Chronometric	ACL-TOP® (Instrumentation Laboratory)
Fibrinogen Clauss Method	STA®-Fibrinogen	Diagnostica Stago, Asnieres, France	Chronometric	STA-R Evolution® (Diagnostica Stago)
Fibrinogen PT-derived	RecombiPlasTin 2G®	Instrumentation Laboratory, Lexington, KY, USA	Chronometric	ACL-TOP® (Instrumentation Laboratory)
Antithrombin	STA®-Stachrom® ATIII	Diagnostica Stago, Asnieres, France	Chromogenic (thrombin- based)	STA-R Evolution® (Diagnostica Stago)
	HemosIL® Liquid Antithrombin	Instrumentation Laboratory, Lexington, KY, USA	Chromogenic (FXa-based)	ACL-TOP® (Instrumentation Laboratory)
Extrinsic Clotting Factors	STA®-C. K. Prest®	Diagnostica Stago, Asnieres, France	Chronometric	STA-R Evolution® (Diagnostica Stago)
Intrinsic Clotting Factors	RecombiPlasTin 2G®	Instrumentation Laboratory, Lexington, KY, USA	Chronometric	STA-R Evolution® (Diagnostica Stago)
Anti-Xa activity	Biophen® Direct Factor Xa Inhibitors	Hyphen Biomed, Neuville-sur-Oise, France	Chromogenic	STA-R Evolution® (Diagnostica Stago)
	Biophen® Heparin LRT			
	STA®-Liquid Anti-Xa	Diagnostica Stago, Asnieres, France		STA-R Evolution® (Diagnostica Stago)
	Technochrom® Anti-Xa	Technoclone, Vienna, Austria		
	HemosIL® Liquid Heparin	Instrumentation Laboratory, Lexington, KY, USA		ACL-TOP® (Instrumentation Laboratory)
Thrombin Generation Assay	PPP-Reagent Low	Thrombinoscope BV, Maastricht, The Nederlands	Fluorimetric	Calibrated Automated Thrombogram Analyser® (Thrombinoscope BV)
	PPP-Reagent			
	PPP-Reagent High			

data is currently available. This is especially of concern since haemodialysis is not an effective mechanism for removal of edoxaban from the blood (6).

It is well recognised that routine biological monitoring of direct factor Xa inhibitors is not required since dose tailoring based on patient's characteristics appeared to be at least as safe as warfarin (1, 7, 8). However, a phase-II trial in patients with non-valvular AF revealed that the bleeding tendency increased with the dose of edoxaban administered (9). The median edoxaban plasma concentration was ± 170 ng/ml (inter-quartile range (IQR): 125 to 245 ng/ml) at C_{max} and ± 25 (IQR: 10 to 40 ng/ml) at C_{trough} following four weeks of treatment with edoxaban 60mg QD (9). In addition, in a subanalysis of patients included in the ENGAGE-AF TIMI 48 study, median (IQR) edoxaban concentrations at trough, i.e at a median time from the preceding dose of 20.0 hours (h) (IQR: 15.4–24.3 h), were 36.1 (19.4–62.0) ng/ml after one month of treatment with edoxaban 60 mg QD. Once the dose is reduced due to one or more of the clinical conditions mentioned above, the 30 mg QD dose in this population showed a median (IQR) plasma concentration of 27.0 (14.6–44.6) ng/ml (4). The high variability of the plasma concentration is clearly a concern since the data support that clinical outcomes, mainly major bleeding and stroke, were closely correlated to the plasma concentration measured at trough. Thus, as for dabigatran etexilate, for which the benefit of a therapeutic drug monitoring in special populations is subject to debate in the literature (10), the implementation of such strategies in frail patients treated with edoxaban might be suitable to ensure a safe use of the product. Importantly, similar data are available for rivaroxaban and apixaban in the "Clinical pharmacology and biopharmaceutics review(s)" published on the Food and Drug Administration website (11, 12).

Therefore, some situations may certainly benefit from an assessment of the anticoagulant activity, at least to investigate the aetiology of clinical outcome or to ensure the safe management of the patient in the perioperative context. Thus, conditions requiring measurement of the treatment include recurrent thrombosis, bleedings, urgent procedure, patients with CrCl between 15 and 30 ml/min, estimation of patient's compliance and bridging with other anticoagulants. In addition, some pharmacological interactions (e.g. P-gp/CYP3A4 inducers or inhibitors) and/or the underlying physiopathological status (severe renal impairment and mild or moderate hepatic impairment) may influence the pharmacokinetic profile of the different factor Xa inhibitors, although no relevant data on drug levels associated with expected therapeutic and harmful ranges are currently available. Finally, measurements may also be useful in extreme body weight, in elderly patients or to know if, and when, an elective surgery may be safely performed since a standardisation of the delay between the last intake of the drug and the surgery is subject to debate in the literature (13).

The aim of the present study is to assess and compare the performance of routinely used and more specific coagulation assays to measure the pharmacodynamics of edoxaban and estimate its plasma concentration. We also aimed at providing good laboratory recommendation for the accurate interpretation of haemostasis diagnostic tests that may be affected by edoxaban.

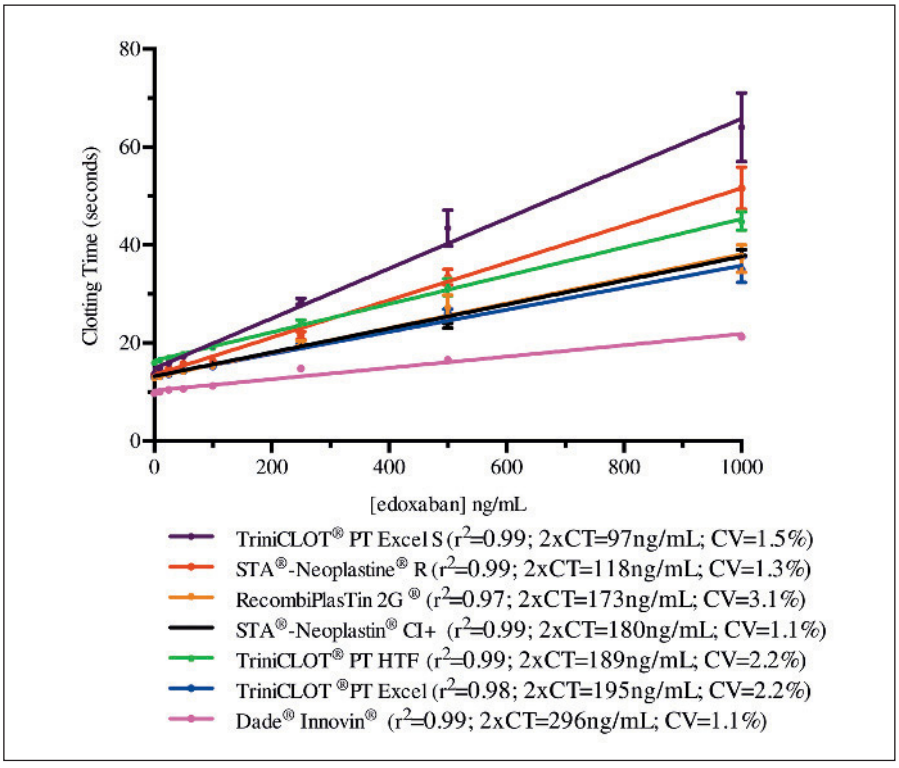

Figure 1: Impact of edoxaban on Prothrombin Time. Edoxaban showed a concentration-dependent prolongation of the prothrombin time. The relation was linear and the sensitivity depended on the reagent. The sensitivity ranged from 97 ng/ml for Triniclot PT Excel S® to 296 ng/ml for Innovin®. (r^2: Correlation Coefficient; 2xCT: 2× Clotting Time (sensitivity) expressed in ng/ml; CV: Coefficient of variation expressed in percentage [%]).

