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Regular Article

Assessment of the impact of rivaroxaban on coagulation assays: Laboratory recommendations for the monitoring of rivaroxaban and review of the literature

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ABSTRACT

Introduction: Rivaroxaban does not require monitoring nor frequent dose adjustment. However, searching for the optimal dose in the individual patient may be useful in some situations.
Aim: To determine which coagulation assay could be used to assess the impact of rivaroxaban on haemostasis and provide guidelines for the interpretation of routine lab tests.
Materials: Rivaroxaban was spiked at concentrations ranging from 11 to 1090 ng/mL in plateletpoor plasma. A large panel of coagulation assays was tested.
Results: A concentration dependent prolongation of aPTT, PT, dPT, PiCT was observed. PT and dPT were the most sensitive chronometric assays but results varied depending on the reagent (Triniclot PT Excel S > Recombiplastin 2 G > Neoplastin R > Neoplastin CI + > Triniclot PT Excel > Triniclot PT HTF > Innovin). FXa chromogenic assays showed the highest sensitivity. In TGA, Cmax was the most sensitive parameter with the tissue factor induced pathway. Rivaroxaban interferes on haemostasis diagnostic tests such the measurement of clotting factors, fibrinogen, antithrombin, proteins C and S, activated protein-C resistance and Xa-based chomogenic assays.
Conclusions: PT may be used as screening test to assess the risk of bleedings. A more specific and sensitive assay such as Biophen DiXaI using calibrators should be used to confirm the concentration of rivaroxaban. We also propose cut-off associated with a bleeding or thrombosis risk based on pharmacokinetic studies. Standardization of the time between the last intake of rivaroxaban and the sampling is mandatory.

Introduction

Rivaroxaban (Xarelto®) is a direct, antithrombin independent and orally active FXa inhibitor that inhibits not only free FXa but also the prothrombinase complex and clot bound FXa [1]. It is approved by the European Medicine Agency (EMA) [2] and the Food and Drug Administration (FDA) [3] for the prevention of thromboembolism in total hip replacement (THR) or total knee replacement (TKR) and to prevent stroke in patients with non-valvular atrial fibrillation (AF). The treatment of acute deep-vein thrombosis (DVT) is an additional indication approved by the EMA [4]. It was also evaluated for secondary prevention after acute coronary syndrome [5] and for thromboprophylaxis in acutely ill medical patients [6]. Rivaroxaban was found statistically superior to enoxaparin (versus both European and North American regimen) in prevention of venous thromboembolism (VTE) and equivalent in term of bleedings in the orthopaedic indications [7]. In patients with non-valvular AF, rivaroxaban was non-inferior to warfarin for the prevention of stroke or systemic embolism and showed a similar rate of major bleeding [8]. The net clinical benefit of rivaroxaban, and other NOACs, versus warfarin in patients with high risk of bleeding and stroke, suggests a wider use of these compounds in the near future [9].

The absolute bioavailability of rivaroxaban is high (80%– 100%). In patients undergoing total hip replacement receiving Xarelto® 10 mg *qd*, median C_{max} reaches 125 μg/mL (5th – 95th percentile: 91 – 196 μg/mL) and median C_{trough} was 9 μg/mL (5th – 95th percentile: 1 – 38 μg/mL) [10]. At the dose of 20 mg *od* in stroke prevention in a simulated population of patients with non-valvular atrial fibrillation, rivaroxaban has a C_{max} of approximately 290 μg/L (5th – 95th percentile ≈ 177 – 409 ng/mL) and a C_{trough} of approximately 32 μg/L (5th – 95th percentile ≈ 5 – 155 ng/mL) [11]. Similar drug levels were found in patients receiving rivaroxaban 20 mg *od* for the treatment of DVT [11].

Thanks to its predictable pharmacokinetic and pharmacodynamic profiles, monitoring is generally not recommended [12]. However,

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clinical surveillance is recommended throughout the treatment period in several subgroups of patients [13]. Thus, in patients with severe renal impairment (creatinine clearance<30 mL/min) rivaroxaban plasma levels may be significantly increased that may lead to a moderate increased bleeding risk [14]. The use of rivaroxaban is not recommended in patients with creatinine clearance<15 mL/min and is to be used with "caution" in patients with creatinine clearance between 15 to 29 mL/min. In addition, Xarelto® is a substrate of P-gp transporter and is partially metabolized by CYP3A4 resulting in some clinically relevant drug interactions [15]. Moreover, it should be used with caution in cirrhotic patients with hepatic impairment (classified as Child Pugh B) and is contraindicated in patients with hepatic disease associated with a coagulopathy [16]. Therefore, biological monitoring would be valuable in acute situations such as recurrent thrombosis, bleedings, before urgent surgery, in case of bridging and in case of at least two risk factors among the following: drug interactions with caution, moderate renal impairment and moderate hepatic impairment; Monitoring may also be useful in infants, pregnant women or in extreme body weights, although no relevant data on drug levels associated with approximate therapeutic and harmful ranges are currently available [17].

The primary aim of the present study is to assess which coagulation assay(s) could be proposed to measure the pharmacodynamic effects of rivaroxaban and to compare our results with those found in the literature. Secondly, we also provide laboratory recommendations for the accurate determination of plasma drug concentration in patients treated by rivaroxaban as well as a correct interpretation of routine lab tests influenced by the presence of rivaroxaban.

