Received: 30 October 2018 | Revised: 17 December 2018 | Accepted: 4 January 2019

DOI: 10.1111/ijlh.12974

ORIGINAL ARTICLE

WILEY ISLH International Journal of Laboratory Hematology

An in vitro study to investigate the interference of enoxaparin on plasma levels of direct oral factor Xa inhibitors measured by chromogenic assays

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Abstract

Introduction: Co-administration of enoxaparin and a direct oral factor Xa inhibitor (xabans: apixaban, edoxaban, rivaroxaban) could give rise to the problem of overlapping the anti-Xa activity when measuring direct oral anticoagulant (DOAC) levels. We aimed to evaluate in vitro the degree of the interference of increasing enoxaparin concentrations on xaban plasma levels measured by different chromogenic anti-Xa assays with drug-specific calibrators and controls.

Methods: Seven plasma samples were spiked with apixaban, edoxaban, or rivaroxaban at fixed concentration, and enoxaparin at increasing concentrations (0, 0.125, 0.250, 0.50, 1.0, 1.50, and 2.0 IU/mL). The evaluated chromogenic assays were as follows: Biophen DiXaI and Biophen Heparin LRT (Hyphen BioMed), Berichrom Heparin and Innovance Heparin (Siemens), STA-Liquid Anti-Xa (Stago Diagnostics), Technochrom anti-Xa (Technoclone), and HemosIL Liquid Anti-Xa (Werfen).

Results: The presence of enoxaparin caused increased DOAC levels, with over-estimation depending on the anti-Xa assay and on the heparin concentration in the sample. The smallest over-estimation was in the sample with enoxaparin 0.125 IU/mL and the greatest in the sample with enoxaparin 2.0 IU/mL (0%, 3.1%, and 7.4% vs 583.8%, 526.1%, and 415.2% for apixaban, edoxaban, and rivaroxaban, respectively). Biophen DiXaI showed lower interference compared to other methods (maximum over-estimation in the presence of enoxaparin 2.0 IU/mL: 56.4% dosing rivaroxaban by Biophen DIXaI vs 583.8% dosing apixaban by Berichrom Heparin).

Conclusion: The presence of enoxaparin interferes with xabans measurement by chromogenic anti-Xa assays causing falsely elevated DOAC levels, the over-estimation being dependent on the anti-Xa assay and on the heparin concentration in the sample.

KEYWORDS

apixaban, edoxaban, enoxaparin, interference, rivaroxaban

1 | INTRODUCTION

Direct oral anticoagulants (DOACs) apixaban, dabigatran, edoxaban, and rivaroxaban have been developed and approved for specific clinical indications.[1-11] Among them, apixaban, edoxaban, and rivaroxaban (xabans) produce their anticoagulant effect by directly inhibiting factor Xa (FXa). Low molecular weight heparins (LMWHs) have been widely used for prevention and management of venous thromboembolism for over 30 years; at variance from xabans, LMWHs are indirect inhibitors of factor Xa and, to a lesser extent, thrombin.[12]

Concomitant administration of LMWH and DOAC is infrequent in clinical practice but in some situations (eg, patients in DOAC treatment who receive LMWH before/after surgery or during hospital stays), or when a change in the type of anticoagulant therapy is needed (from subcutaneous LMWH to DOAC or vice versa), an overlap period may occur due to the time needed for drug elimination.[13-15] Although LMWH or DOAC half-lives are normally short, some factors may influence their pharmacokinetics, such as decreased renal function, severe liver insufficiency, and drug interactions.[16,17]

In a standard clinical scenario, DOACs do not require routine laboratory testing for dose adjustment.[18] However, levels measurement may be necessary in some critical conditions such as invasive procedures, thromboembolic events, life-threatening bleeding, or need for reversal therapy.[19] Laboratory LMWH monitoring is performed by chromogenic anti-Xa assays; after the introduction of DOACs, the same tests have been optimized to assess xabans concentration, using specific calibrators and controls.[19,20] Since anti-Xa assays are able to detect the activity of both xabans and LMWHs, it is likely that the presence of LMWH in the sample may influence the measurement of the direct FXa inhibitor.[21]

Such an interference may give rise to problems when DOAC dosage is necessary because of the presence of a critical clinical condition but a combined administration with LMWH is not known. In that case, the measured anti-Xa activity may be not correctly interpreted and consequently xaban level may be over-estimated.

