Heparin Anti-Xa Activity, a Readily Available Unique Test to Quantify Apixaban, Rivaroxaban, Fondaparinux, and Danaparoid Levels

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BACKGROUND: Despite their usefulness in perioperative and acute care settings, factor-Xa inhibitor-specific assays are scarcely available, contrary to heparin anti-Xa assay. We assessed whether the heparin anti-Xa assay can (1) be used as a screening test to rule out apixaban, rivaroxaban, fondaparinux, and danaparoid levels that contraindicate invasive procedures according to current guidelines (>30 ng·mL^{-1}, >30 ng·mL^{-1}, >0.1 µg·mL^{-1}, and >0.1 IU·mL^{-1}, respectively), (2) quantify the anticoagulant level if found significant, that is, if it exceeded the abovementioned threshold.

METHODS: In the derivation cohort then in the validation cohort, via receiver operating characteristics (ROC) curve analysis, we evaluated the ability of heparin anti-Xa assay to detect levels of factor-Xa inhibitors above or below the abovementioned safety thresholds recommended for an invasive procedure (screening test). Among samples with relevant levels of factor-Xa inhibitor, we determined the conversion factor linking the measured level and heparin anti-Xa activity in a derivation cohort. In a validation cohort, the estimated level of each factor-Xa inhibitor was thus inferred from heparin anti-Xa activity. The agreement between measured and estimated levels of factor-Xa inhibitors was assessed.

RESULTS: Among 989 (355 patients) and 756 blood samples (420 patients) in the derivation and validation cohort, there was a strong linear relationship between heparin anti-Xa activities and factor-Xa inhibitors measured level (r = 0.99 [95% confidence interval {CI}, 0.99–0.99]). In the derivation cohort, heparin anti-Xa activity ≤0.2, ≤0.3, <0.1, <0.1 IU·mL^{-1} reliably ruled out a relevant level of apixaban, rivaroxaban, fondaparinux, and danaparoid, respectively (area under the ROC curve ≥0.99). In the validation cohort, these cutoffs yielded excellent classification accuracy (≥96%). If this screening test indicated relevant level of factor-Xa inhibitor, estimated and measured levels closely agreed (Lin's correlation coefficient close to its maximal value: 95% CI, 0.99–0.99). More than 96% of the estimated levels fell into the predefined range of acceptability (ie, 80%–120% of the measured level).

CONCLUSIONS: A unique simple test already widely used to assay heparin was also useful for quantifying these 4 other anticoagulants. Both clinical and economic impacts of these findings should be assessed in a specific study. (Anesth Analg 2021;132:707–16)

KEY POINTS

- **Question:** How reliable is the test assaying heparin to assay apixaban, rivaroxaban, fondaparinux, and danaparoid levels?
- **Findings:** Heparin anti-Xa activity (1) reliably detected apixaban, rivaroxaban, fondaparinux, and danaparoid anticoagulant intensity above the recommended thresholds allowing an invasive procedure to be performed in safe conditions and (2) accurately quantified this anticoagulant intensity.
- **Meaning:** A wide-scale readily available unique assay was also useful to assay other factor-Xa inhibitors.

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GLOSSARY

aPTT = activated partial thromboplastin time; **AUC_{ROC}** = area under the ROC curve; **CCC** = concordance correlation coefficient; **CI** = confidence interval; **CTAD** = citrate, theophylline, adenosine, and dipyridamole; **DOAC** = direct oral anticoagulant; **FXa** = activated factor X; **IRB** = institutional review board; **LC-MS/MS** = liquid chromatography-mass spectrometry/mass spectrometry; **LMWH** = low molecular weight heparin; **NPV** = negative predictive value; **PPV** = positive predictive value; **ROC** = receiver operating characteristics; **SD** = standard deviation; **Se** = sensitivity; **Sp** = specificity; **UFH** = unfractionated heparin

Direct oral anticoagulants (DOACs) apixaban and rivaroxaban do not require routine measurements of their anticoagulant intensity.[1] However, this may not apply to several situations often encountered in the perioperative and the acute care settings.[2–4] Indeed, when an invasive procedure is considered, DOAC level may indicate its optimal timing. If the procedure is urgent, DOAC level helps at determining whether or not an antidote should be administered. DOAC level is crucial in the decision-making process during active bleeding, during cerebral stroke or in case of persistent/recurrent thrombosis.[5–9] Patients with extreme body weight and/or renal impairment exposed to drug interactions and/or with uncertain compliance with medication may also benefit from the quantification of DOAC level.[10,11] Similar issues are encountered with parenteral factor-Xa inhibitors fondaparinux and danaparoid.[12] Thus, assays enabling measurement of the anticoagulant intensity (ie, the anti-Xa activity) of these anticoagulants should be available on a 24/7 basis in all hospitals providing surgical and/or acute care.[10,13–15]

Routine screening coagulation assays such as activated partial thromboplastin time and prothrombin time are neither suitable for quantifying factor-Xa inhibitors nor reliable to rule out any residual anticoagulant effect.[6,12,16] Liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) serves as reference method[1] but is not suitable as routine or urgent test since this technique is cumbersome, requires expertise, and is not widely available.[4] Reliable assays have been therefore developed to measure the anticoagulant intensity of factor-Xa inhibitors, via a chromogenic kit specifically calibrated for each anticoagulant.[1,14,17] Providing specific testing for apixaban, rivaroxaban, fondaparinux, and danaparoid separately implies the necessity to have at least 4 methodologies implemented on the analyzer and 4 non–out-of-date different sets of calibrators and controls. The inherent cost is therefore hardly affordable for many laboratories. Specific assays are therefore unavailable in many institutions, only intended for research purposes or, at best, rarely available on a 24/7 basis.[14] Importantly, in the perioperative setting or in life-threatening situations such as ongoing bleeding, ignoring the anticoagulant intensity may be harmful to the patient.[10]

The principle of quantification of the anticoagulant effect, that is, the chromogenic anti-Xa method, is similar for all factor-Xa inhibitors, including unfractionated heparin (UFH) and low molecular weight heparin (LMWH). Briefly, the assay relies on the inhibition, by heparin or other anti-Xa anticoagulant, of a constant amount and in excess of activated factor X (FXa). The residual FXa hydrolyzes a specific chromogenic substrate releasing a colored product (paranitroaniline) which amount could be quantified via its light absorbance (at 405 nm). This amount is inversely proportional to the anticoagulant concentration. From one factor-Xa inhibitor to another, assays only differ by the drug-specific calibrator. Since a UFH/LMWH hybrid calibrator is far more accessible than the abovementioned specific assays,[18] adopting it for all factor-Xa inhibitors would be tremendously appealing.

