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ORIGINAL ARTICLE

Assessment of low plasma concentrations of apixaban in the periprocedural setting

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Abstract

Introduction: Estimation of residual apixaban plasma concentrations may be requested in the management of emergencies. This study aims at assessing the performance of specific anti-Xa assays calibrated with apixaban on real-life samples with low apixaban plasma concentrations (<30 ng/mL) and on-treatment ranges, with and without interference of low-molecular-weight heparin (LMWH).

Methods: The performance of the STA®-Liquid Anti-Xa assay (STA® LAX) and the low and normal procedures of the Biophen®Direct Factor Xa Inhibitors (DiXaI) assay was tested on 134 blood samples, collected from patients on apixaban, wherefrom 74 patients received LMWH after apixaban cessation. The results were compared with the liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) measurements.

Results: The Biophen®DiXaI, Biophen®DiXaI LOW, and STA®LAX showed very good correlation with LC-MS/MS measurements in patients without LMWH administration (Spearman *r* .95, .99, and .98, respectively). Their limits of quantitation were defined at 48, 24, and 12 ng/mL, respectively. The Bland-Altman test measured mean bias (SD) at 5.6 (13.1), −2.5 (5.0), and −0.8 (6.1) ng/ml, respectively. The Spearman r of the Biophen®DiXaI decreased to 0.64 in presence of low apixaban concentrations. The Spearman *r* of the Biophen®DiXaI LOW and STA®LAX decreased to 0.39 and 0.26, respectively, in presence of LMWH.

Conclusions: The accuracy of the low methodologies (Biophen®DiXaI LOW and STA®LAX) is slightly improved for low apixaban plasma concentrations, compared with the normal procedure of Biophen®DiXaI. The interference of LMWH on the low methodologies is measurable, however, less important than the previously reported interference of LMWH on rivaroxaban calibrated specific anti-Xa assays.

KEYWORDS

anticoagulants, apixaban, drug monitoring, heparin, low-molecular-weight heparin, perioperative, periprocedural

1 | INTRODUCTION

Apixaban is the third direct oral anticoagulant (DOAC) that has been licensed worldwide to prevent or treat thromboembolic events. Its increasing use has led to manage more patients on apixaban for elective or urgent invasive procedures. Data provided by clinical trials and real-life registries showed that around 30% of patients treated with apixaban had an invasive procedure.[1,2] In the Dresden NOAC registry, the median duration of DOAC interruption before surgery was 2 days.[1] For such short DOAC interruption, the European Heart Rhythm Association does not recommend the use of low-molecular-weight heparin (LMWH) as a bridging therapy before an invasive procedure.[3] However, real-life registries still record longer DOAC perioperative interruption than required and the administration of LMWH at DOAC cessation.[4]

Recent studies on DOAC periprocedural management showed that 2 days of interruption may not be sufficient to achieve a plasma concentration <30 ng/mL, a plasma level proposed by some group of experts as a safe hemostatic threshold before high bleeding risk procedures.[5,6] Expert societies also proposed DOAC plasma concentrations >50 ng/mL to warrant administration of antidotes or efficient hemostatic treatments (ie, factor concentrates) in case of life-threatening bleeding.[7]

As routine coagulation assays, such as the activated partial thromboplastin time (aPTT) and the prothrombin time (PT), are poorly sensitive to apixaban plasma concentrations even within therapy ranges, it occurs regularly that residual effect of apixaban is estimated to guide therapeutic decisions (eg, before thrombolytic therapy). Specific coagulation assays can reliably estimate apixaban plasma concentrations within its therapeutic range.[8] Performances in low DOAC range may be improved by appropriate modifications of the testing protocol, but the clinical relevance of this gain in performances is still debated.[9]

Previous real-life studies have demonstrated the performance of coagulation assays sensitive to low (<30 ng/mL) plasma concentrations of rivaroxaban[10-12] and dabigatran.[13] Similar studies are not available for apixaban. The lack of specificity of chromogenic assays, using the same reagent as for heparin estimation, may lead to overestimation of rivaroxaban or heparin anti-Xa activity during the switch of anticoagulants.[14]

Samama et al developed an optimized chromogenic assay (Biophen®Direct Factor Xa Inhibitors (DiXaI) from Hyphen BioMed®, Neuville-Sur-Oise, France) to avoid this interference. In their experiments, the specific buffer used allowed the measurement of the sole anti-Xa activity from rivaroxaban even in the presence of heparins or fondaparinux.[15] The normal methodology offers a limit of quantitation (LOQ) of around 50 ng/mL of rivaroxaban. An adaptation of the procedure is proposed to allow estimation of lower anti-Xa agents plasma concentrations, but this has shown to be more sensitive to low residual heparin plasma concentrations as the sample is less diluted in the specific buffer. This interference was also shown on a limited number of patients treated by apixaban.[16]

The STA®-Liquid Anti-Xa assay has the advantage to offer an automatic redilution if the plasma concentration of the anti-Xa is over 230 ng/mL, which allows only one calibration for low and high therapy ranges.

The objective of this study was firstly to assess the analytical performances (limit of detection, limit of quantitation and accuracy) of the low and the high procedure of the Biophen®DiXaI assay and the STA®-Liquid Anti-Xa assay from Diagnostica Stago® (Diagnostica Stago®), using real-life samples from patients treated with apixaban. Secondly, we used samples from patients receiving LMWH after apixaban preoperative cessation, to assess heparin interference on the chromogenic assays performance (accuracy).

2 | MATERIALS AND METHODS

The study was performed in accordance with the Declaration of Helsinki and was approved by the Medical Ethics Committee of the CHU UCL Namur, Yvoir, Belgium (NUB: B039201524384). Written informed consent was obtained from each patient.