In the present in vitro study, we aimed to investigate the degree of the interference of different enoxaparin concentrations on the measurement of apixaban, edoxaban, and rivaroxaban plasma levels evaluated by a large panel of commercial chromogenic anti-Xa assays with dedicated calibrators and controls.

2 | MATERIALS AND METHODS

2.1 | Preparation of enoxaparin-spiked plasma samples with/without DOAC

The study was a single-center, in vitro investigation performed in the frame of the activities of Arianna Anticoagulazione Foundation and

TABLE 1 Assays used for apixaban, edoxaban, and rivaroxaban measurement: reagents, instruments, calibrators, and controls are detailed

Assay ID	Reagent	Instrument	Dedicated calibrators and controls	Available low-range application	Xaban dosage		
					Apixaban	Edoxaban	Rivaroxaban
DiXaI-HY	Biophen DiXaI (Hyphen BioMed)	CS-2100 (Sysmex)	Hyphen BioMed	Yes	Yes	Yes	Yes
LRT-HY	Biophen Heparin LRT (Hyphen BioMed)	CS-2100 (Sysmex)	Hyphen BioMed	Yes	Yes	Yes	Yes
BerHep-SI	Berichrom Heparin (Siemens)	BCS (Siemens)	Technoclone (apixaban) Hyphen BioMed (rivaroxaban)	No	Yes	No	Yes
InnHep-SI	Innovance Heparin (Siemens)	BCS (Siemens)	Technoclone (apixaban) Hyphen BioMed (edoxaban, rivaroxaban)	No	Yes	Yes	Yes
AntiXa-STA	STA-Liquid Anti-Xa (Stago Diagnostics)	STA Compact (Stago Diagnostics)	Stago Diagnostics	No	Yes	Yes	Yes
AntiXa-TC	Technochrom anti-Xa (Technoclone)	ACL-TOP (Werfen)	Technoclone	Yes	Yes	No	Yes
AntiXa-WE	HemosIL Liquid Anti-Xa (Werfen)	ACL-TOP (Werfen)	Werfen (apixaban, rivaroxaban) Stago Diagnostics (edoxaban)	No	Yes	Yes	Yes

Hyphen BioMed, Neuville-sur-Oise, France; Siemens Healthcare Diagnostics, Marburg, Germany; Stago Diagnostics, Asnières sur Seine, France; Sysmex Europe GmbH, Norderstedt, Germany; Technoclone GmbH, Wien, Austria; Werfen, Bedford, MA, USA

carried out in the laboratory of the Department of Angiology and Blood Coagulation of the University Hospital S. Orsola-Malpighi, Bologna. A pooled normal plasma (PNP) was prepared from citrated (109 mmol/L) blood of 20 healthy subjects; PNP was divided into three aliquots (one for each xaban), snap frozen within 1 hour from collection and stored at −80°C until analysis, that was performed within 1 week. On each working session, one PNP aliquot was thawed and divided into 2 parts: one was spiked with a DOAC calibrator (apixaban, edoxaban, or rivaroxaban lyophilized calibrator, Hyphen BioMed) in order to achieve a DOAC concentration around 50 ng/mL. Then, both PNP aliquots (with or without DOAC) were spiked with enoxaparin to obtain seven working samples with the following final concentrations: 0, 0.125, 0.250, 0.50, 1.0, 1.50, and 2.0 IU/mL. Testing was carried out immediately after preparation and was completed within 4 hours.