We aimed at assessing whether the widely used heparin anti-Xa assay can (1) rule out apixaban, rivaroxaban, fondaparinux, and danaparoid levels that contraindicate invasive procedures according to the thresholds proposed by current guidelines (>30 $ng \cdot mL^{-1}$, >30 $ng \cdot mL^{-1}$, >0.1 $\mu g \cdot mL^{-1}$, and >0.1 $IU \cdot mL^{-1}$, respectively),[5,19] (2) quantify the anticoagulant level if found significant, that is, if it exceeded the abovementioned threshold.

METHODS

In this bicentric study among hospitalized adult patients, we retrospectively collected all measurements of apixaban, rivaroxaban, fondaparinux, and danaparoid anti-Xa activities performed between March 2015 and January 2018 in Laënnec hospital laboratory (derivation cohort, center no. 1) and Hôtel-Dieu hospital laboratory (validation cohort, center no. 2), the 2 laboratories of the University Hospital of Nantes, France. There was no exclusion criterion.

The study was approved by the appropriate Institutional Review Board (IRB: Groupe Nantais d'Ethique dans le Domaine de la Santé, 17-01-19). The requirement for written informed consent was waived by the IRB (no blood sample has been taken for research purposes and no clinical data were collected).

Measurement of Anti-Xa Activity

Blood samples, in citrate, theophylline, adenosine, and dipyridamole (CTAD) collection tubes underwent centrifugation (within <4 hours from the time of collection) to obtain platelet-free plasma. A chromogenic anti-Xa assay was performed on each plasma according to the manufacturer guidelines including 60 µL of prediluted (1/3) patient's plasma, 100 µL of substrate, and 100 µL of reagent which includes bovine Xa (Biophen Heparin LRT, Hyphen Biomed, Neuville-sur-Oise, France). This kit is widely used because of its ease of use, its stability after opening (14 days at 18°C–25°C), and because it allows—using the same calibration curve—measurements of UFH and LMWH. In addition, the reagent does not contain exogenous antithrombin and this is preferable for rivaroxaban and apixaban measurements.[1] The amount of para-nitroaniline per factor-Xa–specific chromogenic substrate was measured by absorbance at 405 nm and expressed in milliabsorbance per minute ($mAbs \cdot min^{-1}$).

Anti-Xa activities of apixaban, rivaroxaban, fondaparinux, or danaparoid were inferred from a calibration curve which is specific to the tested anticoagulant. This measured anti-Xa activity was expressed in $ng \cdot mL^{-1}$ for apixaban and rivaroxaban, according to the British Committee for Standards in Haematology,[2] in $\mu g \cdot mL^{-1}$ for fondaparinux, and $IU \cdot mL^{-1}$ for danaparoid. The calibrators were Biophen Apixaban, Biophen Rivaroxaban, Biophen Arixtra, and Biophen Orgaran (Hyphen Biomed), respectively. For anti-Xa activities above the upper limit of quantification (250 $ng \cdot mL^{-1}$ for apixaban and rivaroxaban, 1.60 $\mu g \cdot mL^{-1}$ for fondaparinux, 1.75 $IU \cdot mL^{-1}$ for danaparoid), serial manual dilutions in buffer of patients' specimens were performed as needed to obtain anti-Xa activities within the reportable range.[20]

For each sample, heparin anti-Xa activity was similarly inferred from the $mAbs \cdot min^{-1}$ value using UFH/LMWH calibration curve (Biophen heparin calibrator) and expressed in $IU \cdot mL^{-1}$.

To overcome any impact of the use of a single lot of reagent or calibrator, several lots were used. Similarly, different automates were used: ACL TOP 700 and ACL TOP 500 coagulation analyzer (Werfen, Barcelona, Spain) in center no. 1 (derivation cohort) and 2 other ACL TOP 700 (Werfen) in center no. 2 (validation cohort). To ensure the quality of testing, all measurements were performed by adequately trained staff. The 2 centers performed multiple internal quality controls and external quality assessments according to the international standard ISO15189.

Statistical Methods

Performance of Heparin Anti-Xa Activity as a Quick Screening Test to Rule Out Relevant Anticoagulant Intensities of Factor-Xa Inhibitors. In the derivation cohort then in the validation cohort, via receiver operating characteristics (ROC) curve analysis, we evaluated the ability of heparin anti-Xa activity cutoffs to detect levels of factor-Xa inhibitors above or below the relevant safety thresholds recommended for an invasive procedure at high risk of bleeding: 30 $ng \cdot mL^{-1}$ for apixaban and rivaroxaban, 0.1 $\mu g \cdot mL^{-1}$ for fondaparinux, and 0.1 $IU \cdot mL^{-1}$ for danaparoid.[5,19] For each anticoagulant, we calculated the classification accuracy as the number of correct predictions made divided by the number of predictions made (expressed as %). To retain a cutoff, we favored sensitivity over specificity, since we favored a reliable detection of significant anticoagulant intensities. For apixaban and rivaroxaban, the recently proposed cutoff of 50 $\mu g \cdot mL^{-1}$ to allow thrombolysis during ischemic stroke was also tested.[9] To appropriately estimate confidence intervals (CIs) and adjust for within-patient correlation, we used the bootstrap technique. For each anticoagulant, the dataset of concentration levels was used to create a large set (1000 for each cohort): patient identifiers were sampled, with replacement, and all the data from the patient selected were used. This operation was repeated. Therefore, each bootstrap sample has the original number of patient identifiers, but some represented more than once and others not at all.