Material and methods

Edoxaban was spiked at increasing concentrations in pooled citrated normal human platelet-poor plasma (PPP). The selection of the concentration range covered the exposure reported in phase-II clinical trial (median edoxaban plasma concentrations: ± 170 ng/ml (IQR: 125 to 245 ng/ml) at C_{max} and ± 25 (IQR: 10 to 40 ng/ml) at C_{trough}) as well as supra-therapeutic concentrations (9, 14, 15).

Testing solutions of edoxaban

Edoxaban tosylate (molecular weight: 720.26 g/mol – purity=99.07 %) was obtained from Selleckchem (Munich, Germany). A stock solution at 1.0 mg/ml in DMSO was obtained and intermediate solution at 100 ng/ml, 250 ng/ml, 500 ng/ml, 1000 ng/ml, 2500 ng/ml, 5000 ng/ml, and 10,000 ng/ml diluted in phosphate-buffered saline without Ca^{2+} and Mg^{2+} was prepared. Working solutions of 10 ng/ml, 25 ng/ml, 50 ng/ml, 100 ng/ml, 250 ng/ml, 500 ng/ml and 1000 ng/ml of edoxaban were obtained by mixing these stock solutions with normal pooled plasma (NPP) (1:9 v/v). The DMSO concentration in plasma was ≤ 0.05 % (v/v) which does not influence the coagulation (16).

Preparation of platelet-poor plasma

Forty healthy individuals were included in the study. The exclusion criteria were thrombotic and/or haemorrhagic events, antiplatelet and/or anticoagulant medication, hormonal therapy, pregnancy and uptake of drugs potentially affecting the platelet and/or coagulation factor functions during the two weeks prior to the blood drawn. The study protocol was in accordance with the Declaration of Helsinki. Blood was taken by venipuncture in the antecubital vein and collected into 0.109 M sodium citrate (9:1 v/v) tubes (Venosafe®, Terumo, Leuven, Belgium) using a 21-gauge needle (Terumo). PPP was obtained from the supernatant fraction of the blood tubes after a double centrifugation for 15 min at 1500 g at room temperature. Immediately after centrifugation, PPP from the 40 donors was brought together to obtain the NPP which was frozen at – 80 °C without any delay. Frozen NPP samples are thawed and heat to 37 °C for 5 min just before experiment.

Routinely used and specific coagulation assays

A summary of the different assays and their reagents performed in this study is provided in ▶Table 1. To assess the intensity of

Table 2: Expected PT and DRVV-T clotting times for baseline, mean C_{trough} and C_{max} (with their IQR) for patients treated with edoxaban in (A) phase-II trial (60 mg QD) and in (B) phase-III trial (60 mg QD and 30 mg QD after dose reduction). Plasma concentrations and expected clotting times suspected to increase the risk of bleeding is also proposed for the 60 mg QD and the 30 mg QD dose regimen based on the results of the ENGAGE AF-TIMI 48 trial.

A. Phase-II trial													
Reagent	**Baseline time**	**Clotting time corresponding to the lower IQR at C_{max} (i. e. 125 ng/ml)†**		**Clotting time corresponding to the median plasma concentration at C_{max} (i. e. 170 ng/ml)†**		**Clotting time corresponding to the higher IQR at C_{max} (i. e. 245 ng/ml)†**		**Clotting time corresponding to the lower IQR at C_{trough} (i. e. 10 ng/ml)†**		**Clotting time corresponding to the median plasma concentration at C_{trough} (i. e. 25 ng/ml)†**		**Clotting time corresponding to the higher IQR at C_{trough} (i. e. 40 ng/ml)†**	
Prothrombin time	**Sec (± SD)**	**Sec**	**Ratio**	**Sec**	**Ratio**	**Sec**	**Ratio**	**Sec**	**Ratio**	**Sec**	**Ratio**	**Sec**	**Ratio**
TriniCLOT PT Excel S	13.7 ± 0.1	21.2	1.55	23.5	1.72	27.3	1.99	15.3	1.12	16.1	1.18	16.8	1.23
TriniCLOT PT Excel	13.0 ± 0.1	16.1	1.24	17.1	1.32	18.8	1.45	13.5	1.04	13.8	1.06	14.1	1.08
TriniCLOT PT HTF	16.1 ± 0.2	20.0	1.24	21.3	1.32	23.5	1.46	16.7	1.04	17.1	1.06	17.5	1.09
STA®-Neoplastine® R	13.6 ± 0.1	18.2	1.34	19.9	1.46	22.8	1.68	13.8	1.01	14.4	1.06	15.0	1.10
STA®-Neoplastine® CI+	13.0 ± 0.2	16.2	1.25	17.3	1.33	19.2	1.48	13.4	1.03	13.8	1.06	14.2	1.09
Dade® Innovin®	9.7 ± 0.1	11.7	1.21	12.3	1.27	13.1	1.35	10.4	1.07	10.6	1.09	10.8	1.11
RecombiPlasTin 2G®	12.7 ± 0.1	16.2	1.28	17.3	1.36	19.2	1.51	13.3	1.05	13.6	1.07	14.0	1.10
DRVV-T	**Sec (± SD)**	**Sec**	**Ratio**	**Sec**	**Ratio**	**Sec**	**Ratio**	**Sec**	**Ratio**	**Sec**	**Ratio**	**Sec**	**Ratio**
STA®-Staclot® DRVV Confirm	35.2 ± 0.3	64.2	1.82	71.6	2.03	82.1	2.33	41.1	1.17	44.5	1.26	47.8	1.36
STA®-Staclot® DRVV Screen	38.1 ± 0.4	72.2	1.90	81.4	2.14	95.0	2.49	44.0	1.15	48.1	1.26	52.1	1.37

† Plasma concentrations associated with a risk of bleeding is defined as the upper 95th percentile (mean+1.96*SD) at C_{max}. For PT, several reagents were tested and for dRVVT, both the screen and the confirm reagent were assessed. Depending on the reagent, PT cannot be used to assess trough level of edoxaban while dRVVT shows sufficient prolongation to provide information on the presence of edoxaban. (DR: dose reduction; dRVVT: dilute Russell Viper Venom Time; IQR: interquartile range; QD: once daily; PT: prothrombin time; SD: standard deviation).