Materials and methods

Rivaroxaban was spiked at increasing concentrations in pooled citrated normal human platelet poor plasma (PPP) to measure Prothrombin Time (PT), dilute PT (dPT), Prothrombinase-induced Clotting Time (PiCT), Thrombin Generation Assay (TGA), Liquid anti-Xa® (LAX) and Biophen Direct Factor-Xa Inhibitor® (DiXaI). Activated Partial Thromboplastin Time (aPTT), activated clotting time (ACT), Thrombin Time (TT), Ecarin Clotting Time (ECT) and Reptilase Time (RT), Activated Protein C Resistance (APC-R), measurement of clotting factors (XII; XI; IX; VIII; VII; V; X; II), Protein-C and free Protein-S (immunological and clotting method) were also tested and were described in supplementary material. The results presented for each clotting test represent the mean value and standard deviation of the triplicate.

Testing solutions of rivaroxaban

Rivaroxaban was tested at 7 concentrations ranging from 11 to 1090 ng/mL (initial concentrations) in normal pooled plasma (NPP). Rivaroxaban solutions were prepared as mentioned in the supplementary material.

Whole blood and platelet-poor plasma

The protocol for whole blood and platelet-poor plasma is described in the supplementary material.

Prothrombin time

Fifty μL of spiked NPP were incubated at 37 °C during 240 seconds (sec) and mixed with 100 μL of calcium thromboplastin. The different thromboplastin reagents used were Triniclot PT Excel® (Trinity Biotech, Bray, Ireland), Triniclot PT Excel S® (Trinity Biotech), Triniclot PT HTF® (Trinity Biotech), Neoplastin R® (Diagnostica Stago, Asnieres, France), Neoplastin CI Plus® (Diagnostica Stago), Innovin® (Siemens Healthcare Diagnostics, Deerfield, IL, USA) and Recombiplastin® (Instrumentation Laboratory, Lexington, KY, USA).

Triniclot PT Excel®, Triniclot PT Excel S®, Neoplastin R®, Neoplastin CI Plus® are derived from rabbit brain. Recombiplastin® and Innovin® are recombinant human thromboplastin. Triniclot PT HTF® is derived from cultured human cells. Clotting time was measured on STA-R (Diagnostica Stago) for Neoplastin R®, Neoplastin CI Plus®, Triniclot PT Excel®, Triniclot PT Excel S® and Triniclot PT HTF®; on BCS (Siemens Healthcare Diagnostics) for Innovin® and on ACL-TOP (Instrumentation laboratory) for Recombiplastin®.

Dilute prothrombin time (dPT)

Thromboplastin reagents were diluted with $CaCl_2$ 25 mM to obtain an initial clotting time of approximately 30 sec. The dilutions [one part of reagent/ x parts of $CaCl_2$ solution] were: Innovin® diluted 1/100; Neoplastin CI Plus® diluted 1/128; NeoplastinR® diluted 1/256; Recombiplastin® diluted 1/64 and Triniclot PT Excel S® diluted 1/60.

One hundred and fifty μL of spiked NPP were incubated during 120 sec at 37 °C. Thereafter, 150 μL of reagent was added, starting the measurement on KC-10 (Amelung, Germany).

Thrombin generation assay (TGA)

The calibrated automated thrombin generation test (CAT) measurement was performed as follows. Eighty μL of spiked-NPP, and 20 μL of PPP-Reagent High (20pM of Tissue Factor (TF) and 4 μM of phospholipids (PL)), PPP-Reagent (5pM of TF and 4 μM of PL) or PPP-Reagent LOW (1pM of TF and 4 μM of PL) were mixed in a 96-well microtiter plate (Thermo Immulon 2HB, Thermo Labsystems, The Netherlands) and were incubated for 5 min at 37 °C. The plasma clotting was then triggered by the addition of 20 μL of fluorogenic substrate (Z-Gly-Gly-Arg-AMC HCl)/calcium chloride buffered solution at 37 °C. A calibration curve was also performed using 70 μL of NPP, 10 μL of PBS, 20 μL of Thrombin Calibrator and 20 μL of substrate/calcium chloride-buffered solution at 37 °C. The substrate hydrolysis was monitored on a microplate fluorometer Fluoroskan Ascent FL® (Thermo Labsystems, The Netherlands) with a 390/460 nm filter set using the Thrombinoscope software (v 3.0, Thrombinoscope BV).

Chromogenic anti-Xa assays

STA® liquid anti-Xa (LAX) (diagnostica stago)

Thirty μL of spiked-NPP diluted 4-fold in Owren-Koller® were mixed with 150 μL of chromogenic substrate (CBS 02.44 consisting of MAPA-Gly-Arg-pNA .HCl) and incubated during 240 sec. Then, 150 μL of bovine FXa pre-warmed at 37 °C were added, starting the measurement. Results are expressed in OD/min and measurements were performed on STA-R.

Biophen direct Xa inhibitor® (DiXaI) (hyphen biomed)

Two hundred μL of spiked-NPP were diluted 50-fold in Tris-NaCl-EDTA buffer at pH 7.85 with PEG6000 1% and sodium azide. Seventy-five μL of human purified FXa were mixed with tested plasma and incubated during 2 min at 37 °C. Seventy-five μL of, chromogenic substrate (CS-11(65) consisting of -D-Arg-Gly-Arg-pNA, 2HCl) pre-warmed at 37 °C were added starting the measurement on STA-R.