Quantification of the Anticoagulant Intensity of Factor-Xa Inhibitors From Heparin Anti-Xa Activity, if Found Significant by the Screening Test. In the derivation cohort, we determined the individual conversion factor linking apixaban, rivaroxaban, fondaparinux, or danaparoid measured level, on the one hand, and heparin anti-Xa activity, on the other hand:

$$\text{measured anticoagulant level} = \text{conversion factor} \times \text{heparin anti-Xa activity}$$

The individual conversion factor was determined in samples with significant concentration (≥30 $ng \cdot mL^{-1}$ for apixaban and rivaroxaban, ≥0.1 $\mu g \cdot mL^{-1}$ for fondaparinux, and ≥0.1 $IU \cdot mL^{-1}$ for danaparoid), that is, in patients identified with the abovementioned screening test. For each pair (measured anticoagulant level and heparin anti-Xa activity), the individual conversion factor was the ratio between measured factor-Xa inhibitor level and heparin anti-Xa activity.

$$\text{Example: Apixaban conversion factor}_{\text{pair1}} = \left[\text{apixaban}\right]_{\text{pair1}} / \text{heparin anti-Xa activity}_{\text{pair1}}$$

Then, we calculated, for each anticoagulant, the mean conversion factor (subsequently referred to as "conversion factor") as the mean of individual

conversion factors. A linear and strong relationship between the 2 parameters (measured anticoagulant level and heparin anti-Xa activity) was a mandatory prerequisite.

In the validation cohort, for each tested anticoagulant, we used the conversion factor we determined for this anticoagulant in the derivation cohort to infer the estimated level of anticoagulant from heparin anti-Xa activity:

$$\text{estimated anticoagulant level} = \text{conversion factor for this anticoagulant} \times \text{heparin anti} - \text{Xa activity}$$

The agreement between measured and estimated levels of each factor-Xa inhibitor was assessed with the Lin's concordance correlation coefficient. Because our dataset included repeated measures in the same patients, adjustment of this coefficient for the within-subject correlation was made by bootstrapping (sampling patients identifiers with replacement, each bootstrap sample having the original number of patient identifiers, but some represented more than once and others not at all). In addition, as proposed earlier, their interchangeability was deemed acceptable if the estimated level fell within the 80%–120% range of the measured one.[17,21,22] These boundaries take into account both analytical capabilities of the assay and the clinical relevance of the bias between measurements by the 2 methods. Outliers were counted.

A $P < .05$ was considered significant. All statistical analyses were performed on MedCalc Software bv (Ostend, Belgium).

Sample Size Justification. We did not perform a priori sample size calculation. We included all the eligible patients within the study period which started from the beginning of the use of the tested reagent in our institution. Of note, our sample size was sufficient to report, for each anticoagulant in the derivation and validation cohorts, a narrow 95% CI for the area under the ROC curves (AUC_{ROC}), the performance gauge of the screening test: 0.997–1.

RESULTS

In the derivation cohort, 989 blood samples from 362 patients were tested in center no. 1: 145 for apixaban (97 patients), 157 for rivaroxaban (138 patients), 72 for fondaparinux (57 patients), and 615 for danaparoid (70 patients), covering a wide range of values. There