2.2 | Assays for DOACs or enoxaparin measurement

Apixaban, edoxaban, and rivaroxaban anticoagulant activity, expressed as drug concentration equivalent (ng/mL), was measured by chromogenic assays of different manufacturers as detailed in Table 1. When the study was carried out, not all the assays employed for apixaban and rivaroxaban were available for edoxaban determination. Characteristics of the evaluated assays (plasma volume, volume and type of FXa, volume and type of chromogenic substrate, type of buffer) are detailed in Table S1.

Assays were previously calibrated in duplicate with dedicated lyophilized standards according to manufacturer's instructions. For some assays, low-range applications were provided by manufacturers (DiXaI-HY, LRT-HY and AntiXa-TC for apixaban and rivaroxaban; DiXaI-HY, LRT-HY for edoxaban); in that case, if results obtained with the standard protocol were below the value suggested by manufacturers (50 ng/mL), they were repeated using the low-range application. Specific quality control samples were tested in single determination before the study samples and had to comply with the acceptance range, otherwise the calibration was repeated.

Enoxaparin concentration in the seven working samples was checked and confirmed by testing enoxaparin-spiked samples without DOAC by the anti-Xa assay routinely used in the laboratory, calibrated with enoxaparin standards (antiXa-WE).

TABLE 2 Results of DOAC measurement performed in samples with increasing enoxaparin concentrations and spiked with apixaban, edoxaban, or rivaroxaban

Assay	PNP + 0 IU/mL enoxaparin	PNP + 0.125 IU/mL enoxaparin	PNP + 0.25 IU/mL enoxaparin	PNP + 0.5 IU/ml enoxaparin	PNP + 1.0 IU/mL enoxaparin	PNP + 1.5 IU/mL enoxaparin	PNP + 2.0 IU/mL enoxaparin
Spiked with apixaban							
DiXaI-HY*	62.5	59.1 (---)	59.3 (---)	64.9 (3.8%)	63.8 (2.1%)	63.5 (1.6%)	62.5 (---)
LRT-HY*	48.0	61.1 (27.3%)	52.3 (9.0%)	60.4 (25.8%)	77.4 (61.3%)	82.1 (71.0%)	96.8 (101.7%)
BerHep-SI	47.5	54.5 (14.7%)	55.7 (17.3%)	100.5 (111.6%)	152.7 (221.5%)	183.0 (285.3%)	324.8 (583.8%)
InnHep-SI	58.2	70.5 (21.1%)	88.2 (51.5%)	119.3 (105.0%)	162.4 (179.0%)	185.0 (217.9%)	223.9 (284.7%)
AntiXa-STA	43.2	49.9 (15.5%)	53.1 (22.9%)	59.2 (37.0%)	68.3 (58.1%)	85.3 (97.5%)	103.0 (138.4%)
AntiXa-TC*	71.4	95.2 (33.3%)	118.6 (66.1%)	149.3 (109.1%)	191.6 (168.3%)	220.9 (209.4%)	278.4 (289.9%)
AntiXa-WE	55.6	72.7 (30.8%)	98.2 (76.6%)	120.0 (115.8%)	177.5 (219.2%)	211.0 (279.5%)	275.4 (395.3%)
Spiked with edoxaban							
DiXaI-HY*	80.8	84.2 (4.2%)	85.6 (5.9%)	84.6 (4.7%)	101.4 (25.5%)	99.1 (22.6%)	120.8 (49.5%)
LRT-HY*	55.5	57.2 (3.1%)	64.4 (16.0%)	78.4 (41.3%)	99.4 (79.1%)	113.3 (104.1%)	135.8 (137.4%)
InnHep-SI	54.2	71.6 (32.1%)	84.8 (56.5%)	112.6 (107.7%)	148.1 (173.2%)	172.0 (217.3%)	229.7 (323.8%)
AntiXa-STA	46.8	58.8 (25.6%)	69.2 (47.9%)	90.4 (93.2%)	129.0 (175.6%)	156.2 (233.8%)	201.7 (331.0%)
AntiXa-WE	42.6	63.1 (48.1%)	81.7 (91.8%)	120.9 (183.8%)	179.1 (320.4%)	215.1 (404.9%)	266.7 (526.1%)
Spiked with rivaroxaban							
DiXaI-HY*	52.8	56.7 (7.4%)	59.5 (12.7%)	62.4 (18.2%)	68.7 (30.1%)	74.0 (40.2%)	82.6 (56.4%)
LRT-HY*	52.5	57.0 (8.6%)	59.9 (14.1%)	71.8 (36.8%)	84.8 (61.5%)	92.6 (76.4%)	107.0 (103.8%)
BerHep-SI	55.1	66.7 (21.1%)	96.6 (75.3%)	112.2 (103.6%)	193.7 (251.5%)	235.0 (326.5%)	283.9 (415.2%)
InnHep-SI	45.9	67.2 (46.4%)	79.0 (72.1%)	98.8 (115.3%)	128.2 (179.3%)	144.5 (214.8%)	182.4 (297.4%)
AntiXa-STA	40.1	44.6 (11.2%)	48.6 (21.2%)	60.3 (50.4%)	78.8 (96.5%)	88.2 (120.0%)	106.8 (166.3%)
AntiXa-TC*	45.5	62.2 (36.7%)	83.0 (82.4%)	96.9 (113.0%)	109.9 (141.5%)	114.7 (152.1%)	160.0 (251.6%)
AntiXa-WE	44.2	60.2 (36.2%)	77.9 (76.2%)	103.9 (135.1%)	143.9 (225.6%)	162.6 (267.9%)	192.2 (334.8%)