Statistical analysis

Sensitivity and reproducibility of the different assays were compared using GraphPad Prism 5.01® for Windows®.

Sensitivity of a particular assay was defined as the final concentration in rivaroxaban needed to double (or halve)* the analytical

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parameter; (2xCT [CT = Clotting Time]; C_{max} IC_{50}* [The inhibitor concentration reducing the C_{max} of 50%] and 2 x OD/min* [The concentration needed to halve the change in the optical density reported by minute]).

Reproducibility expressed as CV (coefficient variation = [(standard deviation/mean)*100]) of the triplicate for each concentration and each test was determined. The minimum, mean and maximum CV was determined for each test and compared between tests.

For PT, LAX and Biophen DiXaI, the intra- and inter-assays variability, expressed in mean CV, was also assessed by measuring 10 replicates of 5 different concentrations (436; 218; 110; 22 and 0 ng/mL in initial concentrations). The mean CV represented the sum of the CV for the five concentrations divided by 5 (i.e. the number of concentrations). For the inter-assay variability, measures were performed once a day during 10 days with the same lot of reagents.

The lower limit of quantitation was calculated as follow: [(10*standard deviation of Y0)/ slope] where Y0 was the baseline value of the linear regression. The upper limit of quantitation reflects the concentration from which results were unreliable (concentration above 941 ng/mL in the initial sample were not tested). For aPTT and PT, the dynamic range was calculated as the mean of the individual lower and upper limit of quantitation of the different reagents.

Results

Prothrombin time (PT)

Rivaroxaban showed a concentration-dependent prolongation of PT (Fig. 1) depending on on the thromboplastin reagent used. The relation was linear for each reagent. Two-fold CT was respectively 66 ng/mL for Triniclot PT Excel S®; 73 ng/mL for Recombiplastin®; 84 ng/mL for Neoplastin R®; 135 ng/mL for Neoplastin CI+®; 161 ng/mL for Triniclot PT Excel®; 180 ng/mL for Triniclot PT HTF®; and 258 ng/mL for Innovin®. Results in terms of reproducibility are summarized in Supplementary material: Table 2. The intra- and inter-assay variability was respectively ranging from 2.2% and 1.9% for Neoplastin R® to 7.7% and 8.4% for Triniclot PT HTF®.

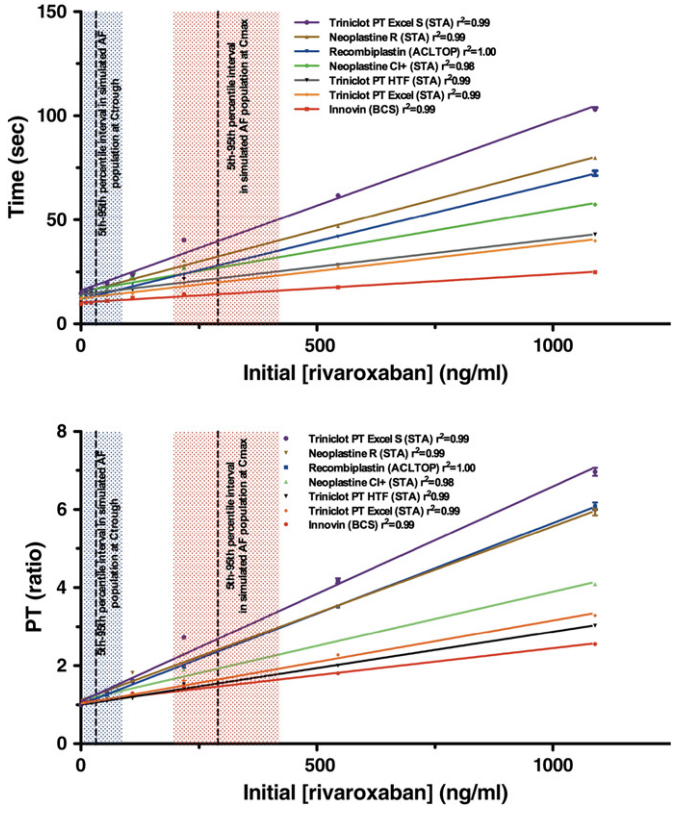

Fig. 1. Influence of rivaroxaban on PT. PT shows a concentration-dependent prolongation of clotting time. The sensitivity depends on the reagent. Triniclot PT Excel S® is the most sensitive reagent while Innovin® seems to be the less affected thromboplastin. The boxes in blue and red represent the therapeutic range in AF (rivaroxaban 20 mg *qd*) at C_{trough} and C_{max} (median; 5th-95th percentile), respectively.

Diluted prothrombin time (dPT)

Dilute Prothrombin Time (dPT) showed a concentration-dependent prolongation of clotting time also depending on the reagent (Fig. 2). Two-fold CT was respectively 56 ng/mL for Recombiplastin® diluted 1/64; 99 ng/mL for Neoplastin CI+® diluted 1/128; 144 ng/mL for Triniclot PT Excel S® and Neoplastin R® diluted 1/256, and 362 ng/mL for Innovin® diluted 1/100. Results in terms of reproducibility were summarized in Supplementary material: Table 2.