Table 2: Continued

B. Phase-III trial: ENGAGE AF-TIMI 48 trial																	
Reagent	**Baseline time**	**Clotting time corresponding to the lower IQR at C_{trough} for 60 mg QD (i.e. 19.4 ng/ml)**		**Clotting time corresponding to the median plasma concentration at C_{trough} for 60 mg QD (i.e. 36.1 ng/ml)**		**Clotting time corresponding to the higher IQR at C_{trough} for 60 mg QD (i.e. 62.0 ng/ml)**		**Clotting time corresponding to a possible risk of bleeding with 60 mg QD at C_{trough} (i.e. 138.3 ng/ml)†**		**Clotting time corresponding to the lower IQR at C_{trough} for 30 mg QD DR (i.e. 14.6 ng/ml)**		**Clotting time corresponding to the median plasma concentration at C_{trough} for 30 mg QD DR (i.e. 27.0 ng/ml)**		**Clotting time corresponding to the higher IQR at C_{trough} for 30 mg QD DR (i.e. 44.6 ng/ml)**		**Clotting time corresponding to a possible risk of bleeding with 30 mg QD at C_{trough} (i.e. 95.2 ng/ml)†**	
Prothrombin time	**Sec (± SD)**	**Sec**	**Ratio**	**Sec**	**Ratio**	**Sec**	**Ratio**	**Sec**	**Ratio**	**Sec**	**Ratio**	**Sec**	**Ratio**	**Sec**	**Ratio**	**Sec**	**Ratio**
TriniCLOT PT Excel S	13.7 ± 0.1	15.8	1.15	16.6	1.21	17.9	1.31	21.8	1.59	15.5	1.13	16.2	1.18	17.1	1.25	19.6	1.43
TriniCLOT PT Excel	13.0 ± 0.1	13.7	1.05	14.1	1.08	14.6	1.12	16.4	1.26	13.6	1.05	13.8	1.06	14.2	1.09	15.4	1.18
TriniCLOT PT HTF	16.1 ± 0.2	17.0	1.06	17.4	1.08	18.2	1.13	20.4	1.27	16.8	1.04	17.2	1.07	17.7	1.10	19.1	1.19
STA®-Neoplastine® R	13.6 ± 0.1	14.2	1.04	14.8	1.09	15.8	1.16	18.7	1.38	14	1.03	14.5	1.07	15.2	1.12	17.1	1.26
STA®-Neoplastine® CI+	13.0 ± 0.2	13.7	1.05	14.1	1.08	14.7	1.13	16.6	1.28	13.6	1.05	13.9	1.07	14.3	1.10	15.5	1.19
Dade® Innovin®	9.7 ± 0.1	10.5	1.08	10.7	1.10	11	1.13	11.9	1.23	10.5	1.08	10.6	1.09	10.8	1.11	11.4	1.18
RecombiPlasTin 2G®	12.7 ± 0.1	13.5	1.06	13.9	1.09	14.6	1.15	16.5	1.30	13.4	1.06	13.7	1.08	14.1	1.11	15.4	1.21
DRVV-T	**Sec (± SD)**	**Sec**	**Ratio**	**Sec**	**Ratio**	**Sec**	**Ratio**	**Sec**	**Ratio**	**Sec**	**Ratio**	**Sec**	**Ratio**	**Sec**	**Ratio**	**Sec**	**Ratio**
STA®-Staclot® DRVV Confirm	35.2 ± 0.3	43.3	1.23	47	1.34	52.4	1.49	66.5	1.89	42.2	1.20	45	1.28	48.8	1.39	58.8	1.67
STA®-Staclot® DRVV Screen	38.1 ± 0.4	46.6	1.22	51	1.34	57.6	1.51	75	1.97	45.3	1.19	48.6	1.28	53.2	1.40	65.5	1.72

† Plasma concentrations associated with a risk of bleeding is defined as the upper 95th percentile (mean+1.96*SD) at C_{max}. For PT, several reagents were tested and for dRVVT, both the screen and the confirm reagent were assessed. Depending on the reagent, PT cannot be used to assess trough level of edoxaban while dRVVT shows sufficient prolongation to provide information on the presence of edoxaban. (DR: dose reduction; dRVVT: dilute Russell Viper Venom Time; IQR: inter-quartile range; QD: once daily; PT: prothrombin time; SD: standard deviation).

anticoagulation with edoxaban, the performances of prothrombin time (PT), activated partial thromboplastin time (aPTT), dilute Russell Viper Venom time (DRVV-T), chromogenic anti-Xa assays and the calibrated automated thrombogram (CAT) were evaluated.

The influence of edoxaban on the following diagnostic tests was also investigated. Thus, in addition to PT, aPTT and DRVV-T, thrombin time (TT), ecarin clotting time (ECT), reptilase time (RT), measurement of protein C and S, measurement of clotting factors, measurement of antithrombin and fibrinogen, as well as activated protein C resistance (APC-R) was also performed.

All procedures were performed according to the recommendation of the manufacturer except for the PT using RecombiPlasTin 2G® (Instrumentation Laboratory, Bedford, MA, USA) and the aPTT using SynthASil® (Instrumentation Laboratory) that were both performed on a STA-R Evolution® coagulometer.

For all chromogenic anti-Xa assays, the results were collected as OD/min. For STA®-Liquid Anti-Xa (Diagnostica Stago, Asnieres, France), Biophen® Direct Factor Xa Inhibitor (DiXaI) (Hyphen BioMed, Neuville-sur-Oise, France) and Technochrom® Anti-Xa (Technoclone, Vienna, Austria) assays, we used the methodology provided by the manufacturer for the assessment of rivaroxaban, but the tests were not calibrated and results were given in OD/min. For the Technochrom® Anti-Xa, two different methodologies were applied (for the high and the low range) differing by the pre-dilution of the sample (1:15 and 1:5 for the high and the low range, respectively).

For the following anti-Xa chromogenic assays, an adapted method was proposed with the aim of increasing the dynamic range of quantitation.

Thus, for Biophen® Heparin LRT (Hyphen BioMed), 50 µl of spiked NPP diluted 1/10 in physiological saline were mixed with 125 µl of chromogenic substrate (SXa-11) and incubated 240 seconds (s) at 37 °C. Then, 125 µl of bovine factor Xa pre-warmed at 37 °C were added, starting the measurement on STA-R Evolution®. For HemosIL® Liquid Heparin (Instrumentation Laboratory), 10 µl of spiked NPP was mixed with 100 µl of chromogenic substrate and was incubated for 180 s at 37 °C. Thereafter, 75 µl of bovine FXa was added, starting the measurement on ACL-TOP®.

The calibrated automated thrombogram (CAT) measurement was performed according to previously reported procedures (17–19).

Statistical analysis

Statistical analyses and graphics were computed using GraphPad Prism 6.0® for Mac OSX® (GraphPad Software, La Jolla, CA, USA).

To compare the sensitivity of the different clotting assays, we use the final concentration of edoxaban (i.e. after addition of the reagents) needed to double the clotting time (2xCT [CT = Clotting Time]). For chromogenic assays, the sensitivity is defined as the final concentration of edoxaban (i.e. after dilution steps and addition of the reagents) needed to halve the analytical parameter (½xOD/min [The concentration needed to halve the change in the optical densitometry reported by minute]). For the CAT, the sensitivity of the different parameters is defined as follow: C_{max} IC_{50} [The final edoxaban concentration reducing the C_{max} of 50 %]; Peak IC_{50} [The final edoxaban concentration reducing the Peak of 50 %]; mVRI IC_{50} [The final edoxaban concentration reducing the mVRI of 50 %]; 2xLT [The final edoxaban concentration needed to double the lag time (LT)]; 2xTTP (The final edoxaban concentration needed to double the Time to Peak (TTP)).

Except for clotting factor activities that were run only once, each test was run in triplicate on the same day. Data on the graphics represent the mean and the standard deviation. The repeatability is defined as the mean of the coefficient of variation ([(standard deviation/mean)*100]) of the triplicate of each concentration for each test.

For aPTT, chromogenic anti-Xa assays, DRVV-T, PT and TGA, the limit of detection (LOD) and quantitation (LOQ) were calculated as follow:

- LOD: [(3*standard deviation of Y0)/ slope]
- LOQ: [(10*standard deviation of Y0)/ slope]

Where Y0 is the baseline value of the linear regression.