For each sample, the measured concentration is reported as ng/ml and the relative over-estimation (calculated vs DOAC level of the sample without enoxaparin) is shown in parenthesis.

*The low-range procedure was used when results obtained with the standard protocol were <50 ng/mL.

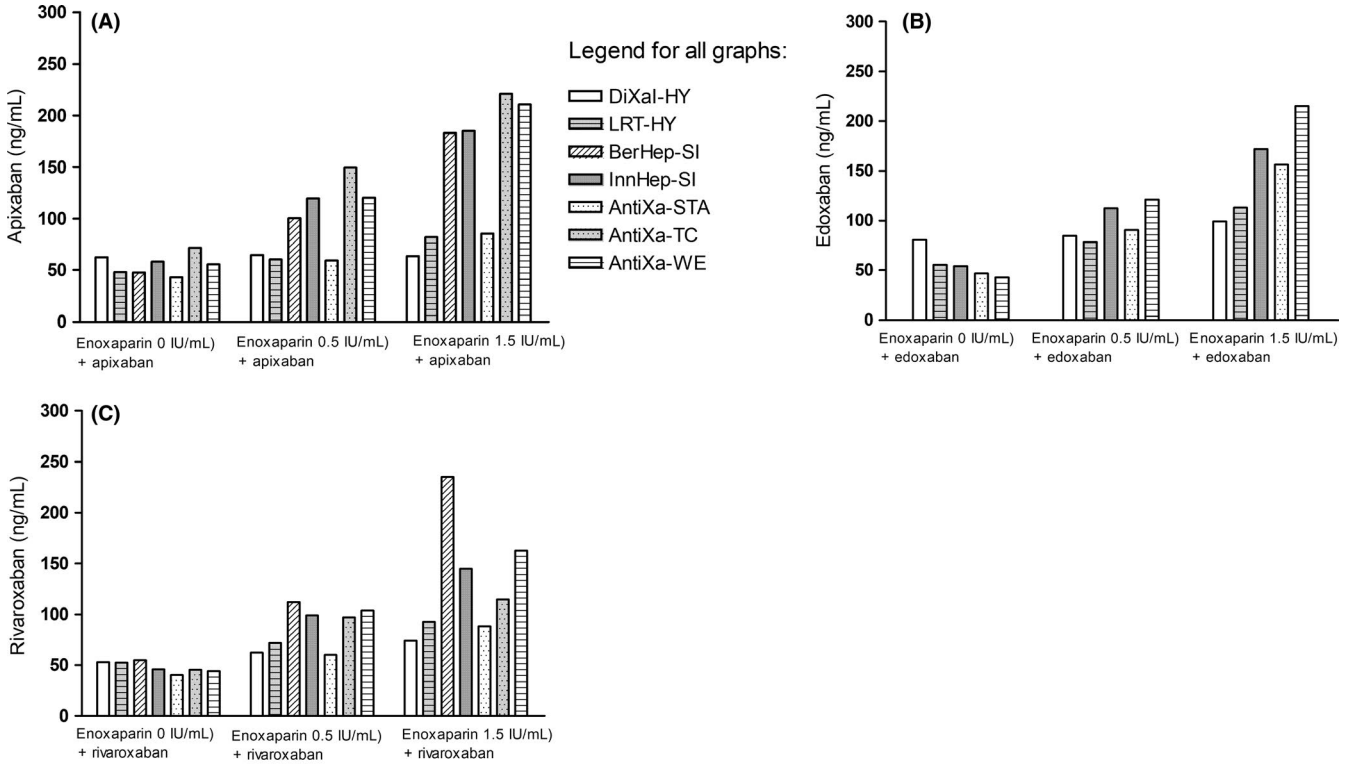

FIGURE 1 DOAC levels measured by dedicated anti-Xa assays in samples spiked with apixaban (A), edoxaban (B), or rivaroxaban (C) without enoxaparin or added with enoxaparin 0.5 or 1.5 IU/mL

2.3 | Statistics

For each working sample, results are presented as measured concentration (ng/mL) and relative over-estimation (%) to express the over-estimation of DOAC levels caused by the presence of enoxaparin, computed as follows:

$$\text{Over - estimation}\,(\%) = [(\text{DOAC}_{\text{enoxaparin}} - \text{DOAC}_{\text{no - enoxaparin}})/ \text{DOAC}_{\text{no - enoxaparin}}] \times 100$$

where $\text{DOAC}_{\text{enoxaparin}}$ and $\text{DOAC}_{\text{no-enoxaparin}}$ are DOAC concentration in the presence or absence of enoxaparin, respectively. Statistical analysis was performed using the GraphPad Prism Software (San Diego, CA, USA).

3 | RESULTS

Apixaban, edoxaban, and rivaroxaban concentrations measured in samples with different enoxaparin concentrations are shown in Table 2, where relative over-estimations are also reported. DOAC levels in the sample without