Chromogenic anti-Xa assays

STA® liquid anti-Xa (LAX)

Liquid anti-Xa showed a concentration dependent decrease of OD/min (Fig. 3). The relation was linear until 224 ng/mL but may be correlated by an exponential model until 1090 ng/mL. Half OD/min was 8 ng/mL and reproducibility (CV %) was 1.0%. The intra- and inter-assay variability was respectively 1.3% and 1.9%.

Biophen direct Xa inhibitor® (DiXaI)

Biophen DiXaI showed a concentration dependent decrease of OD/min (Fig. 3). The relation was linear. Concentration of rivaroxaban required to halve the initial OD/min was 9 ng/mL and reproducibility was 1.3%. The relation was linear until 545 ng/mL with a decrease of sensitivity. The intra- and inter-assay variability was respectively 3.4% and 3.9%.

Thrombin generation assay (TGA)

The Peak and mVRI were the most sensitive CAT parameters with a high sensitivity (Peak IC_{50} was 3 ng/mL with PPP-Reagent Low and PPP-Reagent and was 14 ng/mL with PPP-Reagent High; mVRI IC_{50} was 1 ng/mL with PPP-Reagent Low and PPP-Reagent and 3 ng/mL with PPP-Reagent High) and a low variability (CV < 1.0%).

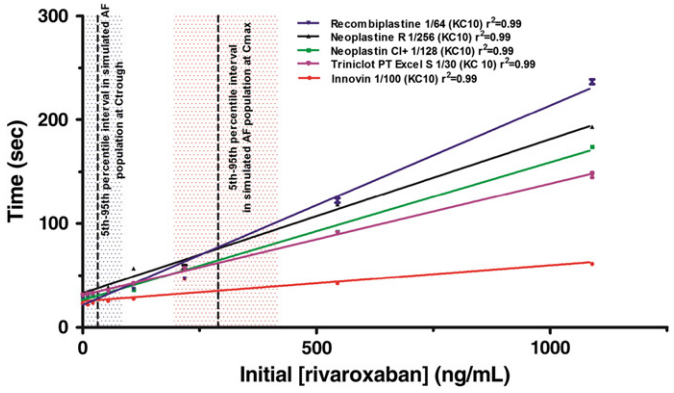

Fig. 2. Influence of rivaroxaban on dPT: dPT shows a concentration-dependent prolongation of clotting time. The sensitivity also depends on the reagent. Recombiplastin® is the most sensitive reagent and Innovin® is less affected by rivaroxaban in comparison with the other thromboplastin. The boxes in blue and red represent the therapeutic range in AF (rivaroxaban 20 mg *qd*) at C_{trough} and C_{max} (median; 5th-95th percentile), respectively.

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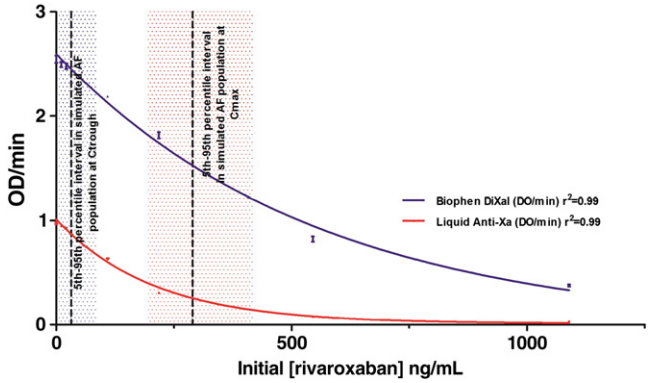

Fig. 3. Influence of rivaroxaban on Liquid anti-Xa (LAX) and Biophen DiXaI: LAX shows a concentration-dependent decrease in OD/min. The relation is linear until 224 ng/mL. LAX is the most sensitive assay with a concentration in rivaroxaban needed to halve OD/min of 8 ng/mL. For Biophen DiXaI, the relation is linear until 545 ng/mL with a concentration needed to halve OD/min of 9 ng/mL. For concentration higher than 545 ng/mL, the relation is no longer linear and a higher dilution (initially 50-fold) of the sample is needed (data not shown). The boxes in blue and red represent the therapeutic range in AF (rivaroxaban 20 mg *qd*) at C_{trough} and C_{max} (median; 5th-95th percentile), respectively.

Activated partial thromboplastin time (aPTT)

aPTT showed a concentration-dependent prolongation of clotting time and also depended on the reagent (Supplementary material: Fig. 1). Two-fold CT was respectively 208 ng/mL for CKPrest®; 234 ng/mL for Actin FS®; 258 ng/mL for Synthasil®; 375 ng/mL for PTT-A® and 420 ng/mL for Cephascreen®. Results in terms of reproducibility were summarized in Supplementary material: Table 2.

Prothrombinase-induced clotting time (PiCT)

PiCT showed a linear regression with 30 sec or 180 sec incubation (Supplementary material: Fig. 2). Two-fold CT was 185 ng/mL for the 180 sec incubation methodology and for the 30 sec incubation methodology 2xCT was different for fast half-life (2 ng/mL) and short half-life (365 ng/mL). Reproducibility (mean CV %) was 0.4% for PiCT 180 sec incubation and 5.6% for PiCT 30 sec incubation.

Activating clotting time (ACT)

ACT showed a concentration-dependent prolongation of clotting time (Supplementary material: Fig. 3). Two-fold CT was 334 ng/mL and reproducibility (mean CV %) was 17,0%.