For assays that did not fit a linear response on their entire range of measurement, the first five points of the calibration curve (until 100 ng/ml) were used to define a linear regression. The slope of this linear regression was then used for the calculation of the LOD and LOQ. The upper limit of quantitation (ULQ) reflects the concentration from which results are unreliable (concentrations above 1000 ng/ml were not tested and are stated as the ULQ when applicable).

Results

Prothrombin time (PT)

A concentration-dependent prolongation of PT was found. The 2xCT depended on the reagent and ranged from 97 ng/ml

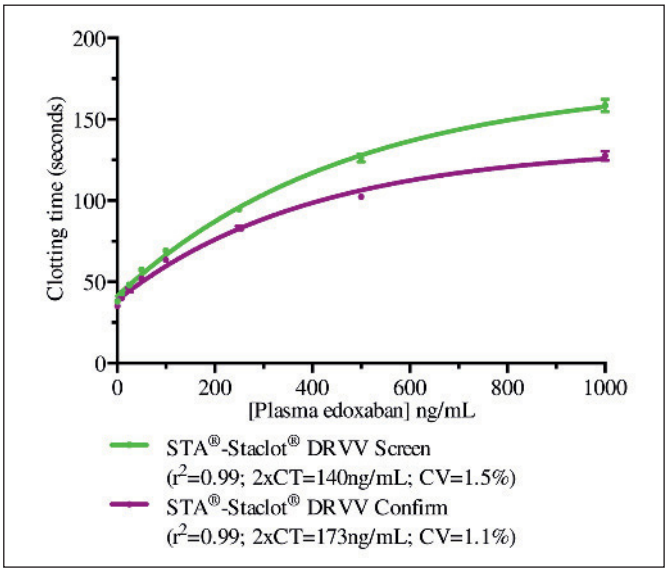

Figure 2: Impact of edoxaban on the dilute Russell Viper Venom Time. Edoxaban prolonged the dilute Russell Viper Venom Time dose-dependently. The relation is curvilinear showing a lower sensitivity at the higher concentrations. However the higher sensitivity at the lower concentrations is interesting since it provides a low limit of detection and quantitation.

Coagulation assay	Reagent	Method	Coagulation Analyzer (Manufacturer)	Influenced (Y/N)	Recommendations
Thrombin Time	STA®-Thrombin	Chronometric	STA-R Evolution® (Diagnostica Stago)	N	/
Reptilase Time	STA®-Reptilase	Chronometric	STA-R Evolution® (Diagnostica Stago)	N	/
Lupus Anticoagulant	STA®-Staclot® DRVV Screen	Chronometric	STA-R Evolution® (Diagnostica Stago)	Y	DRVVT testing should be avoided. Taipan/ecarin testing could be an alternative.
	STA®-Staclot® DRVV Confirm				
Protein C	HemosIL® Protein C	Chromogenic	ACL-TOP® (Instrumentation Laboratory)	N	/
Protein S	STA®-Staclot® Protein S	Chronometric	STA-R Evolution® (Diagnostica Stago)	Y	Not influenced until 100 ng/ml of edoxaban. At higher concentrations (from 250 ng/ml), an overestimation of 30 % was observed. This test should be avoided.
Free Protein S Antigen	HemosIL® Free Protein S	Immunoturbidimetric	ACL-TOP® (Instrumentation Laboratory)	N	/
Activated Protein C Resistance	Pefakit® APC-R Factor V Leiden	Chronometric	ACL-TOP® (Instrumentation Laboratory)	Y	The ratio is slightly influenced (3.54 for baseline and 3.82 for 100 ng/ml). It can provide false negative results.
Fibrinogen Clauss Method	STA®-Fibrinogen	Chronometric	STA-R Evolution® (Diagnostica Stago)	N	/
Fibrinogen PT-derived	RecombiPlasTin 2G®	Chronometric	ACL-TOP® (Instrumentation Laboratory)	N	There is an underestimation of the fibrinogen rate at on-therapy concentrations (± 100 ng/ml)
Antithrombin	STA®-Stachrom® ATIII	Chromogenic thrombin-based	STA-R Evolution® (Diagnostica Stago)	N	FXa-based chromogenic assays should be avoided (increase of 10 % per 100 ng/ml) and thrombin-based chromogenic assays should be preferred.
	HemosIL® Liquid Anti-thrombin	Chromogenic FXa-based	ACL-TOP® (Instrumentation Laboratory)	Y	
Extrinsic Clotting Factors	STA®-C.K. Prest®	Chronometric	STA-R Evolution® (Diagnostica Stago)	Y	Higher dilution (> 1:40) of the sample should be performed.
Intrinsic Clotting Factors	RecombiPlasTin 2G®	Chronometric	STA-R Evolution® (Diagnostica Stago)	Y	Use of less sensitive reagents could be preferred when feasible (e.g. Dade® Innovin® for extrinsic pathway). Higher dilution (> 1:40) of the sample should be performed.
Ecarin Clotting Time	Ecarin	Chronometric	STA-R Evolution® (Diagnostica Stago)	N	/

aPTT: activated Partial Thromboplastin Time; dRVVT: Dilute Russell Viper Venom Time; ECT: Ecarin clotting time; PT: Prothrombin Time; RT: reptilase time; TT: thrombin time). † 140 and 173 ng/ml for STA®-Staclot®-DRVV Screen and Confirm, respectively. ‡ Classification of the chromogenic anti-Xa assays based on their limit of quantitation (from the lower to the higher): Technochrom® Anti-Xa (1:5) (10 ng/ml) < HemosIL® Liquid Heparin (15 ng/ml) < Technochrom® Anti-Xa (1:15) (24 ng/ml) < Biophen® Direct Factor Xa Inhibitors and STA®-Liquid Anti-Xa (41 ng/ml) < Biophen® Heparin LRT (62 ng/ml). Technochrom® Anti-Xa (1:5) and HemosIL® Liquid Heparin were enable to measure accuretely plasma concentration above 500 ng/ml (no more decrease of the OD/min).

Table 3: Summary of recommended assays for the measurement of edoxaban in plasma.

(TriniCLOT® PT Excel S) to 296 ng/ml (Dade® Innovin®) (►Figure 1). Prothrombin time may be normal (ratio< 1.2) with on-therapy concentration of edoxaban depending on the reagent (►Table 2). The repeatability ranged from 1.1% (Dade® Innovin® and STA®-Neoplastin® CI+) to 3.1% (RecombiPlasTin 2G®). The LOD and LOQ ranged from 5 to 19 ng/ml and from 20 to 63 ng/ml, respectively.

Activated partial thromboplastin time (aPTT)

The aPTT showed a concentration-dependent prolongation of clotting time (Suppl. Figure 1, available online at www.thrombosis-online.com). The 2xCT ranged from 304 ng/ml (Actin® FS) to 400 ng/ml (STA®-PTT Automate). The repeatability was always below 3%. The LOD and LOQ ranged from 5 to 89 ng/ml and from 18 to 297 ng/ml, respectively.

Thrombin time, ecarin clotting time and reptilase time

All these tests were not influenced by the presence of edoxaban at the concentration tested in this study.