2.1 | Clinical samples

From November 2014 until May 2017, we collected 156 plasma samples from patients treated with apixaban at the CHU UCL Namur, Yvoir, Belgium.

The inclusion criteria were patients receiving apixaban for prevention of thromboembolic events in atrial fibrillation, or treatment and secondary prevention of venous thromboembolism. Collection of blood samples took place after a preanesthetic assessment (n = 25), just before an invasive procedure (n = 118), or during a hospitalization with recent administration of apixaban (n = 13).

The only exclusion criterion was the absence of result for one of the chromogenic assays. Therefore, 22 blood samples were excluded from the study, which was then realized on the remaining 134 blood samples collected.

Blood was taken by venipuncture in the antecubital vein using a 21-gauge needle (Greiner Bio-One™) or through a peripheral venous catheter (BD Insyte-W®, 18- or 16-gauge) and collected into 0.109 M sodium citrate (9:1v/v) tubes (Greiner Bio-One™).

Platelet-poor plasma (PPP) was obtained from the supernatant fraction after double centrifugation at 1500 g for 15 minutes at room temperature. Samples were then aliquoted and frozen immediately at −80°C. Plasma sample aliquots were thawed and heated to 37°C for at least 5 minutes before running the experiments.

We have measured the glomerular filtration rate (GFR) with the Cockcroft-Gault equation (C-G), using the actual body weight.

2.1.1 | The blood samples were divided into two groups depending on LMWH interference

Group A included blood samples from patients who received LMWH after interruption of apixaban within (n = 73) and outside (n = 1) a perioperative setting.

To assess solely the impact of residual LMWH concentrations on the chromogenic assays, we have excluded all samples with apixaban levels >2 ng/mL which correspond to the limit of detection of the liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

Group B included blood samples from patients who did not receive LMWH within (n = 23) and outside (n = 37) a perioperative setting.

2.2 | Chromogenic anti-Xa assays

We have used two coagulometers from Diagnostica Stago, the STA-R Evolution® coagulometer (until September 2016 included) and the STA-R MAX® (since October 2016) to carry the study.

The chromogenic assays were prepared according to manufacturers' recommendations and using manufacturers' apixaban calibrators: the Biophen®Direct Factor Xa Inhibitors with calibration for normal and low plasma concentrations of apixaban (Hyphen BioMed) and the STA®-Liquid Anti-Xa (Diagnostica Stago) (Table S1).

For anti-Xa activity of LMWH, we have used the Biophen®Heparin Liquid Reagent Technology (LRT) assay (Hyphen BioMed), calibrated with Biophen®Heparin Calibrators (Hyphen BioMed). The LOQ of the anti-Xa activity was 0.05 IU/mL, according to the manufacturer.

The normal pooled plasma (NPP) was prepared at the University of Namur (Namur, Belgium) in order to measure the limit of blank (LOB), the limit of detection (LOD) and of quantitation (LOQ). The LOB was calculated using the equation: LOB = $mean_{blank}$ + 1.645 (SD_{blank}). The mean and standard deviation (SD) were calculated from 20 consecutive runs of a sample of NPP containing no analyte, with the anti-Xa assays on the STA-R Max® analyzer. The LOD was calculated using the equation: LOD = LOB + 1.645 ($SD_{low\ concentration\ sample}$).[17] For the determination of the LOQ, we referred to the method proposed by the latest FDA Guidelines for Industry for Bioanalytical Method Validation.[18] Briefly, three different samples containing low levels of apixaban were measured in 20 consecutive runs. The nominal (theorical) concentrations of the standards were 48, 24, and 12 ng/mL. For each of these concentrations, the acceptance criteria were an accuracy of ±25% of the nominal value and a precision ≤25% of CV. If the test met these two validation criteria in at least one of the given three different standards, then the concentration of the lower standard was defined as the LOQ for the test.

For the LC-MS/MS (University of Namur), weighted (1/x) linear calibration was done using calibrators prepared by spiking blank plasma at 1, 3, 10, 50, 130, 250, and 400 ng/mL. Quality controls (QCs) were prepared by spiking blank plasma at 1, 3, 25, 250, and 400 ng/mL. Validation with the QCs on five different days showed a repeatability relative standard deviation (RSD) between 1.8% and 2.4%, an intermediate precision RSD between 1.8% and 5.5%, and a relative bias between −3.0% and 2.3% for the concentration in the interval of 1 to 400 ng/mL. The method was validated according to FDA Guidelines for Industry for Bioanalytical Method Validation.[18]

2.3 | Statistical analyses

We used GraphPad Prism® version 6.0c for MacOSx (GraphPad Software, www.graphpad.com) for statistical analyses. Results for the apixaban anti-Xa assays (Biophen®DiXaI, Biophen®DiXaI LOW and STA®LAX) and those obtained using LC-MS/MS were compared by Spearman's correlation, linear regression, and Bland-Altman analyses.

The Spearman correlation coefficients of group A were compared with those of group B. A *P*-value lower than .05 was considered as statistically significant. Bland-Altman plots were constructed by plotting the differences A-B against the averages (A + B)/2, where A was the result of the corresponding coagulation test and B was the result of the LC-MS/MS. Limits of agreement of the Bland-Altman analyses were calculated as the mean difference – or +1.96*standard deviation for the 5th and the 95th limit of agreement, respectively.