Fibrinogen assay (clauss method); thrombin time (TT); reptilase time (RT) and ecarin clotting time (ECT)

Rivaroxaban had no effect on the Clauss method fibrinogen assay (Supplementary material: Fig. 4) as well as on TT (Supplementary material: Fig. 5), RT (Supplementary material: Fig. 6) and ECT (Supplementary material: Fig. 7).

Discussion

Rivaroxaban (Xarelto®) is an orally, direct FXa inhibitor approved by the EMA [2] and FDA [3] in the prevention of DVT and pulmonary embolism in TKR or THR and in stroke prevention in patients with non-valvular atrial fibrillation. Moreover, it has received the market authorization in the treatment of acute DVT and prevention of symptomatic VTE, in Europe only [2–4]. Thanks to its predictable kinetics, therapeutic monitoring is generally not required. Nevertheless, this statement is debated [18,19]. As clearly highlighted by different authors, patients in clinical trials were carefully selected patients, excluding those with assumed poor- compliance, renal insufficiency and/or an increased bleeding risk. However, bleeding and other side effects were still encountered at significant percentages [17].

Thus, the opportunity to further improve the efficacy and safety of new anticoagulants including rivaroxaban by searching for the optimal dose in specific patients may require laboratory monitoring. Moreover, such monitoring can be helpful in acute situations such as recurrent thrombosis, bleedings, before urgent surgery, in case of bridging, in infants, in pregnant women, in extreme body weight and in case of at least two risk factors among the following: drug interactions with caution, moderate renal impairment and moderate hepatic impairment; Point measurement may also be useful in the management of bleeding.

Aim of the study

Several studies have already been performed to suggest which assay could be used to monitor patients on rivaroxaban but only some of them compared the different reagents in terms of sensitivity, reproducibility, linearity and/or specificity [20–22]. Nevertheless, in clinical settings, practical approach is essential and no guidelines have been provided to perform this measurement.

The primary objective of the present study is to specify which coagulation assays may be recommended to measure the effects of rivaroxaban among a large range of tests and reagents (Table 1) and to compare the results with those already published (Table 2). The secondary objective is to propose recommendations about how to perform this monitoring in clinical routine practise and how to interpret the influence of rivaroxaban on routinely used laboratory assays. We also propose cut-off associated with a bleeding or thrombosis risk based on pharmacokinetic studies but further investigation in the field and confirmation are required.

Assessment of the pharmacodynamic effects of rivaroxaban: advantages and drawbacks of different coagulation assays

Prothrombin time is usually used to assess vitamin K antagonist therapy using the international normalized ratio (INR) and the international sensitivity index (ISI) specific to each reagent. Nevertheless, INR using ISI_{VKA} cannot be used for rivaroxaban. Authors have proposed to use specific $ISI_{rivaroxaban}$ showing a reduction of the coefficient of variation between the slopes of the dilution curves and the ratios of the thromboplastin reagents, but up to now this method required further investigations and standardization [23,24]. Thus, PT showed a concentration dependent prolongation of clotting time with a linear regression (Fig. 1). Two-fold CT depended on the clotting reagent used (2xCT was ranging from 66 ng/mL to 258 ng/mL in our study). The most sensitive reagent in this study was Triniclot PT Excel S®. Results obtained for PT reagents showed an important variability between studies in terms of 2xCT. This may be explained by the fact that, in the other studies, results in terms of sensitivity were not expressed in final concentration (in Table 2 our results are expressed in initial concentration in order to allow direct comparison between studies). By calculating sensitivity using the initial plasma drug concentration instead of the final plasma drug concentration, results were consistent with previous publications. A recent multicenter trial has demonstrated that results expressed in rivaroxaban concentration (ng/mL) did not show significant difference ($P > 0.05$) in the interlaboratory variations of the PT measurement [25], suggesting that the use of the widely available PT assay, in conjunction with rivaroxaban calibrators, may be useful for the measurement of peak plasma levels of rivaroxaban. Nevertheless, results were obtained from spiked apheresis citrated-pooled plasma originating from transfusion blood banks and further experiments in patient's plasma should be investigated to know whether the interindividual

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Table 1
Summary of assays for the monitoring of rivaroxaban in plasma. (PT: Prothrombin Time; Biophen DiXaI: Biophen Direct Factor Xa Inhibitor; LAX: Liquid anti-Xa; dPT; dilute Prothrombin Time; TGA: Thrombin Generation Assay; aPTT: activated Partial Thromboplastin Time; PiCT: Prothrombinase-induced Clotting Time; ACT: Activated Clotting Time; TT: Thrombin Time; ECT: Ecarin Clotting Time).