Dilute Russell Viper Venom Time

STA®-Staclot®-DRVV Screen and Confirm were also prolonged dose-dependently (►Figure 2). The 2xCT were 140 and 173 ng/ml for STA®-Staclot®-DRVV Screen and Confirm, respectively. Despite a non-linear prolongation of the clotting time, the low LOQ of DRVV-T can ensure sufficient performances in the lower concentrations (from 25 ng/ml) making DRVV-T useful to screen the relative intensity of on-therapy and supra-therapeutic edoxaban concentrations, even at C_{trough} (►Table 2).The LOD was

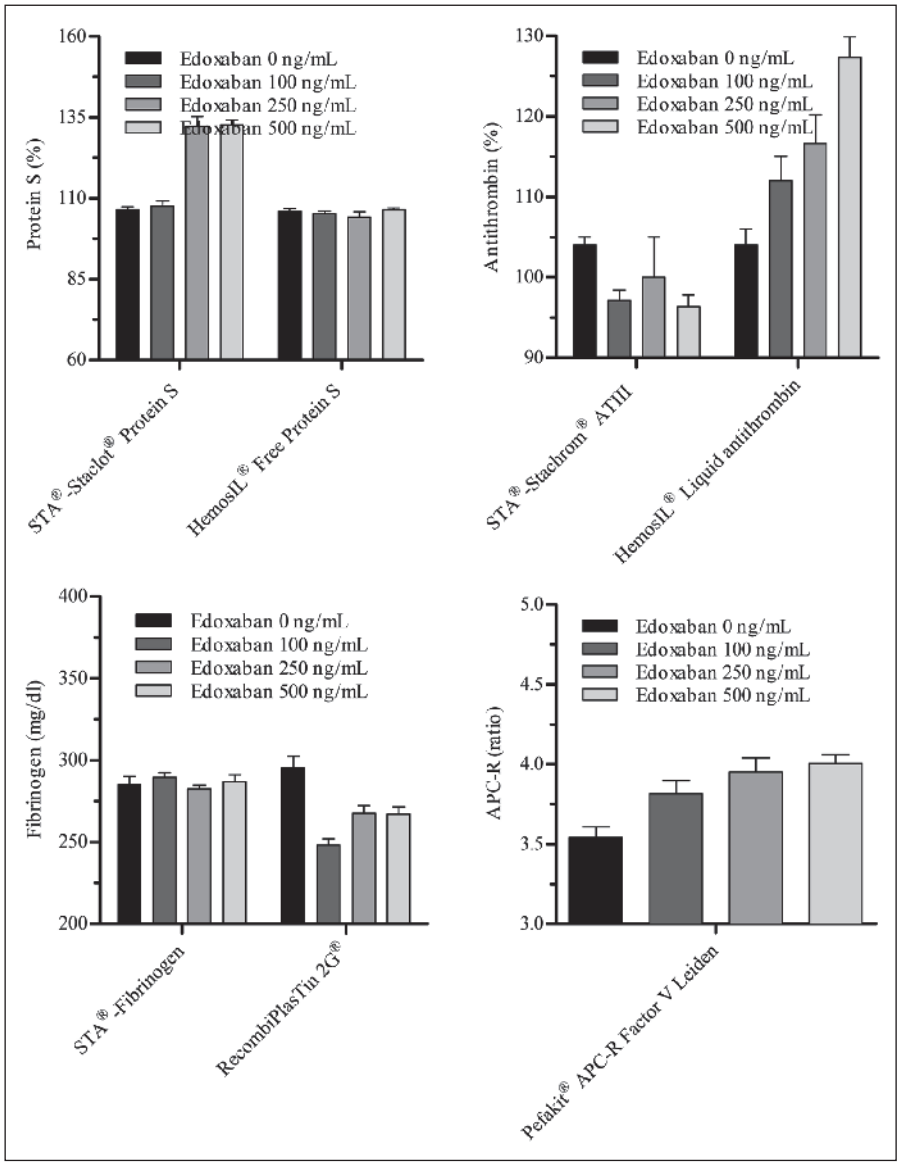

Figure 3: Impact of edoxaban on activated protein C resistance, protein S, antithrombin and fibrinogen measurements. Assays that involve FXa or upstream coagulation factors are more likely to be influenced by edoxaban than immunological or thrombin-based assays. Results are the means of the triplicate ± SD.

7 ng/ml with both the screen and the confirm reagents, and the LOQ ranged from 22 to 24 ng/ml.

Activated protein C resistance, antithrombin measurement, fibrinogen measurement (Clauss and PT-derived methods), clotting factor measurement, protein-C and protein-S measurement

The impact of edoxaban on these tests is summarised in ►Table 3. ►Figure 3 shows the impact of edoxaban on APC-R, protein S, antithrombin and fibrinogen measurements. For factors of the intrinsic pathway (FVIII, FIX, FXI, and FXII), the aPTT-based clotting method showed a mean decrease of ± 35% at 100 ng/ml of edoxaban, 55% at 250 ng/ml and 75% at 500 ng/ml. The impact was more pronounced for FVII and FIX as presented in Suppl. Table 1 and Suppl. Figure 2 (available online at www.thrombosis-online.com). For FV, FVII and FX, a mean decrease of ± 18% at 100 ng/ml of edoxaban; 26% at 250 ng/ml and 43% at 500 ng/ml was observed, while prothrombin measurement seemed to be less affected (maximal decrease of 19%) (Suppl. Table 1 and Suppl. Figure 2, available online at www.thrombosis-online.com).

Chromogenic anti-Xa assays

A concentration-dependent decrease in OD/min was observed. The reactions were fitted by an exponential model (►Figure 4). Otherwise, for Biophen® DiXaI, the relation was linear until 500 ng/ml. The ½ OD/min ranged from 4 ng/ml (HemosIL® Liquid Heparin) to 14 ng/ml (Biophen® DiXaI). The repeatability was from 0.9% (Technochrom® Anti-Xa 1/5) to 3.4% (STA®-Liquid Anti-Xa). The LOD and LOQ ranged from 3 to 18 ng/ml and from 10 to 62 ng/ml, respectively.

Thrombin generation assay (TGA)

The most influenced parameters were the peak (C_{max} IC_{50}: 32 to 91 ng/ml) and the mVRI (mVRI IC_{50}: 15 to 46 ng/ml), while the lag time (2xLT: 195 to 384 ng/ml), the time to peak (2xTTP: 166 to 241 ng/ml) and the ETP (ETP IC_{50}: 79 to 210 ng/ml) were less affected, as reported in ►Figure 5. The prolongation/inhibition of all parameters decreased inversely to the amount of tissue factor (TF) in the reagent. The CV of the triplicate was always below 5%. The LOD and LOQ for the peak ranged from 10 to 14 ng/ml and from 33 to 48 ng/ml, respectively. For the mVRI, the LOD and LOQ ranged from 8 to 16 ng/ml and from 28 to 55 ng/ml, respectively.

Discussion

Our study aimed at investigating the impact of edoxaban on a series of routine or more specific coagulation tests in order to provide good recommendations to estimate the intensity of the treatment as well as to correctly interpret the results of diagnostic tests which may be altered by the presence of edoxaban.