enoxaparin ranged from 43.2 to 71.4 ng/mL (apixaban), from 42.6 to 80.8 ng/mL (edoxaban), and from 40.1 to 55.1 ng/mL (rivaroxaban). The presence of enoxaparin increased measured levels of all xabans, with the degree of over-estimation depending on enoxaparin concentration and anti-Xa assay used. As expected, the smallest interference was found in the sample with the lowest enoxaparin concentration 0.125 IU/mL (0%, 3.1%, and 7.4% for apixaban, edoxaban, and rivaroxaban, respectively); the greatest interference was observed in the sample with the highest enoxaparin concentration 2.0 IU/mL (583.8%, 526.1%, and 415.2% for apixaban, edoxaban, and rivaroxaban, respectively).

In Figure 1, the analysis was focused on two working samples spiked with xabans and enoxaparin 0.5 or 1.5 IU/mL, compared to the sample without heparin, in order to better evaluate the behavior of different assays for xabans measurement at enoxaparin doses ranging from prophylactic to supratherapeutic. In the sample added with enoxaparin 0.5 IU/mL, DOAC levels ranged from 59.2 to 149.3 ng/mL (apixaban), from 78.4 to 120.9 ng/mL (edoxaban), and from 60.3 to 112.2 ng/mL (rivaroxaban) depending on the test used. Higher DOAC concentrations were measured in the sample spiked with enoxaparin 1.5 IU/mL: from 63.5 to 220.9 ng/mL (apixaban), from 99.1 to 215.1 ng/mL (edoxaban), and from 74.0 to 235.0 ng/mL (rivaroxaban).