3 | RESULTS

Group A and Group B had different plasma concentrations ranges which are mainly due to the different timing of last apixaban intake. Table 1 shows the demographic and statistical characteristics of the apixaban plasma concentrations in the different groups. Group B has been divided in two other subgroups which enable the comparison of chromogenic assays' performance in a normal therapeutic range and two trough therapy ranges (<100 ng/mL and <50 ng/mL). All the patients had a GFR above 30 mL/min.

3.1 | Limit of blank, detection, and quantitation

The LOB was calculated at 26 ng/mL for Biophen®DiXaI, 11 ng/mL for Biophen®DiXaI LOW, and 2 ng/mL for STA®LAX.

The LOD was calculated at 46 ng/mL for Biophen®DiXaI, 14 ng/mL for Biophen®DiXaI LOW, and 4 ng/mL for STA®LAX.

The LOQ was measured at 48 ng/mL for Biophen®DiXaI, 24 ng/mL for Biophen®DiXaI LOW, and 12 ng/mL for STA®LAX (Table S2).

3.2 | Spearman's correlation and linear regression

For Group A, the Spearman coefficient and the linear regression showed only a weak/moderate correlation between chromogenic assays and LC-MS/MS measurements (Biophen®DiXaI LOW > STA®LAX > Biophen®DiXaI) (Table 1).

TABLE 1 Demographic and statistical characteristics of the study population on apixaban

	Biophen®DiXaI	Biophen®DiXaI LOW	STA®-Liquid Anti-Xa
Group A: Apixaban + LMWH			
Range of apixaban plasma concentration (ng/mL) (n = 74) median interquartile range	from 0 to 23.6 1.1 (0.4–3.2)		
Last administration (h) (n = 71) median (IQR)	85 (72-108)		
GFR†median (IQR) (mL/min) (n = 70)	74 (61-100)		
Bland-Altman mean bias (ng/mL)	9.7 [11%]	2.4 [−12%]	6.7 [59%]
SD (ng/mL)	15.3 [169%]	5.7 [155%]	9.8 [146%]
95% Limits of agreement (ng/mL)	-20.3 to 39.8 [−320 to 341%]	-8.8 to 13.6 [−316 to 293%]	-12.4 to 25.8 [−227 to 346%]
R square	0.04	0.54	0.26
Spearman r	0.22	0.39	0.26
95% CI	-0.02 to 0.43	0.17 to 0.57	0.03 to 0.47
Group B: Apixaban			
Range of apixaban plasma concentration (ng/mL)(n = 60) median interquartile range	from 0 to 382.9 41.20 (11.0 −142.8)		
Last administration median (IQR) (h) (n = 59)	18 (7-48)		
GFR† median (IQR) (mL /min) (n = 54)	75 (61-99)		
Bland-Altman mean bias (ng/mL)	10.6 [−0.47%]	-17.6 [−39%]	-8.2 [−20%]
SD (ng/mL)	18.4 [92%]	36.7 [67%]	12.4 [72%]
95% Limits of agreement (ng/mL)	-25.6 to 46.7 [−181 to 180%]	-89.4 to 54.3 [−170 to 93%]	-32.5 to 16.0 [−160 to 121%]
R square	0.96	0.90	0.98
Spearman r	0.95	0.99	0.98
95% CI	0.91 to 0.97	0.98 to 0.99	0.97 to 0.99
Group B: Apixaban <100 ng/mL			
Range of apixaban plasma concentration < 100 ng/mL (n = 40) median (ng/mL) interquartile range (ng/mL)	23 (4.6 - 50.2)		
Bland-Altman mean bias (ng/mL)	6.7 [−6%]	-2.1 [−45%]	-3.0 [−23%]
SD (ng/mL)	14.4 [113%]	5.5 [81%]	9.4 [88%]
95% Limits of agreement (ng/mL)	-21.5 to 34.9 [−228 to 215]	-13.0 to 8.7 [−204 to 113%]	-21.3 to 15.4 [−196 to 149%]
R square	0.80	0.96	0.89
Spearman r	0.83	0.96	0.94
95% CI	0.69 to 0.1	0.92 to 0.8	0.89 to 0.97
Group B: Apixaban			
Range of apixaban plasma concentration < 50 ng/mL (n = 31) median (ng/mL) interquartile range (ng/mL)	12.9 (2.4 - 27.6)		
Bland-Altman mean bias (ng/mL)	5.6 [−11%]	-2.5 [−59%]	-0.8 [−25%]

(Continues)

TABLE 1 (Continued)

	Biophen®DiXaI	Biophen®DiXaI LOW	STA®-Liquid Anti-Xa
SD (ng/mL)	13.1 [129%]	5.0 [88%]	6.1 [100%]
95% Limits of agreement (ng/mL)	-20.0 to 31.2 [−263 to 241%]	-12.4 to 7.4 [−232 to 114%]	-12.8 to 11.3 [221 to 171%]
R square	0.39	0.86	0.79
Spearman r	0.64	0.91	0.90
95% CI	0.37 to 0.82	0.82 to 0.96	0.79 to 0.95

Note: The number in square brackets represents the % difference (100x (A-B)/average) vs average.

For Group B, the Spearman coefficient and the linear regression had a strong correlation, with variation following high or low apixaban plasma concentration. The Biophen®DiXaI LOW and STA®LAX had the strongest correlation compared with Biophen®DiXaI in the subgroup with low trough plasma concentrations ($P < .05$ for Spearman *r* comparison) (Table 1).

Table 2 reports the accuracy of the chromogenic assays in the guidance of emergency management of patients on DOACs. Group B has a total of 27 plasma samples ≤30 ng/mL, as measured by LC-MS/MS, wherefrom apixaban plasma concentrations were measured >30 ng/mL in seven samples with Biophen®DiXaI (26%), one with Biophen®DiXaI LOW (4%), and two with STA®LAX (7%).