Several reagents and methodologies have been assessed in order to provide good laboratory practice for the management on edoxaban at the laboratory level. We used normal pooled plasma (NPP) from healthy volunteers spiked with increasing amount of edoxaban. This technique has already been used in the past for the assessment of other direct oral anticoagulants (DOAC) but this is also one limitation of these studies (17–19). It has been found that we can reliably apply these results in real-life samples. Indeed, some studies revealed similarities between *in vitro* and *ex vivo* data for rivaroxaban (20–22), and this might be applicable to edoxaban, but further investigations are required to confirm these data and

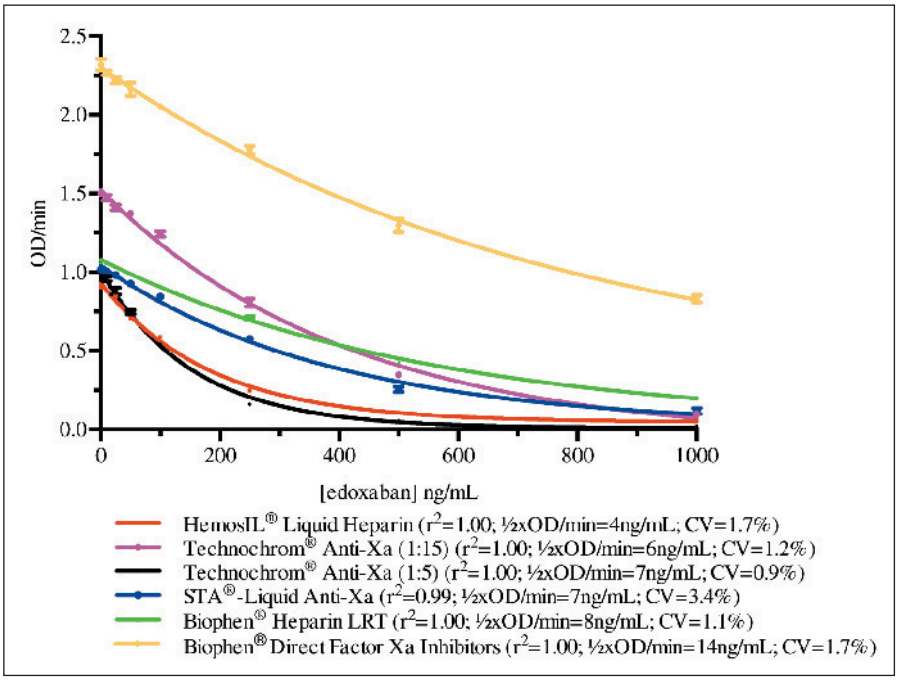

Figure 4: Measurement of edoxaban pharmacodynamics with chromogenic anti-Xa assays. There was a concentration-dependent decrease of the OD/min. Tests with higher baseline OD/min had a wider range of quantitation compared to those starting at lower OD/min at baseline. (r^2: Correlation Coefficient; ½xOD/min: Halve in optical density by minute (sensitivity) expressed in ng/ml; CV: Coefficient of variation expressed in percentage [%]).

evaluate the inter-individual variability. However, the "Clinical and biopharmaceutics review(s)" of the FDA, reported the impact of edoxaban administered in healthy subject on PT (23). Nevertheless, the reagent is not reported and the study was only performed in 10 healthy patients but it supports our results with PT. This is also in agreement with a recent systematic review in the field (24), but we should keep in mind that some global assays, such as PT or aPTT, proposed for the estimation of the intensity of some DOAC, were found to be more influenced by inter-individual variability than other assays, precluding in some cases the generalisability of the results obtained in *in vitro* studies (25–29).

In addition, by testing only edoxaban, we have no idea about the impact of the pharmacodynamically active M4-metabolite, but it represents less than 10% of the total edoxaban in plasma (30). One can therefore expect that this contribution is limited and that these data can be generalised in patients treated with edoxaban. One should also note that the present study is mono-centric and several studies already reported inter-laboratory variability in the *in vitro* assessment of dabigatran, rivaroxaban and apixaban (31–35). Therefore, even if the results of this study provide important insight on the measurement of edoxaban in plasma and reports an overall assessment of its impact on laboratory testing, it seemed more realistic that each laboratory estimates the sensitivity of its own reagent/coagulometer combination towards edoxaban using preferably home-made or commercially available calibrators, when they will become available (31, 36). Particularly, it is strongly advised to avoid calibration with low-molecular-weight heparin calibrators or with rivaroxaban or apixaban calibrators since the pharmacodynamics towards factor Xa is not strictly similar, precluding the extrapolation of the results into edoxaban equivalent.

Delay between the drug intake and the blood sampling

An important point to consider before aiming at measuring the effect of edoxaban in patients is the delay between the last intake of the drug and the blood sampling since assays that are impacted by edoxaban are influenced proportionally to the concentration. For edoxaban, the C_{max} is between 120–250 ng/ml and is reached within 1 to 2 h for a 60 mg QD dose regimen (14, 15). The plasma concentration at C_{trough} is between 15 to 60 ng/ml for the same dose regimen. For an elective assessment of the therapeutic response, trough sampling should be recommended since plasma concentrations are less variable in the late elimination phase compared to the absorption phase, as suggested with the other DOAC (37).

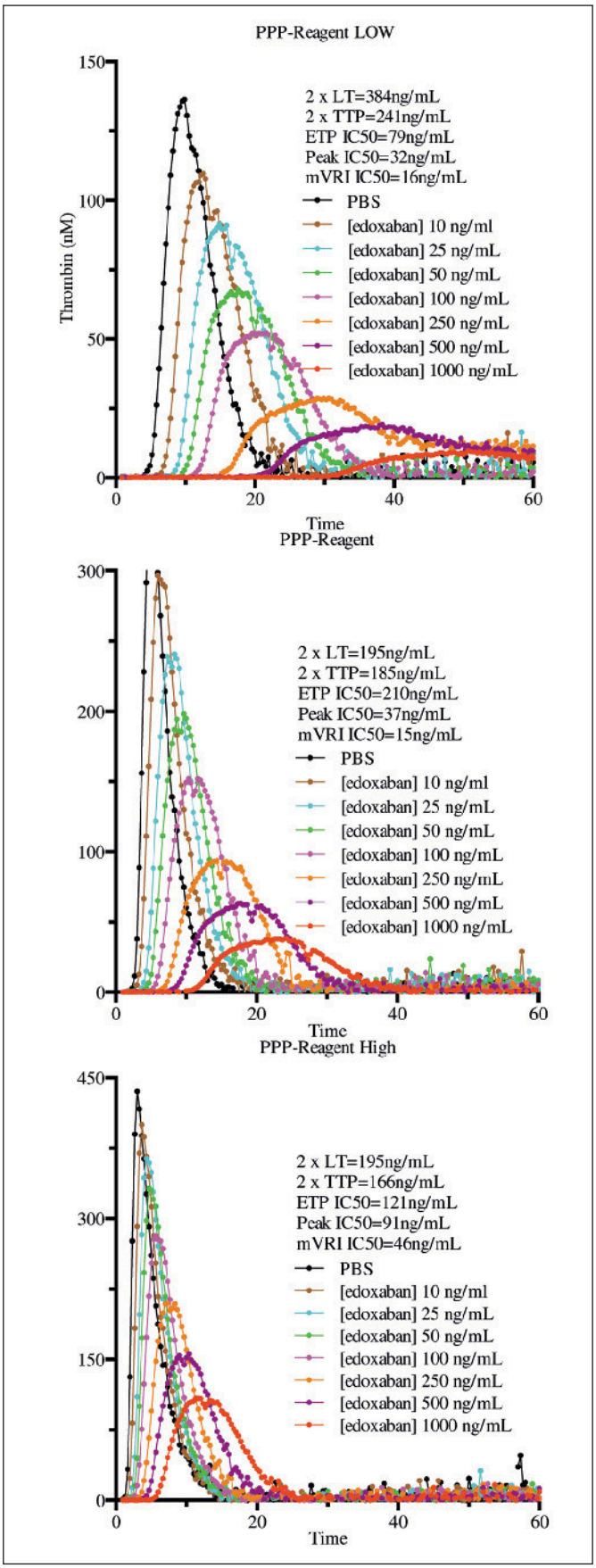

Figure 5: Impact of edoxaban on Calibrated Automated Thrombogram® (CAT). The most influenced CAT® parameters are the peak and the mean velocity rate index. There is an inter-reagent variability. Thanks to their high sensitivity, the better resolution and their large range of application, PPP-Reagent and PPP-Reagent High seemed to be the best reagents to monitor patients on edoxaban. ETP: Endogenous Thrombin Potential; IC_{50}: half-maximum inhibitory concentration; LT: Lag Time; mVRI: mean Velocity Rate Index; TTP: Time to Peak. mVRI was defined as follow: (Peak) / (Time to Peak – Lag Time).