Our results showed that for some anti-Xa assays, the extent of the enoxaparin interference was different depending on the type of xaban. The over-estimation of edoxaban levels measured by antiXa-STA and antiXa-WE assays was almost double compared to the interference on apixaban or rivaroxaban at the same enoxaparin concentration. On the other hand, DiXaI-HY showed less interference when dosing apixaban compared to other xabans (maximum over-estimation 3.8%, 49.5%, and 56.4% for apixaban, edoxaban, and rivaroxaban, respectively; Table 2).

4 | DISCUSSION

Different studies in literature dealt with the matter of the management of DOAC patients during invasive procedures, but the benefit of heparin bridging at DOAC arrest in reducing perioperative thromboembolism without increasing bleeding risk has not been clearly established. In most studies, heparin bridging and anticoagulant interruption did not follow a standardized protocol, although LMWH at therapeutic or prophylactic doses was the main choice for bridging therapy.[17,22,23] Although DOAC treatment should have already been stopped before prescribing heparin treatment, the high intra- and interindividual variability of DOAC levels[24] and the potential presence of factors influencing DOAC metabolism and elimination, such as creatinine clearance <50 mL/min and antiarrhythmic treatment,[25] could increase DOAC elimination half-life, otherwise short in standard conditions. An overlapping presence of DOAC and LMWH is therefore possible.

Many chromogenic anti-Xa assays are currently available to measure the activity of xabans; they are all based on the inhibition of exogenous FXa by the anti-Xa drug present in the plasma patient and use FXa-directed chromogenic substrates to detect the residual FXa activity. Although these assays employ dedicated calibrators to convert the residual FXa activity into DOAC concentration, they are not drug-specific since they may be affected by the presence of other agents which display anti-Xa activity, such as LMWH.[21]

Previous studies investigated the influence of heparins on direct FXa inhibitors measurement. In vitro studies on spiked samples showed that the addition of LMWH or unfractionated heparin (UFH) had an additive effect on the anti-Xa assays for rivaroxaban and apixaban,[26,27] but it did not affect DOAC pharmacokinetic.[28] Other studies investigated the effect of co-administration of apixaban and enoxaparin[13] or rivaroxaban and enoxaparin[14] in healthy subjects, showing an association with increased anti-Xa activity. Similar results were also obtained in patients taking apixaban or rivaroxaban and UFH during periprocedural hemostasis,[29] and in real-life samples from patients treated with rivaroxaban with or without bridging with LMWH.[15]

The present in vitro study was designed by some of the participants in the START-Register. Whereas the effect of enoxaparin on DOAC levels is already well recognized, the aim of the study was to investigate the extent of interference between different anti-Xa assays when dosing a fixed xaban concentration (50 ng/mL) in the presence of increasing enoxaparin concentrations.

In a clinical scenario of combined use of LMWH and xabans, unknowingly or during transition of drug therapy, it is likely that the stopped drug is almost completely eliminated before starting the new treatment. Therefore, in the present study, samples were spiked with a low concentration (50 ng/mL) of apixaban, rivaroxaban, or edoxaban, and enoxaparin was added at various concentrations (from prophylactic to supratherapeutic, from 0 to 2.0 IU/mL) that can be achieved in clinical practice.

The seven anti-Xa assays employed in the study to measure DOAC levels with dedicated standards were the assays used in the majority of the centers participating in the START-Register. These kits differ in some factors (ie, the type of buffer, the reagent, or sample dilution) that could determine varying performances when dosing DOACs. One of the analyzed assays, Biophen DiXaI (Hyphen BioMed), was reported in literature as having no interference of heparin or fondaparinux when dosing rivaroxaban by using a high ionic strength buffer, but a recent study did not confirm that data.[30] At the time of our study, most of the evaluated anti-Xa assays or specific calibrators and controls did not report in their package insert any information regarding the possible influence of heparins on xabans measurement. Only the package inserts of InnHep-SI reagent (Siemens) and STA-Apixaban calibrator (Stago Diagnostics) stated a possible interference of other anticoagulants on the assay, but no data on heparins were given. None of the evaluated methods included neutralizing heparin reagents.