3.3 | Bland-Altman analyses

Despite potential presence of LMWH in the blood samples with very low apixaban plasma concentrations (<25 ng/mL) in Group A, the mean difference between the chromogenic assays and LC-MS/MS was less than 10 ng/mL (performance of Biophen®DiXaI LOW > STA®LAX > Biophen®DiXaI) (Figure 1 and Table 1).

In Group B (no presence of LMWH and higher apixaban plasma concentrations), the mean difference between the chromogenic assays and LC-MS/MS was less than 20 ng/mL with performance for STA®LAX (−8.2 ng/mL)> Biophen®DiXaI (10.6 ng/mL)> Biophen®DiXaI LOW (−17.6 ng/mL). When plasma concentrations were >100 ng/mL, the STA®LAX and especially the Biophen®DiXaI LOW tended to underestimate systematically the apixaban plasma concentration, while the Biophen®DiXaI overestimated it.

When apixaban plasma concentration were <100 ng/mL, the mean difference was less than 5 ng/mL for Biophen®DiXaI LOW and STA®LAX and less than 10 ng/mL for Biophen®DiXaI (Figure 2 and Table 1).

3.4 | Interference of residual LMWH with Biophen®DiXaI, Biophen®DiXaI LOW, and STA®LAX

Residual LMWH plasma concentration was measured in 70 patients, wherefrom 49 received therapeutic doses (1 mg/kg LMWH twice a day) with last administration at 24 (16-27) hours (median, IQR), 6 patients received half-therapeutic doses (1 mg/kg LMWH once a day) at 27 (14-32) hours, 14 patients received preventive doses at 21 (16-25) hours, and one patient for whom this information was not available. In samples collected minimum 24 hours or 12 hours since last LMWH administration of therapeutic and preventive doses, respectively, we have measured residual LMWH plasma concentrations from 0.03 to 0.48 IU/mL.

Figure 3 shows the interference of residual LMWH on the chromogenic assays calibrated with apixaban. It included only blood samples (n = 48) with apixaban plasma concentrations ≤2 ng/mL (measured with LC-MS/MS).

For Biophen®DiXaI, there was no interference with LMWH up to 0.65 IU/mL (LC-MS/MS measured 3.5 ng/mL and Biophen®DiXaI

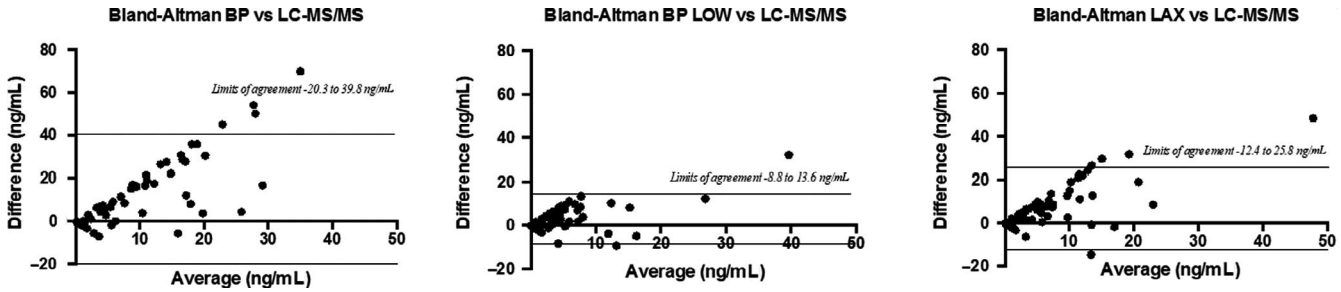

FIGURE 1 Bland-Altman of apixaban plasma concentrations measured by the chromogenic assays and LCMS/MS in group A. Bland-Altman plots were constructed by plotting the differences A-B against the means (A + B)/2, where A was the result of the corresponding coagulation test and B was the result of the LC-MS/MS. BP: Biophen®DiXaI; LAX: STA®LAX; BP LOW: Biophen®DiXaI LOW