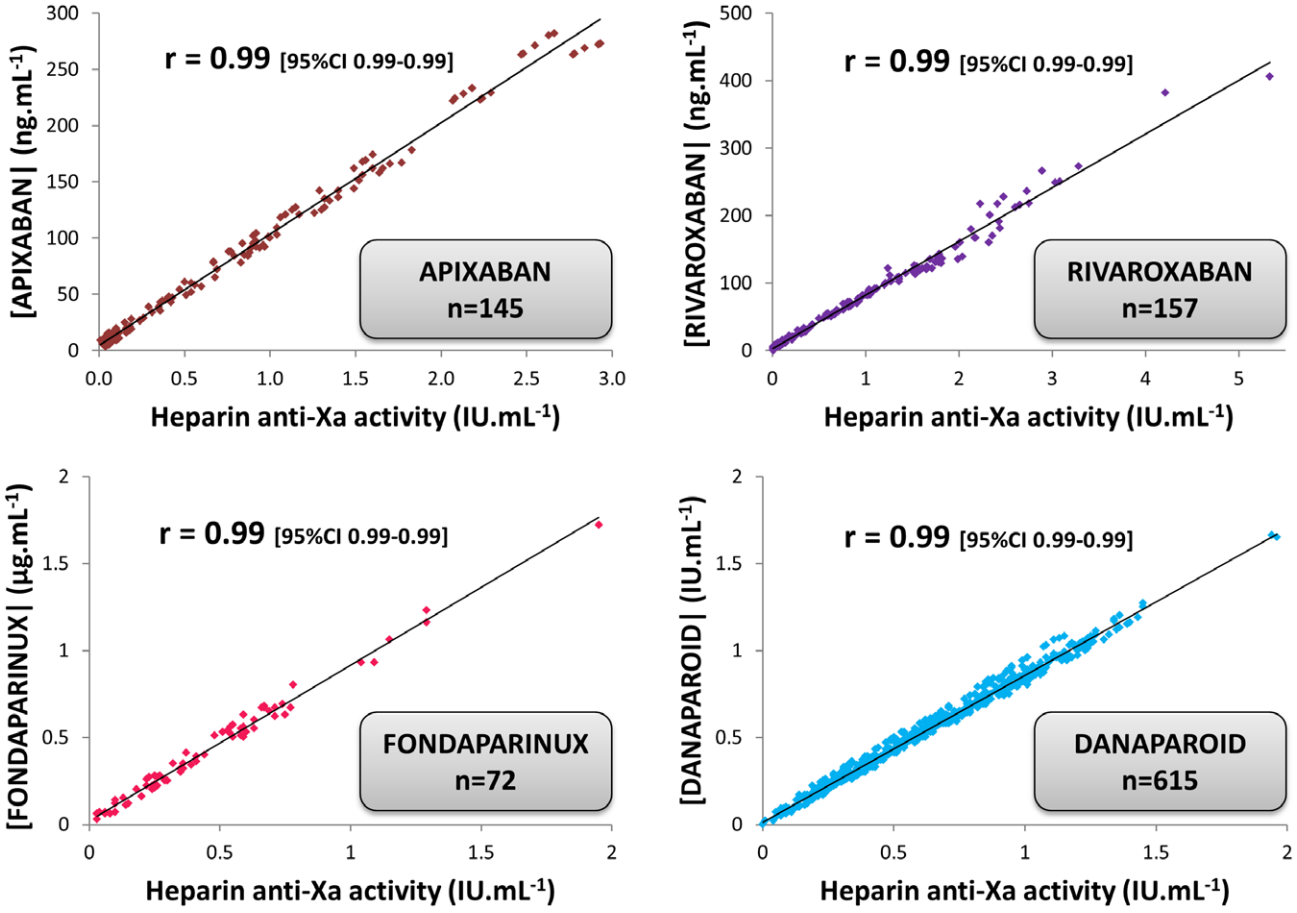

Figure 1. Derivation cohort: relationship between measured level of anticoagulants and heparin anti-Xa activity. *r*: Pearson coefficient. CIs were refined by bootstrapping. CI indicates confidence interval.

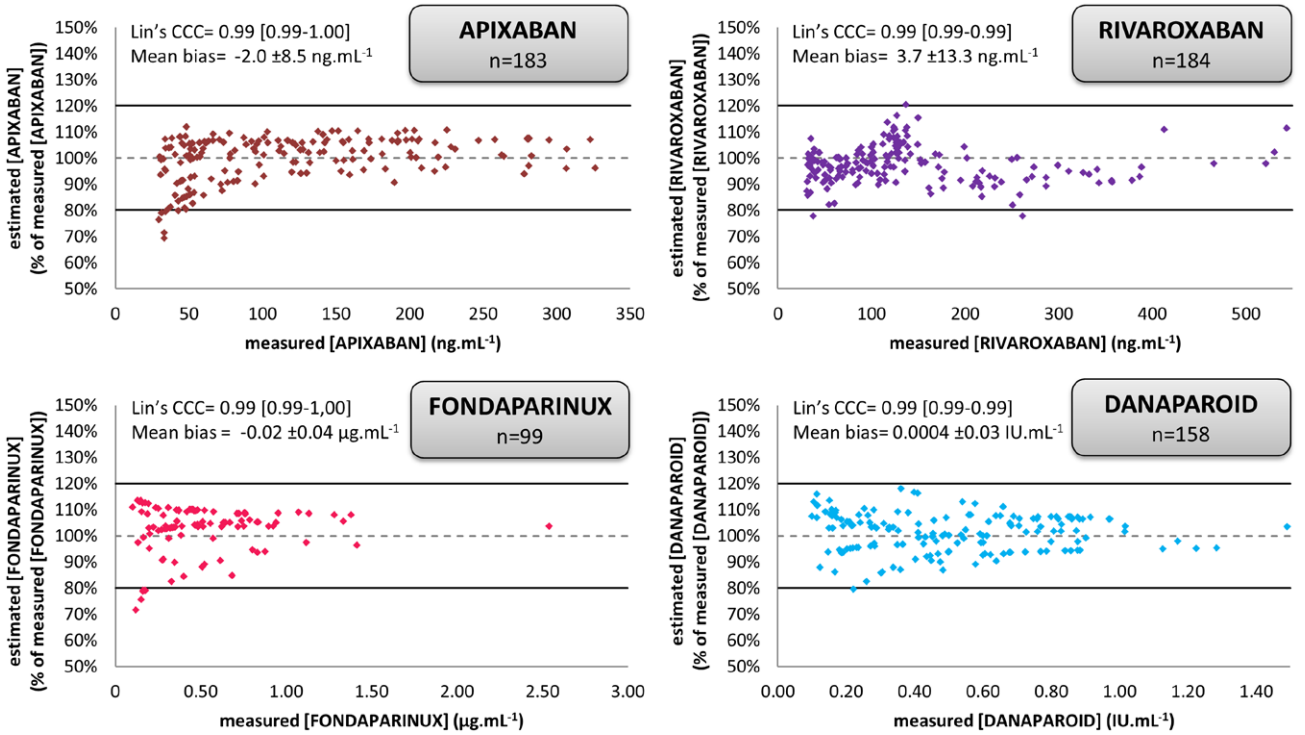

Figure 2. Validation cohort: agreement between the estimated and the measured level for each tested anticoagulant, in patients in whom the screening test is positive. CIs were refined by bootstrapping. The estimated level was calculated by simply multiplying heparin anti-Xa activity and the conversion factor (which is specific to each anticoagulant, see Figure 4). The solid lines delineate the range of acceptability (80%–120%). Values below the lower limit of quantification of anticoagulant level measurements (ie, <30 ng·mL^{-1} for apixaban and rivaroxaban, <0.1 µg·mL^{-1} for fondaparinux, and <0.1 IU·mL^{-1} for danaparoid) were excluded from this analysis. Indeed, as illustrated in Figure 4, determining the level of anticoagulant from heparin anti-Xa activity is the second step, the first step being the detection of a relevant concentration via the use of the cutoffs provided in the Table ("screening test"). CCC indicates concordance correlation coefficient; CI, confidence interval.

was a strong linear relationship between heparin anti-Xa activity and apixaban, rivaroxaban, fondaparinux, or danaparoid measured levels, respectively (all r = 0.99 [95% CI, 0.99–0.99]; Figure 1).