Overall, our study confirmed that the presence of enoxaparin had pronounced effect on direct FXa inhibitors plasma levels, as shown in previous works.[13-15,28] Our data showed that the degree of levels over-estimation caused by heparin was different depending on the commercial anti-Xa assay and on enoxaparin concentration. The DiXaI-HY assay, reported to be not influenced by heparin, revealed a moderate sensitivity to enoxaparin for rivaroxaban and edoxaban detection, as also reported in a recent study regarding rivaroxaban.[30] However, this effect was smaller than that observed for the other evaluated assays (maximum over-estimation: 56.4% for DiXaI-HY vs 583.8% for BerHep-SI). For some assays, also prophylactic enoxaparin concentrations might determine a moderate over-estimation of the measured DOAC levels (183.8% in the sample spiked with enoxaparin 0.5 IU/mL). However, the extent of over-estimation was highly considerable especially for therapeutic or supratherapeutic enoxaparin concentrations (583.8% in the presence of 2.0 IU/mL). Moreover, our data showed variable assays performance for apixaban, edoxaban, and rivaroxaban dosage. Various factors might influence the analytical difference among methods: direct factor Xa inhibitors may have different sensitivity toward activated factor X, or the affinity may be altered by the ionic environment given by the buffer composition. Moreover, the initial dilution may not be the same among different methodologies and the low-range procedure requires a lower dilution of the sample.

Some limitations of the study should be pointed out. First, the present study is an in vitro experiment and thus should be confirmed ex vivo. We focused our investigation on enoxaparin since it is the most used LMWH in the hospital where the experiments were carried out; for that reason, our results should not be generalized to different heparins. Moreover, the effect of heparin neutralizers was not examined since they were not available in the laboratory when the study was carried out. Finally, we did not use the gold standard mass spectrometry to measure DOAC concentrations since its limited availability and long turnaround time make it an unpractical test for emergency situations.

Strengths of this study are the analysis of the effect of a wide range of enoxaparin concentrations on xaban levels and the use of

a large panel of commercially available anti-Xa assays. However, it should be pointed out that currently some of the evaluated assays have been modified and contain a heparin neutralizer in the buffer.

In conclusion, this study confirms the hypothesis that the presence of enoxaparin interferes with apixaban, edoxaban, or rivaroxaban measurement by dedicated chromogenic assays, causing falsely elevated xabans levels, the over-estimation being dependent on the anti-Xa assay and the enoxaparin concentration. The results of this study may have a practical relevance for managing potential problems due to concomitant use of xabans and enoxaparin. Though DOACs have been approved for fixed-dose administration, with no requirement for routine laboratory monitoring, in some emergency situations, the assessment of their anticoagulant levels is useful or even necessary. When DOAC measurement is required, it is important that the laboratory be aware of a concomitant or recent enoxaparin treatment to avoid results misinterpretation and erroneous clinical management decisions.

ACKNOWLEDGEMENTS

The authors would like to thank Dasit (for Hyphen BioMed reagents and CS-2100 instrument), Siemens Healthcare Diagnostics, Stago Diagnostics, Technoclone GmbH, and Werfen for supplying reagents needed for this study.

CONFLICT OF INTERESTS

None of the authors have any potential conflict of interest to report.

AUTHORS' CONTRIBUTION

LC, TS, TA, CB, and PG designed the study; LC and CM performed laboratory measurements and analyzed the data; CM wrote the paper; LC, TS, TA, CB, and PG revised the paper; all the authors revised and accepted the final version of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Cini M, Legnani C, Testa S, Tripodi A, Cosmi B, Palareti G. An in vitro study to investigate the interference of enoxaparin on plasma levels of direct oral factor Xa inhibitors measured by chromogenic assays. *Int J Lab Hematol*. 2019;41:309–315. https://doi.org/10.1111/ijlh.12974