	Useful for monitoring		Reliable but requires laboratory experience			Not recommended				
	PT	Biophen DiXaI	LAX	dPT	TGA (Peak IC 50)	aPTT	PiCT	ACT	TT	ECT
Sensitivity (ng/mL)[†]	66 to 258	9	8	56 to 362	3 to 14	208 to 420	185	334	Not Influenced	Slightly Influenced
Dynamic range of quantitation (ng/mL) [‡]	80 – 1090	29 – 545	13 – 224	141 – 1090	N.D	164 – 1090	N.D	N.D	N.D	N.D
Reproducibility (%) [††]	0.5 to 1.3	1.3	0.9	1.1 to 1.9	1.0	0.9 to 4.4	0.4 to 5.6	17	N.D	N.D
Dependence of reagent	Yes	No	No	Yes	Yes	Yes	No	Yes	No	No
Linearity of the response	Yes	Yes	Yes	Yes	Yes	No	No	No	Not Influenced	Yes

[†] Sensitivity expressed the concentration needed to double or halve the evaluated parameter (2 x clotting time (CT); ½ x OD/min; Peak IC_{50}).
[‡] The lower limit of quantitation was calculated as follow: [(10*standard deviation of Y0)/ slope]. The upper limit of quantitation reflects the concentration from which results were unreliable (concentration above 1090 ng/mL in initial concentration were not tested).
[††] Reproducibility was expressed as the coefficient of variation [CV (standard deviation/mean*100)] of the triplicate for each concentration and each test.

variables of PT impact on the results. CoaguCheck XS, a PT test that used a specific thromboplastin reagent that enables the measurement on whole blood was also assessed in a previous study. It showed a 2xCT of approximately 50 ng/mL as well as a low inter-individual variation [22]. Thus, CoaguCheck XS may be one good opportunity for monitoring but results have to be expressed in ng/mL.

As suggested previously, dPT may increase the sensitivity by mimicking physiological conditions [22]. In this study, dPT was found slightly more sensitive than PT only for Recombiplastin® (2xCT = 73 vs. 56 ng/mL) and Neoplastin CI + ® (2xCT = 135 vs. 99 ng/mL). However, according to Samama et al., the relation for each dPT reagent was also linear (Fig. 2). Reproducibility was lower than PT test probably due to the manual method (KC-10) used in our study. Base on the same method (e.g. the addition of calcium chloride), another proposal for assessing the anticoagulant activity of oral FXa inhibitors is to modify a PT assay by adding calcium chloride at a different concentration (final dilution: 1:2,25) and NaCl, as appropriate, to the thromboplastin reagent to increase assay dynamic range and improve sensitivity but this method has not yet been investigated with rivaroxaban [26].

As presented in previous studies [20–22,27,28], aPTT showed a concentration-dependent prolongation of clotting time (Supplementary material: Fig. 1). This relation is curvilinear suggesting that the affinity decreases for higher rivaroxaban concentrations. As for PT and dPT, 2xCT depends on the reagent. These results clearly showed that aPTT is less sensitive than PT or dPT as mentioned in earlier publications [22,29] but were not in agreement with results obtained by Hillarp et al. [21] who demonstrated that aPTT was generally more influenced by the presence of rivaroxaban than PT, especially with Owren method. Thus, one can conclude that PT is preferable to ensure a quantitation of rivaroxaban due to its linearity in a broad range of concentrations and its higher sensitivity.

At lower concentration of rivaroxaban (<200 ng/mL), there was a shortening in clotting time using the two-step (180 sec incubation) PiCT (Supplementary material: Fig. 2). This was in agreement with previous study [22] and a reduction of incubation time (30 sec) was voluntary performed to avoid this shortening. This may be explained by an interaction between endogenous antithrombin (AT) and rivaroxaban as this artefact was removed using AT-deficiency plasma [22]. Nevertheless, results in terms of sensitivity were not consistent with those previously published but the two graphics looked alike (Supplementary material: Fig. 2) [22]. The explanation was that in this study we used a two-phase association exponential relation expressing the 2xCT in low (<54 ng/mL) and high (>54 ng/mL) final concentration in rivaroxaban. In conclusion, due to its lack of linearity, this test is not useful to measure rivaroxaban.

Ecarin clotting time (ECT) was almost insensitive to rivaroxaban. Ecarin converts prothrombin in meizothrombin [30], and rivaroxaban, by inhibiting FXa, acts only on the capacity of FXa to generate meizothrombin but not on the ecarin activity.

ACT, a bedside test currently used to monitor heparin therapy during cardiac interventions, showed a linear prolongation of the clotting time until 545 ng/mL (Supplementary material: Fig. 3). The sensitivity increases proportionally with the concentration of rivaroxaban. However, 2xCT was 334 ng/mL that is less sensitive than PT, dPT or aPTT (depending on the reagent). Moreover, its low reproducibility (mean CV% = 17.0%) is a limiting parameter already described in other clinical applications [31,32]. Moreover, the sensitivity towards FXa or thrombin inhibition depends on the composition of the reagent [31]. All of these limitations preclude the use of ACT to assess rivaroxaban drug levels.

Thromboeslastography (TEG) was previously assessed [22]. Rivaroxaban induced a concentration dependent prolongation of the TEG clotting parameters (R: the time of clot formation in min and K: velocity of fibrin formation in min) without any modification in the amplitude making this test promising to assess the impact of rivaroxaban, but further studies are required [22].

In this study, two chromogenic anti-Xa assays were used and compared with those already tested in the literature. Liquid anti-Xa® (LAX) showed a very high sensitivity with a concentration of rivaroxaban required to halve OD/min of 8 ng/mL (Fig. 3). This chromogenic assay is also very reproducible with a mean CV of 1.0%. Its use in routine may be valuable to monitor patients on rivaroxaban. The relation is described by a one-phase decay equation. However, the relation is linear for concentration in rivaroxaban in the initial sample <224 ng/mL, suggesting that a more important dilution for samples presenting an OD/min <0.25 units should be performed (1/8 instead of 1/4). In addition, one should also keep in mind that LAX may be influenced by the presence of heparin and pentasaccharides (i.e. fondaparinux, idraparinux) and a more specific test is then required when clinicians will face in unconscious patients without information on the nature of the anticoagulant.