The validation cohort included 756 blood samples (from 420 patients) tested in center no. 2: 251 for apixaban (from 136 patients), 215 for rivaroxaban (146 patients), 106 for fondaparinux (79 patients), and 184 for danaparoid (59 patients), also covering a wide range of values (Figure 2). A strong linear relationship between heparin anti-Xa activity and factor-Xa inhibitors measured level was also found (all r = 0.99 [95% CI, 0.99–0.99]).

None of the analyzed samples came from a patient with contemporary exposure (within 24 hours) to both nonheparin factor-Xa inhibitor and heparin.

Performance of Heparin Anti-Xa Activity as a Quick Screening Test to Rule Out Relevant Anticoagulant Intensities of Factor-Xa Inhibitors

In the derivation cohort, the detection, by heparin anti-Xa activity, of a measured level <30 ng·mL^{-1} and <50 ng·mL^{-1} of apixaban and rivaroxaban,[5,9,19,23] <0.1 µg·mL^{-1} of fondaparinux, and <0.1 IU·mL^{-1} of danaparoid was excellent: the respective AUC_{ROC}s were almost perfect, from 0.997 to 1 (Table; Figure 3). The cutoffs for heparin anti-Xa activity we determined in the derivation cohort were then successfully validated in the validation cohort since they were associated with an excellent classification of samples with measured level of factor-Xa inhibitors below and above the relevant thresholds (classification accuracy from 96% to 99%; Table; Figure 3). For instance, a heparin anti-Xa activity below the cutoff of 0.2 IU·mL^{-1} allowed ruling out a significant level of apixaban (excellent negative predictive value of 1 [95% CI, 0.99–1], no false-negative case) and would have therefore permitted an invasive procedure to be performed in safe conditions. To detect an apixaban level above 30 ng·mL^{-1}, the positive predictive value was good (0.96 [0.92–0.99]). Cutoffs for other anticoagulants are provided in the Table.

Quantification of the Anticoagulant Intensity of Factor-Xa Inhibitors From Heparin Anti-Xa Activity, if Found Significant by the Screening Test

In the derivation cohort, the conversion factors linking heparin anti-Xa activity and the level of each factor-Xa inhibitor were 104, 82, 0.97, and 0.87 for apixaban, rivaroxaban, fondaparinux, and danaparoid, respectively.

Table. Performance of Heparin Anti-Xa Assay as a Quick Screening Test to Rule Out a Clinically Relevant Level of Factor-Xa Inhibitors

		Derivation Cohort				Validation Cohort						
	Threshold	Samples/ Patients	Samples With Concentration Under the Threshold	AUC_{ROC}	Cutoff ($IU \cdot mL^{-1}$)	Samples/ Patients	Samples With Concentration Under the Threshold	AUC_{ROC}	Se	Sp	PPV	NPV	Classification Accuracy
Apixaban	30 $ng \cdot mL^{-1}$	145/97	35	1 (0.99–1)	≥0.2	251/136	68	1 (0.99–1)	1 (0.99–1)	0.90 (0.85–0.97)	0.96 (0.92–0.99)	1 (0.99–1)	96% (95–99)
	50 $ng \cdot mL^{-1}$		48	1 (0.99–1)	≥0.4		108	1 (0.99–1)	1 (0.99–1)	0.91 (0.91–0.92)	0.93 (0.90–0.97)	1 (0.99–1)	96% (94–98)
Rivaroxaban	30 $ng \cdot mL^{-1}$	157/138	55	1 (0.99–1)	≥0.3	215/146	31	1 (0.99–1)	1 (0.97–1)	0.94 (0.87–0.99)	0.99 (0.96–1)	1 (0.99–1)	98% (97–100)
	50 $ng \cdot mL^{-1}$		65	1 (0.99–1)	≥0.55		62	1 (0.99–1)	0.99 (0.99–1)	0.95 (0.87–0.97)	0.98 (0.94–0.99)	0.98 (0.99–1)	98% (96–99)
Fondaparinux	0.1 $\mu g \cdot mL^{-1}$	72/57	8	1 (0.99–1)	≥0.1	106/79	7	1 (0.99–1)	0.99 (0.99–1)	1 (0.67–1)	1 (0.99–1)	0.88 (0.91–0.93)	99% (99–100)
Danaparoid	0.1 $IU \cdot mL^{-1}$	615/70	19	1 (0.99–1)	≥0.1	184/59	26	1 (0.99–1)	1 (0.99–1)	0.93 (0.92–1)	0.99 (0.99–1)	1 (0.99–1)	99% (99–100)

Confidence intervals were determined after bootstrapping for their accurate determination and adjusting for within-patient correlation. Cutoff refers to the cutoff proposed for heparins anti-Xa activity to rule out a significant concentration of anticoagulant (<30 $ng \cdot mL^{-1}$ for apixaban and rivaroxaban, <0.1 $\mu g \cdot mL^{-1}$ for fondaparinux, and <0.1 $IU \cdot mL^{-1}$ for danaparoid). A positive test is the detection of a significant anticoagulant concentration. Hence, a false-negative case is a patient in whom the anticoagulant concentration was erroneously considered to be irrelevant, therefore exposing him to hemorrhagic complications if an invasive procedure would have been performed. Of note, the rate of false-negative cases was remarkably low (from 0% to 1%). False-positive cases may be seen as less problematic since they expose patients to unnecessary antidote administration (if an invasive procedure is urgent) or to postponement of the invasive procedure.

Abbreviations: AUC_{ROC}, area under the receiver operating characteristics curve; NPV, negative predictive value; PPV, positive predictive value; Se, sensitivity; Sp, specificity.