Table 4: Summary of recommended assays for the measurement of edoxaban in plasma.

	Useful for measurement	Reliable but requires laboratory experience			Not recommended			
	Chromogenic anti-Xa assays	PT	TGA	DRVV-T	aPTT	TT	ECT	RT
Sensitivity (ng/ml)	4 to 14	97 to 296	Peak: 32 to 91 mVRI: 15 to 46	140 to 173†	304 to 400	Insensitive		
Dynamic range of quantitation (ng/ml)	10 to 1000‡	65 to 1000*	Peak: 12 to 1000* mVRI: 12 to 1000*	23 to 1000*	61 to 1000*	N.A		
Repeatability (%)	0.9 to 3.4	1.1 to 3.1	Peak : 0.6 to 16.3 mVRI : N. A.	1.2 to 1.5	1.0 to 2.5	N.A		
Dependence of reagent	Yes	Yes	Yes	Yes	Yes	No		
Linearity of the response	Yes/No	Yes	No	Yes	No	N.A		

† 140 and 173 ng/ml for STA®-Staclot®-DRVV Screen and Confirm, respectively. ‡ Classification of the chromogenic anti-Xa assays based on their limit of detection/quantitation (from the lower to the higher): Technochrom® Anti-Xa (1:5) (3/10 ng/ml) < HemosIL® Liquid Heparin (5/15 ng/ml) < Technochrom® Anti-Xa (1:15) (8/24 ng/ml) < Biophen® Direct Factor Xa Inhibitors and STA®-Liquid Anti-Xa (14/41 ng/mL) < Biophen® Heparin LRT (21/62 ng/ml). Technochrom® Anti-Xa (1:5) and HemosIL® Liquid Heparin were unable to measure accurately plasma concentration above 500 ng/ml (no more decrease of the OD/min).
* For aPTT, dRVVT, PT and TGA, the dynamic range presented is the mean of the individual lower and upper limit of quantitation of the different reagents. For dRVVT, the limit of detection/quantitation were 7/22 and 8/24 ng/ml for STA®-Staclot®-DRVV Screen and Confirm. For PT, the lowest limit of detection/quantitation were obtained with STA®-Neoplastine® R and TriniCLOT® PT Excel S (5/15 and 6/19 ng/ml, respectively). aPTT: activated Partial Thromboplastin Time; dRVVT: Dilute Russell Viper Venom Time; ECT: Ecarin clotting time; PT: Prothrombin Time; RT: reptilase time; TT: thrombin time.

However, in emergency settings when the assessment of the intensity of anticoagulation is required, it is important to keep in mind that these tests should be interpreted in relation to the delay since last intake of the drug, at least in case of recurrence of thrombosis. For a haemorrhagic event, the current plasma concentration will be the main factor guiding the physician. Therefore, normal, sub- and supra-therapeutic pharmacokinetic curves for the different populations would help the clinician and the biologist to correctly interpret the results when facing a random sampling.

Practical recommendations

What test should be recommended for an accurate measurement of edoxaban concentrations?

The accurate measurement of edoxaban plasma concentrations should be performed with chromogenic anti-Xa assays specifically dedicated for the measurement of direct factor Xa inhibitors using specific calibrators. Indeed, even if PT and DRVV-T can be of interest in some situations, chromogenic anti-Xa assays performed better (►Table 4). To date, edoxaban calibrators are not commercially available, and the majority of pharmacokinetic and pharmacodynamics studies in healthy volunteers or patients used chromogenic assays dedicated to the measurement of heparins (4, 15, 38, 39). Thus, results are expressed in anti-Xa units that are probably not the most appropriate unit to quantify edoxaban activity. Therefore, specific chromogenic anti-Xa assays, developed for the quantitative measurement of direct FXa inhibitors, using NPP sample spiked with edoxaban at different concentrations as calibrators should be recommended as the most feasible and reliable assays for the assessment of edoxaban plasma concentrations. Another advantage of chromogenic anti-Xa assays is that they are less sensitive than clotting tests to sample collection conditions and variations of the amounts of clotting factors among patients, allowing a reduction of the inter-individual variability (40).

The sensitivities of the different chromogenic assays tested in this study were higher for HemosIL® Liquid Heparin and Technochrom® Anti-Xa (4 and 6–7 ng/ml, respectively). This reveals that some methodologies, but also the chromogenic substrate, the exogenous factor Xa and the ratio between these reagents influence the sensitivity (41). The ionic force and the pH of the buffer solution could also be parameters that impact on the sensitivity of a particular assay.

Compared to other chromogenic anti-Xa assays, Biophen® DiXaI is specific to direct FXa inhibitors such edoxaban. Thanks to its Tris/EDTA/NaCl buffer at pH=7.85, this assay is insensitive to the presence of antithrombin-dependent FXa inhibitors (42).

Nevertheless, confirmation of the accuracy of these chromogenic anti-Xa assays using specific calibrators and controls should be confronted to the reference quantitative measurement method, i. e. liquid chromatography coupled with tandem-mass spectrometry, in patients treated with edoxaban.

What test should be recommended in a patient with a recurrence of thrombosis or when plasma concentrations are suspected to be low such in the perioperative setting?

In the ENGAGE AF-TIMI 48 study, patients with the more frequent recurrence of thrombosis were those in which the plasma

concentration of edoxaban seemed to be below 10–20 ng/ml at trough (4). Consequently, only some chromogenic anti-Xa assays tested in this study (i.e. Technochrom® Anti-Xa (1:5) and HemosIL® Liquid Heparin with an LOD of 3 and 5 ng/ml and an LOQ of 10 and 15 ng/ml, respectively) are sufficiently sensitive to assess these very low plasma concentrations. Compare to the systematic review of Cuker et al. (24) we found that some chromogenic anti-Xa assay are not sensitive enough to assess these trough level, showing the interest of having dedicated methodologies for the assessment of direct factor Xa inhibitors. However, the absence of anti-Xa activity can likely exclude on-therapy concentrations of edoxaban but in the perioperative context, the accurate assessment of very low concentrations of edoxaban is of interest, as reported for dabigatran (43). Thus, to date, further effort are required to develop dedicated chromogenic anti-Xa assays with a LOQ below 10 ng/ml to ensure an accurate estimation of concentrations below the on-therapy range at trough,

Regarding the clotting assays, PT will still be normal at these low concentrations as mentioned in ►Table 2 and supported by the results of Cuker et al. (24) while DRVV-T could be useful to assess plasma concentration only from 25 ng/ml, making these tests irrelevant in this context.