Biophen Direct Factor Xa Inhibitors® (DiXaI) showed, as LAX, a high sensitivity (9 ng/mL). One of the strength of this chromogenic assay is the use of Tris-EDTA-NaCl buffer making this test insensitive to the presence of heparin or fondaparinux [33] and thus highly interesting in case of switching therapy or with unconscious patients in emergency for example. The relation was linear (Fig. 3). Thanks to its specificity, its high sensitivity and the fact that it covers the therapeutic range, Biophen DiXaI seems to be the more accurate assay to monitor patients on rivaroxaban. Nevertheless, specific calibration for other direct FXa inhibitors marketed (or under development) will be required to correctly assess the plasma drug level. Other

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Table 2
Summary of assays performed in the different *in-vitro* studies: Results show the sensitivity as the expression of the concentration in rivaroxaban **in the initial sample** needed to double (or halve) the evaluated coagulation parameter. (LT: Lag Time; TTP: Time to Peak; ETP: Endogenous Thrombin Potential; mVRI: mean Velocity Rate Index).*

		Perzborn *et al.* 2005 [29]	Gerotziafas *et al.* 2007 [47]	Barret *et al.* 2010 [20]	Hillarp *et al.* 2010 [21]	Samama *et al.* 2010 [33,48]	Asmis *et al.* 2011 [27]	Detail of this study
TGA	PPP-Reagent High	NA	NA	NA	NA	NA	NA	LT: 73 ng/mL TTP: 84 ng/mL ETP: 767 ng/mL Peak: 20 ng/mL mVRI: 4 ng/mL
	PPP-Reagent	NA	LT: 4 ng/ml TTP : 4 ng/mL ETP : 15 ng/mL (Measure performed on PRP)	NA	NA	LT : 51 ng/mL TTP : 45 ng/mL ETP: 305 ng/mL Peak: 21 ng/mL mVRI: 9 ng/mL	NA	LT: 80 ng/mL TTP: 60 ng/mL ETP: 141 ng/mL Peak: 4 ng/mL mVRI: 2 ng/mL
	PPP-Reagent LOW							LT: 93 ng/mL TTP: 98 ng/mL ETP: 98 ng/mL Peak: 5 ng/mL mVRI: 1 ng/mL
PT	Neoplastin CI + ®	229 ng/mL Reagent is not specified	NA	546 ng/mL	506 ng/mL	300 ng/mL	NA	405 ng/mL
	Neoplastin CI®			580 ng/mL	NA	420 ng/mL	NA	NA
	Innovin®			828 ng/mL	591 ng/mL	700 ng/mL	Results not expressed in term of sensitivity	774 ng/mL
	Recombiplastin®			642 ng/mL	498 ng/mL	300 ng/mL		219 ng/mL
	Thromborel S®			NA	885 ng/mL	500 ng/mL	NA	NA
	Technoplastin HIS®			NA	557 ng/mL	NA	NA	NA
	SPA + ®			NA	1375 ng/mL	NA	NA	NA
	Nycotest PT®			NA	1300 ng/mL	NA	NA	NA
	PT Owren®			NA	891 ng/mL	NA	NA	NA
	Simple Simon PT®			NA	871 ng/mL	NA	NA	NA
	Triniclot PT Excel®			NA	NA	450 ng/mL (type of Triniclot is not specified)	NA	483 ng/mL
	Triniclot PT Excel S®			NA	NA		NA	198 ng/mL
	Triniclot PT HTF®			NA	NA		NA	540 ng/mL
	Neoplastin R®			NA	NA	NA	NA	252 ng/ml
aPTT	Actin FSL®	NA	NA	NA	435 ng/mL	NA	aPTT were performed to show the influence of the ingestion of rivaroxaban on coagulation test.	NA
	PTT-A®				491 ng/mL	750 ng/mL		1125 ng/ml
	aPTT SP IL®				530 ng/mL	NA		NA
	Triniclot aPTT HS®				617 ng/mL	NA		NA
	aPTT DG®				389 ng/mL	NA		NA
	CKPrest®				NA	550 ng/mL		624 ng/mL
	PTT-LA®				NA	650 ng/mL		NA
	Actin FS®				NA	500 ng/mL		702 ng/mL
	Synthasil®				NA	NA		774 ng/mL
	Cephascreen®				NA	NA		1260 ng/mL
dPT	Recombiplastin® 1/64	NA	NA	NA	NA	NA	NA	168 ng/mL
	Recombiplastin®1/32					450 ng/mL		NA
	Neoplastin CI® 1/32					250 ng/mL		NA
	Neoplastin CI + ® 1/32					280 ng/mL		NA
	Innovin® 1/64					700 ng/mL		NA
	Thromborel S® 1/64					200 ng/mL		NA
	Triniclot 1/8 (reagent not specified)					280 ng/mL		NA
	Neoplastin CI + ® 1/128					NA		297 ng/mL
	Triniclot PT Excel S® 1/30					NA		432 ng/mL
	Neoplastin R® 1/256					NA		333 ng/mL
	Innovin® 1/100					NA		1086 ng/mL

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Table 2 (*continued*)