When applying these anticoagulant-specific conversion factors to the validation cohort, there was a strong relationship for all the tested factor-Xa inhibitors between the estimated and the measured levels (all $r = 0.99$ [95% CI, 0.99–0.99]). Indeed, the mean bias ± standard deviation (SD) was of −2.0 ± 8.5 $ng \cdot mL^{-1}$, 3.7 ± 13.3 $ng \cdot mL^{-1}$, −0.02 ± 0.04 $\mu g \cdot mL^{-1}$, and 0.0004 ± 0.03 $IU \cdot mL^{-1}$ for apixaban, rivaroxaban, fondaparinux, and danaparoid, respectively. Lin's concordance correlation coefficient was close to the maximal value of 1, the lower boundary of its 95% CI being of 0.99 (Figure 2). Furthermore, 97%, 98%, 96%, and 99% of apixaban, rivaroxaban, fondaparinux, and danaparoid estimated levels fell into the predefined range of acceptability (ie, within the 80%–120% range of the corresponding measured level; Figure 2).

In the validation cohort, 15 apixaban, 25 rivaroxaban, 1 fondaparinux, and no danaparoid samples had to undergo dilution because their level was above the upper limit of quantification (250 $ng \cdot mL^{-1}$ for apixaban and rivaroxaban, 1.60 $\mu g \cdot mL^{-1}$ for fondaparinux, and 1.75 $IU \cdot mL^{-1}$ for danaparoid). Among these samples having undergone dilution, all the estimated levels fell into the range of acceptability.

DISCUSSION

The main findings of this study are (1) the highly reliable detection, by heparin anti-Xa activity, of apixaban, rivaroxaban, fondaparinux, and danaparoid anticoagulant intensity above the recommended thresholds allowing an invasive procedure to be performed in safe conditions; and (2) the accurate quantification of the anticoagulant intensity. The estimated level of factor-Xa inhibitors was simply calculated: it is the product of heparin anti-Xa activity and the conversion factor we determined. Hence, a simple test already widely used to assay heparin was also useful to assay these factor-Xa inhibitors.

To the best of our knowledge, the present study is the first addressing the relationship between heparin and fondaparinux or danaparoid anti-Xa activities. It is noteworthy that, for fondaparinux, the conversion factor between heparin anti-Xa activity and fondaparinux-specific test was almost 1. In other words, fondaparinux anti-Xa activity is already displayed by the widely used heparin anti-Xa activity assay. This questions the utility of a more expensive and less widespread fondaparinux-specific assay.

For the other anticoagulants, a few previous studies reported, as we did, a linear relationship between chromogenic antifactor-Xa assays calibrated with either UFH or LMWH on the one hand and, on the other hand, rivaroxaban or apixaban anticoagulant intensity (determined using specific chromogenic assays[18,24–27] or by LC-MS/MS[20,28–30]). In fewer of these studies, attempts were made to rule out relevant

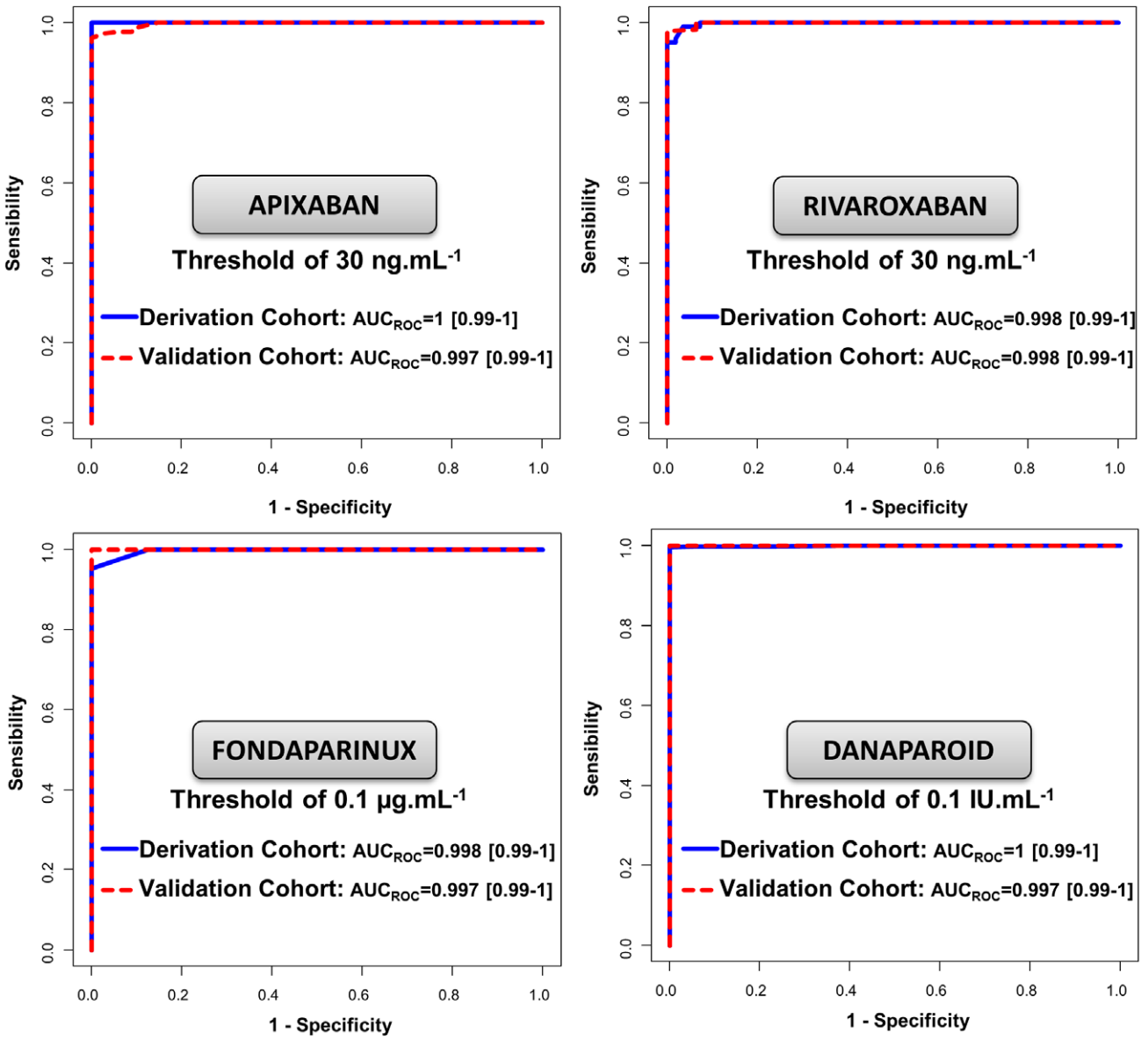

Figure 3. Performance of heparin anti-Xa activity as a quick screening test to detect or to rule out a clinically relevant level of anticoagulant, in the derivation cohort (solid line) and the validation cohort (broken line). AUC_{ROC} indicates area under the receiver operating characteristics curve.