What test should be recommended in a patient facing a haemorrhagic event or when plasma concentrations are suspected to be high/supra-therapeutic?

The majority of the patients facing a haemorrhagic event will probably have supra-therapeutic blood concentrations of edoxaban. However, to date, harmful ranges are not yet defined and we have to extrapolate results from the plasma concentrations measured during the course of the different clinical trials. We can consider that patients outside the mean plasma concentration + 1.96*SD at trough (i.e. ± 140 ng/ml for 60 mg QD and ± 100 ng/ml when the dose is reduced at 30 mg QD due to clinical conditions) could be at risk of bleeding. At ± 100 ng/ml, chromogenic anti-Xa assays, DRVV-T and the most sensitive PT reagents (i.e. TriniCLOT® PT Excel S® and STA®-Neoplastine®R) could be informative while the less sensitive PT reagents are not sufficiently prolonged (ratio< 1.2). At ± 140 ng/ml, chromogenic anti-Xa, DRVV-T and the majority of PT reagents can be useful (►Table 2). However, one should keep in mind; when interpreting the results, that PT is subject to inter-individual variabilities but it can grossly estimate the intensity of anticoagulation in this setting. Importantly, the inter-reagent variability will prevent the establishment of a similar threshold even expressed in ratio as demonstrated in (►Table 2). For DRVV-T, the confirm reagent should be preferred to the screen, since the larger amount of phospholipids can reduced the inter-patient variability. At C_{max}, all tests recommended for the measurement of edoxaban (►Table 4) can be useful due to their sufficient dynamic range of application.

Which diagnostic tests are influenced by edoxaban?

As other anticoagulant drugs, edoxaban may affect the results of a series of coagulation assays routinely used in case of thrombophilia or in the exploration of a haemorrhagic event (►Table 3).

As previously mentioned, the impact of edoxaban on PT and aPTT depends on the reagents. Therefore, some PT reagents will practically not be influenced by edoxaban (e.g. Dade® Innovin®) while other (e.g. TriniCLOT® PT Excel S®) can be influenced by trough on-therapy concentrations (►Table 2). The aPTT is less influenced by edoxaban and trough on-therapy concentrations will probably have no effect on the results. However, concentrations encountered at C_{max} might prolong the aPTT. Therefore, it is of particular importance that the laboratories estimate the sensitivities of their reagents and their combination with the coagulometer (31).

The Pefakit® APC-R Factor V Leiden, a clot-based assay for the assessment of activated protein C resistance, shows an increase in the ratio between the two conditions (in the presence or absence of aPC) in presence of edoxaban and can provide false negative results.

The antithrombin measurement using FXa-based chromogenic assays is overestimated in presence of on-therapy concentration of edoxaban (►Figure 3). Thus, in patients treated with edoxaban, thrombin-based chromogenic assays should be used for the assessment of antithrombin rate.

Measurement of protein S using the STA®-Staclot® Protein S does not seem to be influenced until 100 ng/ml but shows an overestimation of approximately 30 % at 250 ng/ml of edoxaban (►Figure 3). In clinical routine practise it is preferable to use immunological assays or assays that do not involve FX since they are not influenced by edoxaban.

Clinically relevant concentration of edoxaban may interfere with the measurement of clotting factors. Dilution of the sample tends to reduce the drop of clotting factors between baseline and high edoxaban concentrations, as already stated for rivaroxaban and apixaban (17, 44) (Suppl. Table 1 and Suppl. Figure 2, available online at www.thrombosis-online.com). Importantly, the sensitivity of the PT or aPTT reagents must be taken into account and for laboratories that use several aPTT and PT reagents, we also recommend using the less sensitive aPTT and PT reagents to minimise the influence of edoxaban on clotting factor measurement.

As stated above, DRVV-T is affected by edoxaban and sensitivity depends on the amount of phospholipids (Screen is more sensitive than Confirm). Therefore, DRVV-T should be avoided to assess a lupus anticoagulant in patients treated with edoxaban since the ratio between the Screen and the Confirm will be increased, giving false positive results. Thus, as for rivaroxaban (45), a specific test using Taipan venom snake or Ecarin clotting time could be proposed to assess lupus anticoagulant even if international standardisation of the procedure is still required. Finally, measurement of fibrinogen using the dFib method shows an underestimation in presence of edoxaban (►Figure 3).

What about the thrombin generation test?

The calibrated automated thrombogram® gives more information than traditional coagulation assays and is also more sensitive (46, 47). By its mode of action, edoxaban mainly acts on the amplification phase of the thrombin generation (▶Figure 5), affecting mostly the peak and the mean velocity rate index, as already reported for rivaroxaban and apixaban (17, 18). The sensitivity towards the lag time and the time to peak is similar between the different reagents. Similarly to the results obtained with rivaroxaban and apixaban, PPP-Reagent Low cannot provide sufficient resolution on the entire range of concentration tested in this study. In addition, it has been shown that variability was more important at low TF concentrations (i.e. 1 pM) or with hypocoagulable plasma (48), a condition encountered in the presence of edoxaban. Our results also confirmed this higher variability (results not shown), and thus we recommend using PPP-Reagent or PPP-Reagent High to assess edoxaban plasma samples with the CAT analyser. Based on the results of several studies assessing the reversal of DOAC using PCC, FVIIa or specific reversal agents, this test is probably the most promising in this context (49–54). However, the turnaround time, the lack of standardisation and the inter-individual variability are still limitations that restrict its use in clinical practice (48).

Conclusions

In this study, we showed that chromogenic anti-Xa assays are the most appropriate assays to measure the pharmacodynamics of edoxaban. PT can be useful but each laboratory should be aware on the sensitivity and limitations of its own reagent while this test is not appropriate to estimate plasma drug concentration due to several limitations and a lack of sensitivity. DRVV-T could be informative in certain circumstances due to its good sensitivity and its low LOQ. TGA gives further information on the coagulation process but its use in clinical setting is limited. Edoxaban interferes with chronometric or chromogenic assays that involve FXa or upstream coagulation factors on different manner and therefore, immunological assays, thrombin-based or less sensitive assays should be used, when applicable, for diagnostic purposes. As for all DOAC, the delay between the last drug intake and the blood sampling is mandatory to avoid misinterpretation.

What is known about this topic?

- Therapeutic monitoring is generally not necessary with edoxaban but may be required in some clinical situations like invasive procedure or recurrence of stroke and bleedings.
- Anti-Xa activity correlates well with edoxaban concentration.
- Other DOACs have shown interferences with routinely used coagulation assays leading to misdiagnosis.

What does this paper add?

- Our study provides a comparison of the impact of broad plasma concentrations of edoxaban on specific and routinely used coagulation assays with a large panel of reagents. We have investigated the performance of these tests in terms of sensitivity, limit of detection, limit of quantitation, dynamic range of measurement and repeatability.
- We recommend chromogenic anti-Xa assays for the measurement of edoxaban using appropriate calibrator and controls in order to express the results in ng/ml.
- Edoxaban interferes with chronometric or chromogenic assays that involve FXa or upstream coagulation factors on different manner and therefore, immunological assays, thrombin-based or less sensitive assays should be used.

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Conflicts of interest

J.D., B.C., C.C., and J-M.D. have nothing to disclose. F.M. has received speaker fees for Boehringer Ingelheim, Bayer Healthcare and Bristol-Myers Squibb-Pfizer.

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