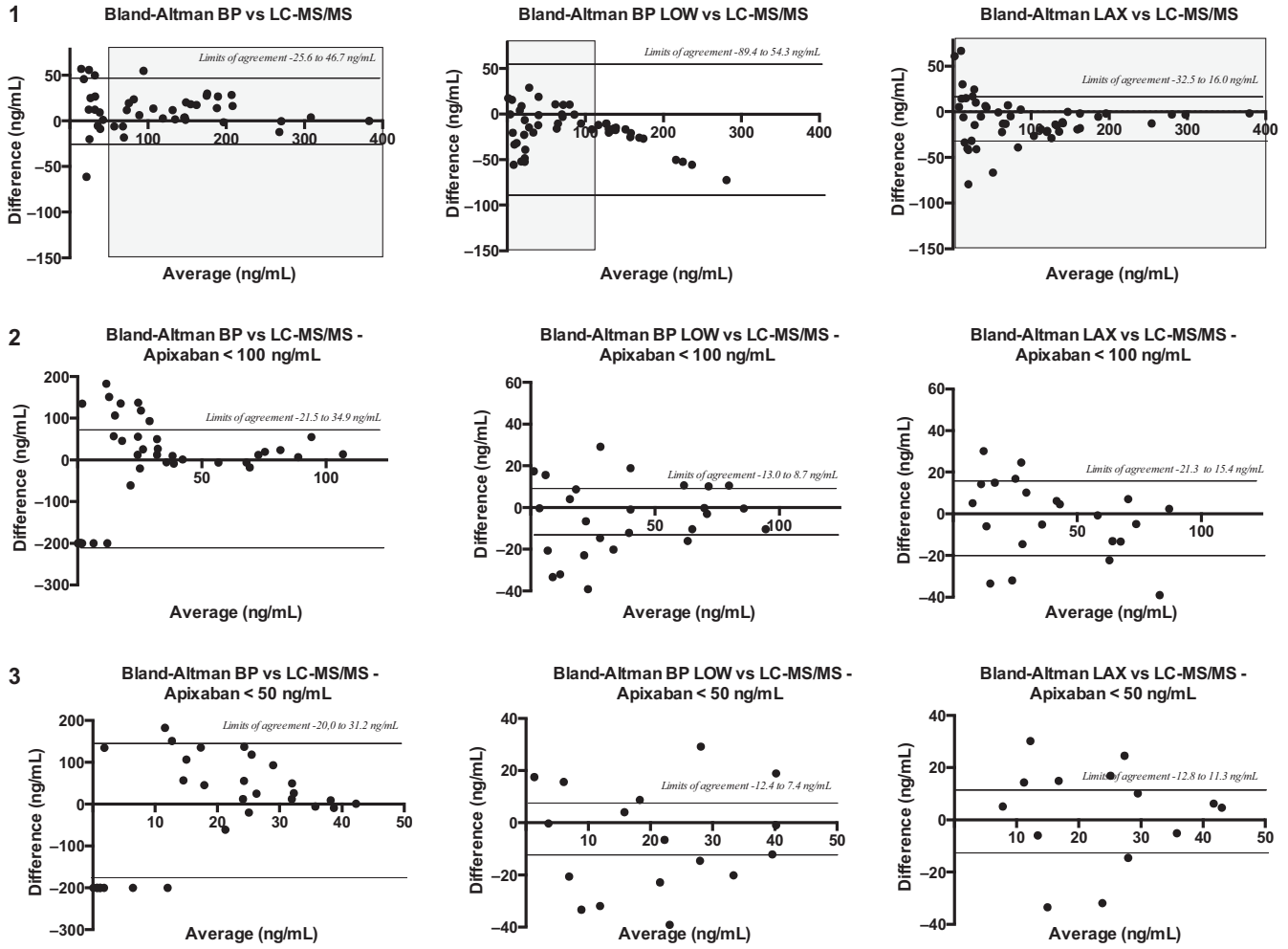

FIGURE 2 Bland-Altman of apixaban plasma concentrations measured by the chromogenic assays and LCMS/MS in group B. Group B (1) presents two subgroups with apixaban plasma concentration <100 ng/mL (2) and <50 ng/mL (3). The gray zones in group B (1) represent the plasma concentrations of the calibrators used for each methodology

0 ng/mL apixaban plasma concentration) which was the highest residual LMWH plasma concentration measured in the study samples. For Biophen®DiXaI LOW, the interference of residual LMWH was poor, increasing with higher LMWH concentrations. For STA®LAX, the interference with residual LMWH was greater, with a maximum of 37 ng/mL for a LMWH concentration of 0.65 IU/mL. We have compared each anti-Xa assay from group A with its counterpart from group B and have found statistically significant difference ($P < .05$) in their respective Spearman's coefficient.

4 | DISCUSSION

In the perioperative setting, the residual plasma concentrations of DOACs vary with the elective or urgent nature of the invasive procedure. The clinicians in charge of the patient may be confronted with high residual DOAC concentrations, especially in case of recent intake or deterioration in renal function. In elective invasive procedure, the DOAC plasma concentration will usually be less than 50 ng/mL when a DOAC cessation of 48-72 hours is requested.[6,19] Many publications have listed clinical and pharmacological causes that can increase DOAC's half-lives.[3,6,20] If it is admitted that systematic DOAC plasma concentration assessment is not required before an elective invasive procedure with adequate DOAC preoperative cessation and a stable clinical situation, this may be different for emergencies and clinical deterioration of the patients. In those situations, knowing the residual DOAC plasma concentration can help clinicians in the management of the patient (eg, bleeding patient or hemorrhagic vs ischemic stroke).

In this study, we have tested commonly used anti-Xa assays calibrated with apixaban. The inclusion of patients at distance or close to an invasive procedure enabled us to collect blood samples with higher (>100 ng/mL) apixaban concentrations and lower apixaban concentrations (<100 ng/mL and <50 ng/mL), representing a panel of plasma concentrations encountered in real-life situations such as before elective and urgent invasive procedures (Table 1).

The results of group B (no interference with LMWH) showed that for high therapeutic apixaban concentrations, the Biophen®DiXaI LOW tended to underestimate the plasma concentrations when measured >100 ng/mL with the gold standard method, the LC-MS/MS. Therefore, we suggest the use of Biophen®DiXaI and STA®LAX for

TABLE 2 Classification of apixaban plasma concentration estimation following recommended thresholds for emergency management

Group B			
Samples with apixaban levels ≤30 ng/mL - LC-MS/MS (n = 27)		**Samples with apixaban levels >30 and ≤50 ng/mL - LC-MS/MS (n = 4)**	
Apixaban >30 ng/mL BP	7/27 (26%)	Apixaban >50 ng/mL BP	0/0 (0%)
Apixaban >30 ng/mL BP LOW	1/27 (4%)	Apixaban >50 ng/mL BP LOW	0/0 (0%)
Apixaban >30 ng/mL LAX	2/27 (7%)	Apixaban >50 ng/mL LAX (	0/0 (0%)
Samples with apixaban levels >30 and ≤50 ng/mL - LC-MS/MS (n = 4)		**Samples with apixaban levels >50 and ≤100 ng/mL - LC-MS/MS (n = 10)**	
Apixaban ≤30 ng/mL BP	0/4 (0%)	Apixaban ≤50 ng/mL BP	0/10 (0%)
Apixaban ≤30 ng/mL BP LOW	0/4 (0%)	Apixaban ≤50 ng/mL BP LOW	0/10 (0%)
Apixaban ≤30 ng/mLLAX	1/4 (25%)	Apixaban ≤50 ng/mL LAX	1/10 (10%)

Note: Estimation of apixaban plasma concentration has been classified following the 30 ng/mL and 50 g/mL tresholds recommended by expert's societies to manage emergencies in patients on DOAC.