anticoagulant intensities from the level of heparin anti-Xa activity, with varying success.[18,26–28] These previous studies paved the way for the present study since this is the first study demonstrating that heparin anti-Xa activity could be used not only to rule out relevant anticoagulant intensity of apixaban, rivaroxaban, fondaparinux, or danaparoid (our so-called screening test) but also to reliably quantify this intensity (Table; Figure 4). Especially in the perioperative and acute care settings, quantifying the anticoagulant intensity is often of utmost importance, to decide whether the invasive procedure should be postponed by a few hours or a few days. Quantification also guides the antidote dosage in the event of an urgent invasive procedure or ongoing bleeding.[14]

Our findings are in line with Maier et al[18] study, both studies using the same assay. For the detection of critical levels of apixaban (121 samples) and rivaroxaban (120 samples)—the 2 anticoagulants tested—Maier et al[18] reported that heparin anti-Xa activity <0.16 IU·mL^{-1} (0.2 in our population) and <0.21 IU·mL^{-1} (0.3 in our population) reliably ruled out a drug level above 30 ng·mL^{-1} for apixaban and rivaroxaban, respectively. In 37 samples, Billoir et al[27] also reported reliable discrimination but found different thresholds, as expected owing to the use of a different assay.

Besides the abovementioned novelty of the present study, other major strengths are its large size (1745 tested samples from 823 patients), the evaluation of

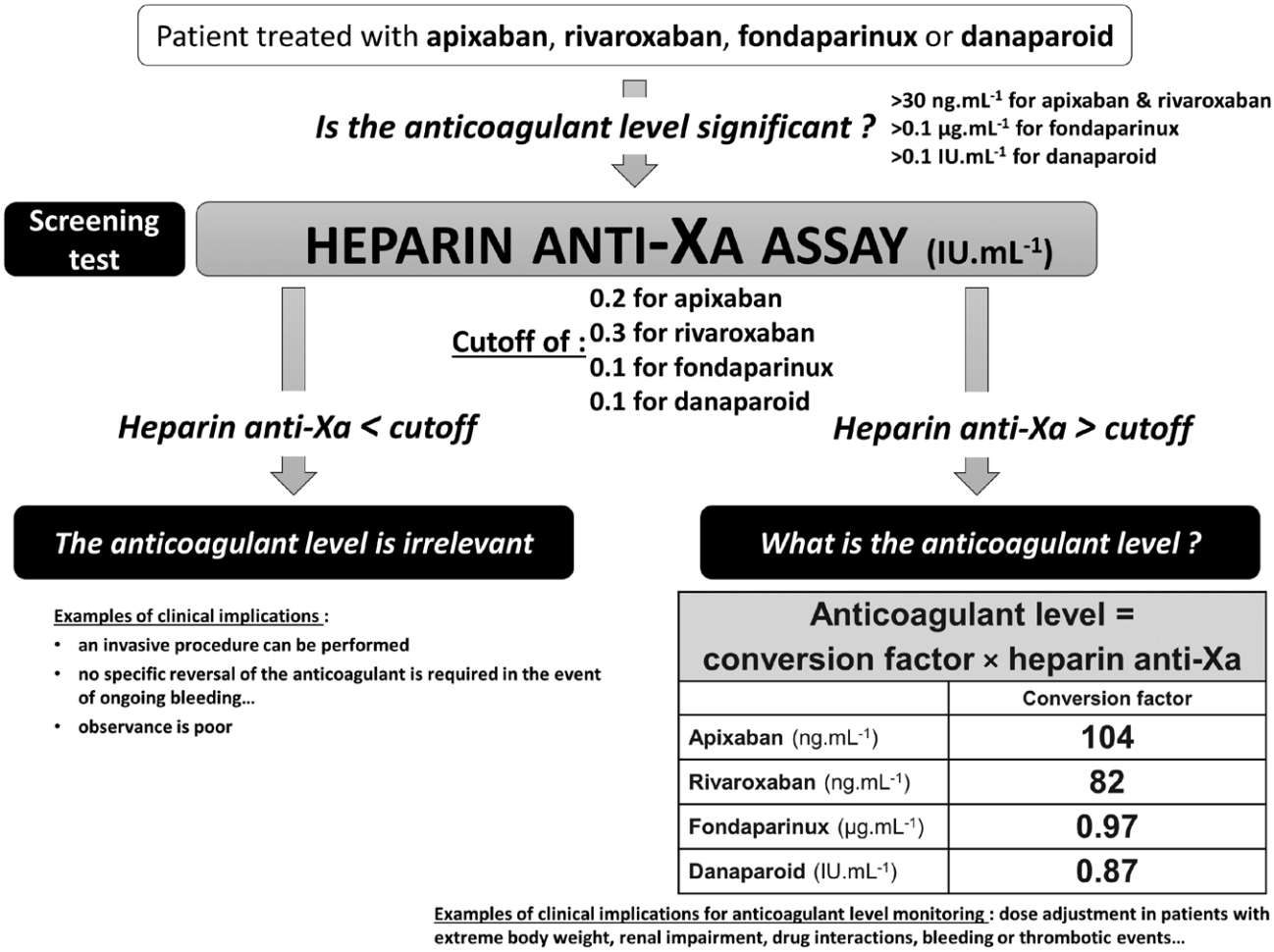

Figure 4. Clinical algorithm helping for the decision making, on the basis of heparin anti-Xa activity, when caring for a patient treated with apixaban, rivaroxaban, fondaparinux, or danaparoid. The conversion factor is provided, for each anticoagulant, allowing inferring anticoagulant estimated level from heparin anti-Xa activity. These conversions factors apply if the screening test indicated that the level of anticoagulant is significant, that is, if heparin anti-Xa activity is above the cutoffs herein provided. Example of a patient treated with apixaban. His heparin anti-Xa = 0.45. In this patient, estimated [apixaban] = 0.45 × 104 = 47 ng·mL^{-1}.

4 anticoagulants, the analysis of samples from adult patients actually treated with the tested anticoagulant rather than drug-enriched plasmas as used in some reports,[29,31] and the use of several lots of coagulometers, reagents, and calibrators. In addition, it is the sole study which adopted the rigorous design of a derivation and a validation cohort. Furthermore, for the "screening test," we determined the optimal cutoff of heparin anti-Xa activity ruling out a residual relevant anticoagulant intensity (Table) rather than applying a predetermined cutoff which would expose to a non-negligible rate of false-positive cases: 14% and 10%, for apixaban and rivaroxaban, respectively, with the cutoff of 0.10 IU·mL^{-1} in our study[24,28,31] and up to 50% in a previous one.[26] Such false positivity exposes to undue postponement of an invasive procedure or unnecessary administration of an expansive antidote. In addition, we tested a wide range of heparin anti-Xa activities including high levels by means of preliminary dilution, whereas some authors preferred to exclude those high levels from their analysis.[18,27] However, such high levels are usual (Figure 1), even in the absence of factor-Xa inhibitor overdose. Indeed, during treatment, peak levels of apixaban and rivaroxaban are 132 (59–302) and 270 (189–419) ng·mL^{-1}, respectively,[4] whereas the upper limit of quantification is 250 ng·mL^{-1}, making preliminary dilution, as we did, desirable.

Study Limitations

First, we did not test all reagents, all calibrators, and all coagulometers. The sensitivity of commercially available chromogenic anti-Xa assays may substantially differ depending on the manufacturer, the methodology, and the calibrator.[27,28,31]

Second, we did not perform a priori study power calculation. We collected all measurements of anti-Xa activities for each drug over a 34-month period. Hence, our findings are less robust for fondaparinux: in the validation cohort, there were only 7 patients below the threshold of 0.1 µg·mL^{-1}. Therefore, one

misclassified patient downplayed the negative predictive value (0.88). In this patient, heparin anti-Xa activity was 0.09 IU·mL^{-1}, while the fondaparinux-specific test indicated a slightly higher anti-Xa activity of 0.12 µg·mL^{-1}. Of note, the uncertainty related to the method itself (8%) is high for a value of 0.12 µg·mL^{-1}, that is, close to the limit of quantification. Importantly, even if the quasi-perfect AUC_{ROC} (0.997) indicated excellent discrimination for fondaparinux, we could not choose a lower cutoff for heparin anti-Xa activity since 0.1 IU·mL^{-1} is its limit of quantification.

Third, we did not use LC-MS/MS, the gold standard method, for measuring DOACs concentration.[1] However, as proposed by the International Council of Standardization in Haematology, we used chromogenic kits specifically calibrated for each factor-Xa inhibitor for which measurements highly correlate with LC-MS/MS measurements.[17]

Fourth, this study lacks clinical data about the included patients. Of note, all eligible patients of our regional university hospital were included over the study period and there was no exclusion criterion. Yet, a selection bias can theoretically not be excluded and whether our findings apply to all case mixes remains unproven.

Finally, this study has assessed neither the clinical nor the economic impact of the use of the heparin assay as an indicator of apixaban, rivaroxaban, fondaparinux, and danaparoid levels.

Implications

The already widely used, simple, and fast test assaying low molecular weight and UFH was also reliable and suitable to simply assay apixaban, rivaroxaban, fondaparinux, and danaparoid. If confirmed, these findings could have major clinical implications in the field of perioperative or acute care medicine and even beyond. Indeed, quantifying anticoagulant intensity is the cornerstone of the decision making in several clinical situations.[2,5,6,23] The need for specific tests may dramatically increase over the coming years[13] and most laboratories could not afford the monitoring of all anticoagulants and if so, scarcely on a 24/7 basis. At our institution as at others,[18] the turnaround time for heparin anti-Xa activity is less than half an hour, 24/7. It could be easily shortened by the reduction of the centrifugation time[32] or the use of end-centrifugation brake.[33]

Owing to the cost of drug-specific assays, of antidotes unnecessarily used during patient management blindly to the anticoagulant intensity, of the potential complications related to a blind/delayed approach with regard to anticoagulant intensity, we believe that using heparin anti-Xa assay as an indicator of factor-Xa inhibitors levels could be cost-effective, even if this remains to be proven in a specific study. Cost-savings may be particularly possible in institutions already using the heparin anti-Xa assay instead of activated partial thromboplastin time (aPTT) to monitor the anticoagulant intensity of heparins. Indeed, in our 2 centers taken together, 10,000–15,000 heparin anti-Xa assays are performed each year for a unit cost of nearly 2$ (including the cost of quality controls). We can roughly estimate that, in a laboratory performing only 2000 heparin anti-Xa assays (also on a 24 hours a day basis), this cost could approximately 5$.

Future studies should assess the clinical impact and the cost-effectiveness of the use of heparin assay as an indicator of factor-Xa inhibitors levels, in various settings (perioperative setting, emergency room, outpatients, etc). Clinical outcomes could be, inter alia, blood transfusion requirements, hemorrhagic and thrombotic events, length of stay (in the emergency room pending surgery, for instance), appropriateness of antidotes use, mortality. The present study should be seen as a mandatory prerequisite before the conduction of such future studies.

DISCLOSURES

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