Abbreviations: BP LOW, Biophen®DiXaI LOW; BP, Biophen®DiXaI; LAX, STA®LAX.

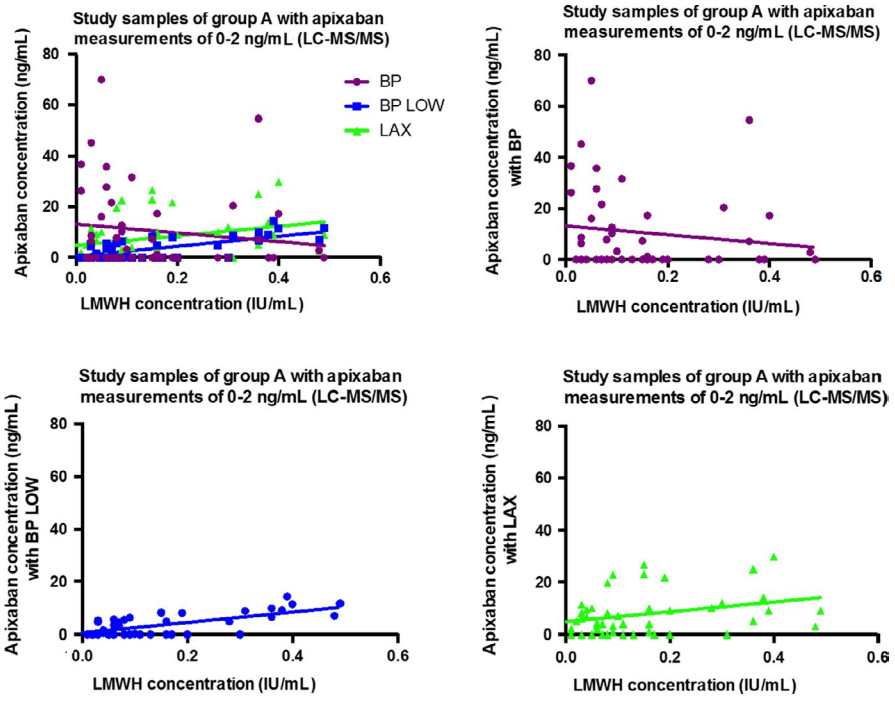

FIGURE 3 Interference of residual LMWH on the performance of the chromogenic assays calibrated for apixaban. Measurements of apixaban concentrations with the specific chromogenic anti-Xa assays in study samples containing max 2 ng/mL of apixaban measured with LC-MS/MS. The Figure 3 illustrates the specificity of the Biophen®DiXaI (BP) to apixaban and its imprecision in very low apixaban concentrations (LOD = 46 ng/mL)

the measurement of apixaban plasma concentrations in patients with recent oral intake (<12 hours), such as in urgent situations (Figure 2).

In the subgroup analysis containing apixaban plasma concentrations <100 ng/mL and <50 ng/mL, the Bland-Altman analyses showed that the Biophen®DiXaI LOW and STA®LAX were more accurate than Biophen®DiXaI (Figure 2). Due to its automatic redilution of the blood samples when apixaban concentration is >230 ng/mL, the anti-Xa assay STA®LAX is accurate in low and high therapeutic concentrations, making it an assay of choice in urgent situations. However, the Biophen®DiXaI with a LOB, LOD, and LOQ of 26, 46, and 48 ng/mL, respectively, estimated falsely apixaban concentrations >30 ng/mL in 26% (7/27) of samples containing apixaban concentrations ≤30 ng/mL. Therefore, this assay should be used cautiously if clinical decision based on residual plasma concentration around 30 ng/mL should be given.[5] Indeed, a considerable number of patients included in group B (7/60), if requiring non-life-threatening urgent procedure carrying a high bleeding risk, might be wrongly delayed if the Biophen®DiXaI was used. Results with the Biophen®DiXaI LOW are more reassuring, that is, only 1 sample out of 27 (4%) samples with apixaban plasma concentration <30 ng/mL was wrongly classified as having more than 30 ng/mL, which is quite acceptable in everyday practice. This supports the interest of having two sets of calibrators for this test even if these results have to be considered in light of the small number of samples analyzed. In case of life-threatening bleeding that cannot be managed with

adequate supportive care, the threshold of DOAC plasma concentration over 50 ng/mL is suggested to warrant antidote administration.[7] The performance of the three chromogenic assays seems to be adequate for such higher threshold. Now, there is still a lack of evidence whether these thresholds are clinically valid to manage patients undergoing high bleeding risk procedures or with life-threatening bleeding.

This imprecision for very low apixaban concentration is illustrated by the poor Spearman correlation (0.22) of Biophen®DiXaI in group A (interference with LMWH). Despite its specificity to apixaban, all the plasma concentrations of apixaban were all <30 ng/mL (median (IQR) 1.1 [0.4 −3.2] ng/mL) (Table 1). Regarding the poor Spearman correlations of Biophen®DiXaI LOW (0.39) and STA®LAX (0.26), it translates the interference of both assays with residual LMWH concentrations. This was similar to our previous results with perioperative measurements of rivaroxaban.[12] Nevertheless, the interference of LMWH on both assays was smaller than for rivaroxaban.[14] Residual LMWH up to 0.25 IU/ml can be found after 24 h of therapeutic LMWH last administration.[12,21] The use of Biophen®DiXaI LOW with such low residual LMWH concentrations could be feasible as long as the residual apixaban plasma concentration is assessed minimum 24 hours after last LMWH therapeutic administration.

For the STA®LAX, the interference with small residual LMWH concentrations is such that it prevents its use in clinical situations where low plasma concentrations are expected or required.

This study has several limitations. Firstly, the range of plasma concentrations between the group A and B is different. Therefore, the subgroup analyses of group B enabled us to have a closer range of apixaban plasma concentrations to group A. Also, this study has analyzed only three anti-Xa assays, preventing extrapolation of the results to other anti-Xa assays calibrated with apixaban.

The recent study of Cini et al, using spiked NPP, has demonstrated that the Biophen®DiXaI calibrated with apixaban was insensitive to increasing plasma concentration of enoxaparin (up to 2.0 IU/mL). This was not the case for samples with rivaroxaban and edoxaban, where increased enoxaparin presence led to false elevation of DOAC measurements. These false elevations were however smaller compared with other anti-Xa assays. For the STA®LAX, false elevation of apixaban was found with small enoxaparin presence (up to 22.9% for enoxaparin concentration = 0.25 IU/mL). Our results on real-life samples confirm the findings of this in vitro study.

Recently, several anti-Xa assays were investigated for measurement of apixaban and rivaroxaban levels in patients enrolled in the START-Register (Cini et al). Six chromogenic methods showed good concordances with the quantitative high-performance liquid chromatography with ultraviolet detection, especially for apixaban concentration <100 ng/mL (bias − 21.3-4.1 ng/mL).[22]

However, there is still a need for evidence regarding accuracy of the measure of anti-Xa agents in patients on DOACs bridged with LMWH.[23]

This is the first perioperative study with real-life samples containing both apixaban and LMWH. Despite such blood samples will be less encountered in the future due to the non-necessity to bridge patients with LMWH when a perioperative DOAC cessation is planned (except for patients at high thrombotic risk),[24] not all clinicians respect current perioperative DOAC recommendations.[4] Furthermore, patients may be switched to other anticoagulants and develop during the switch complications that can require assessment of the anticoagulation status. Therefore, studies evaluating the interference of different anticoagulants on the coagulation assays may guide the laboratories in using the right tests. Note that the interference of apixaban on anti-Xa assays calibrated with LMWH is also problematic and may be greater than LMWH's interference on apixaban measurements depending on the chromogenic kit used. In the present study, Biophen®LRT (anti-Xa calibrated with LMWH) showed excellent correlation (>0.99) with apixaban LC-MS/MS measurement's (data not shown). Such correlation has already been reported with other chromogenic assays.[25,26] However, a previous report showed that residual levels of apixaban in the 30-50 ng/mL range will not provide anti-Xa levels above 0.1 IU/mL with all chromogenic kits. Therefore, in light of the different sensitivities of the heparin calibrated methods to a similar apixaban level, the general guidance that all methodologies may be used to rule out the presence of these drugs is misleading. In this case, and in order to provide more reliable results, the interference of apixaban may be removed by the use of the DOAC-STOP or the DP-Filter.[27,28]

There is actually no clear evidence that the use of low methodologies improves the perioperative management of the patients.[8] The choice of the anti-Xa assay and its methodology should depend on the question the clinician needs to answer: is it important to exclude DOAC plasma concentration <30 ng/mL (where low methodologies are more accurate) or to manage a patient with severe bleeding requiring potentially specific/ nonspecific reversal therapy (warranted with DOAC plasma concentration >50 ng/mL)? The Biophen®DiXaI LOW shows accurate results for apixaban plasma concentrations up to 100 ng/mL, while the STA®LAX has the advantage to be accurate in low and normal therapy ranges avoiding the use of two different calibrations and dilutions. However, in case of concomitant LMWH presence, the Biophen®DiXaI methodologies should be preferred, with a dosage if possible, at distance from the last LMWH administration (12-24 hours).

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CONFLICT OF INTEREST

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REFERENCES

1. Beyer-Westendorf J, Gelbricht V, Forster K, et al. Peri-interventional management of novel oral anticoagulants in daily care: results from the prospective Dresden NOAC registry. *Eur Heart J*. 2014;35(28):1888-1896.
2. Garcia D, Alexander JH, Wallentin L, et al. Management and clinical outcomes in patients treated with apixaban vs warfarin undergoing procedures. *Blood*. 2014;124(25):3692-3698.
3. Steffel J, Verhamme P, Potpara TS, et al. The 2018 European Heart Rhythm Association Practical Guide on the use of non-vitamin K antagonist oral anticoagulants in patients with atrial fibrillation. *Eur Heart J*. 2018;39(16):1330-1393.
4. Flaker GC, Theriot P, Binder LG, Dobesh PP, Cuker A, Doherty JU. Management of periprocedural anticoagulation: a survey of contemporary practice. *J Am Coll Cardiol*. 2016;68(2):217-226.
5. Albaladejo P, Bonhomme F, Blais N, et al. Management of direct oral anticoagulants in patients undergoing elective surgeries and invasive procedures: Updated guidelines from the French Working Group on Perioperative Hemostasis (GIHP) - September 2015. *Anaesth Crit Care Pain Med*. 2017;36(1):73-76.
6. Godier A, Dincq AS, Martin AC, et al. Predictors of pre-procedural concentrations of direct oral anticoagulants: a prospective multicentre study. *Eur Heart J*. 2017;38(31):2431-2439.
7. Levy JH, Ageno W, Chan NC, et al. When and how to use antidotes for the reversal of direct oral anticoagulants: guidance from the SSC of the ISTH. *J Thromb Haemost*. 2016;14(3):623-627.
8. Gouin-Thibault I, Freyburger G, de Maistre E, et al. Evaluation of dabigatran, rivaroxaban and apixaban target-specific assays in a multicenter French study. *Thromb Res*. 2017;158:126-133.
9. Gosselin RC, Adcock DM, Bates SM, et al. International Council for Standardization in Haematology (ICSH) Recommendations for Laboratory Measurement of Direct Oral Anticoagulants. *Thromb Haemost*. 2018;118(3):437-450.
10. Mani H, Rohde G, Stratmann G, et al. Accurate determination of rivaroxaban levels requires different calibrator sets but not addition of antithrombin. *Thromb Haemost*. 2012;108(1):191-198.
11. Konigsbrugge O, Quehenberger P, Belik S, et al. Anti-coagulation assessment with prothrombin time and anti-Xa assays in real-world patients on treatment with rivaroxaban. *Ann Hematol*. 2015;94(9):1463-1471.
12. Lessire S, Douxfils J, Pochet L, et al. Estimation of rivaroxaban plasma concentrations in the perioperative setting in patients with or without heparin bridging. *Clin Appl Thromb Hemost*. 2018;24(1):129-138.
13. Douxfils J, Lessire S, Dincq AS, et al. Estimation of dabigatran plasma concentrations in the perioperative setting. *Thromb Haemost*. 2015;113(04):862-869.
14. Wong WH, Kaur H, Tan CW, Lee LH, Ng HJ. Cross-interference of rivaroxaban and enoxaparin on Berichrom anti-Xa heparin and Biophen direct Xa inhibitor assays. *Int J Lab Hematol*. 2018;40(4):e63-e65.
15. Samama MM, Amiral J, Guinet C, Perzborn E, Depasse F. An optimised, rapid chromogenic assay, specific for measuring direct factor Xa inhibitors (rivaroxaban) in plasma. *Thromb Haemost*. 2010;104(5):1078-1079.
16. Eller T, Flieder T, Fox V, et al. Direct oral anticoagulants and heparins: laboratory values and pitfalls in 'bridging therapy'. *Eur J Cardiothorac Surg*. 2017;51(4):624-632.
17. Armbruster DA, Pry T. Limit of blank, limit of detection and limit of quantitation. *Clin Biochem Rev*. 2008;29(suppl 1):S49-52.
18. Food and Drug Administration. Bioanalytical method validation guidance for industry. 2018; https://www.fda.gov/regulatory-information/search-fda-guidance-documents/bioanalytical-method-validation-guidance-industry. Accessed 27 Februar 2020.
19. Douketis JD, Wang G, Chan N, et al. Effect of standardized perioperative dabigatran interruption on the residual anticoagulation effect at the time of surgery or procedure. *J Thromb Haemost*. 2016;14(1):89-97.
20. Dubois V, Dincq AS, Douxfils J, et al. Perioperative management of patients on direct oral anticoagulants. *Thromb J*. 2017;15:14.
21. Eijgenraam P, Ten Cate H, Henskens Y, van den Ham R, Ten Cate-Hoek A. Effects of peri-operative bridging with low molecular weight heparins on coagulation during interruption of vitamin K antagonists: a mechanistic study. *Thromb Res*. 2016;140:59-65.
22. Cini M, Legnani C, Padrini R, et al. DOAC plasma levels measured by chromogenic anti-Xa assays and HPLC-UV in apixaban- and rivaroxaban-treated patients from the START-Register. *Int J Lab Hematol*. 2020;42(2):214-222.
23. Gosselin RC, Adcock DM, Douxfils J. An update on laboratory assessment for direct oral anticoagulants (DOACs). *Int J Lab Hematol*. 2019;41(suppl 1):33-39.
24. Garwood CL, Korkis B, Grande D, Hanni C, Morin A, Moser LR. Anticoagulation bridge therapy in patients with atrial fibrillation: recent updates providing a rebalance of risk and benefit. *Pharmacotherapy*. 2017;37(6):712-724.
25. Billoir P, Barbay V, Joly LM, Fresel M, Chretien MH, Le Cam DV. Anti-Xa Oral Anticoagulant Plasma Concentration Assay in Real Life: Rivaroxaban and Apixaban Quantification in Emergency With LMWH Calibrator. *Ann Pharmacother*. 2019;53(4):341-347.
26. Margetic S, Celap I, Delic Brkljacic D, et al. Chromogenic anti-FXa assay calibrated with low molecular weight heparin in patients treated with rivaroxaban and apixaban: possibilities and limitations. *Biochem Med (Zagreb)*. 2020;30(1):010702.
27. Favresse J, Lardinois B, Sabor L, et al. Evaluation of the DOAC-Stop(R) Procedure to Overcome the Effect of DOACs on Several Thrombophilia Screening Tests. *TH Open*. 2018;2(2):e202-e209.
28. Bouvy C, Evrard J, Siriez R, Mullier F, Douxfils J, Gheldof D. *P220: Removal of DOACs from plasma: performance comparison and pre-analytical considerations of three different devices*. Marseille, France: European Congress on Thrombosis and Haemostasis; 2018.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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