		Perzborn *et al.* 2005 [29]	Gerotziafas *et al.* 2007 [47]	Barret *et al.* 2010 [20]	Hillarp *et al.* 2010 [21]	Samama *et al.* 2010 [33,48]	Asmis *et al.* 2011 [27]	Detail of this study
PiCT®	Incubation 30 sec Incubation 180 sec	NA	NA	NA	NA	40 ng/mL >1000 ng/mL		Fast 2xCT: 6 ng/mL 555 ng/mL
ACT	NA	NA	NA	NA	NA	NA	418 ng/mL	
TT	NA	NA	NA	NA	NA	NA	Not influenced	
RT	NA	NA	NA	NA	NA	NA	Not influenced	
Liquid anti-Xa® Biophen Direct Factor Xa Inhibitor®	NA NA	NA NA	NA NA	NA NA	NA Sensitivity not explored (graph is not expressed in final concentration)	NA NA	352 ng/mL 788 ng/mL	
Dilute Russell's Viper Venom Time (dRVVT)	NA	NA	NA	NA	80 ng/mL and 60 ng/mL for dRVV Confirm	NA	NA	
Rotachrom LMWH®	NA	NA	31 ng/mL	NA	Decrease in OD/min	NA	NA	
Stachrom LMWH®	NA	NA	NA	NA	Decrease in OD/min	NA	NA	
Biophen Heparin 6®	NA	NA	NA	NA	NA	They just specified that this test give the same [rivaroxaban] than HPLC/MS-MS	NA	
Fibrinogen (Clauss method)	STA-Fibrinogen® FibriPrest® Fibrinogen C® Dade Thrombin® Multifibren U®	NA	NA	NA	NA Almost unaffected			Not influenced NA NA NA NA
Antithrombin	Berichrom ATIII® Stachrom ATII® Coamatic LR®	NA	NA	NA	Not influenced Not influenced Influenced	NA	NA	NA Not Influenced Influenced
APC Resistance assay	Coatest APC Resistance V® Pefakit APC resistance Factor V Leiden®	NA	NA	NA	Influenced Not influenced	NA	NA	Influenced
HepTest (30 sec incubation)		NA	NA	NA	NA	50 ng/mL	NA	NA
Thromboelastography	NA	NcA	NA	NA	Modification of clotting parameters (R and K)	NA	NA	

* Results in term of sensitivity are expressed in final concentration to allow between test comparisons.

chromogenic assays have already been performed also showing good sensitivity but the requirement of calibrator sets is mandatory as mentioned in a previous study [34]. In conclusion, anti-Xa chromogenic assays are preferable to PT assays to perform monitoring of rivaroxaban due to the higher sensitivity, the flexibility, the lower intra- and inter-assay variability, the similar inter-laboratory precision [20,35] and, for Biophen DiXaI, its specificity against other antithrombin-dependent inhibitors [33]. Nevertheless, anti-Xa chromogenic assays are not widely available and their use could be difficult in emergency situations.

The TGA gives more information than traditional coagulation assays (chronometric or chromogenic) [36]. Rivaroxaban, by its mode of action, acts on the amplification phase of the coagulation process as showed by its TGA profile (Fig. 4). Between study's results showed the same tendency: Peak and mVRI are the most influenced parameters. Indeed, in our study, TGA shows a Peak IC_{50} of 3 ng/mL with PPP-Reagent and a 2 x Lag Time (LT) of 55 ng/mL. PPP-Reagent Low is too sensitive. Thus, we recommend using PPP-Reagent or PPP-Reagent High to assess rivaroxaban plasma samples with the CAT analyser. These results show that TGA might be an accurate assay to assess rivaroxaban but in clinical practise, the turnaround time, the interindividual variability and the lack of standardisation will be limitations [37].

Interference of rivaroxaban on haemostasis diagnostic tests

In the case of the exploration of a haemorrhagic event, specific tests such as reptilase time (RT), fibrinogen (Clauss and PT-derived

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method (dFib)), TT and clotting factor activity may be used. In case of thrombophilia, an activated protein C (APC) resistance, AT, protein C, protein S and clotting factor assays may be required and thus, it is of particular importance for the clinician to have information about how these tests may be influenced by rivaroxaban.

Further tests were performed to evaluate the influence of rivaroxaban on the rate of fibrinogen, using the Clauss method. In our study, using STA-Fibrinogen® (Diagnostica Stago) rivaroxaban almost did not affect the rate of fibrinogen, except for higher concentration (>545 ng/mL) where a decrease of approximately 10% in the rate of fibrinogen was noted as mentioned by a previous study [21]. The dFib assay is also widely used and Mani et al. stated that the effect of rivaroxaban on dFib varies significantly depending on the PT reagent used [38]. Logically, neither RT nor TT was influenced by rivaroxaban. The influence on AT was evaluated in a previous study [21] and showed that the choice of AT assay is of importance to correctly evaluate the rate of AT. Indeed, as mentioned by Khor et al. [39] AT deficiency may be measured by either FXa-based assay or by thrombin-based assay. Berichrom ATIII® and Stachrom ATIII®, two thrombin-based AT assays were not influenced by the presence of rivaroxaban in comparison with Coamatic LR® based on FXa (increase of 0.09 IU/mL per 100 ng/mL rivaroxaban) investigated in a previous study [21] and confirmed by our personal unpublished data.

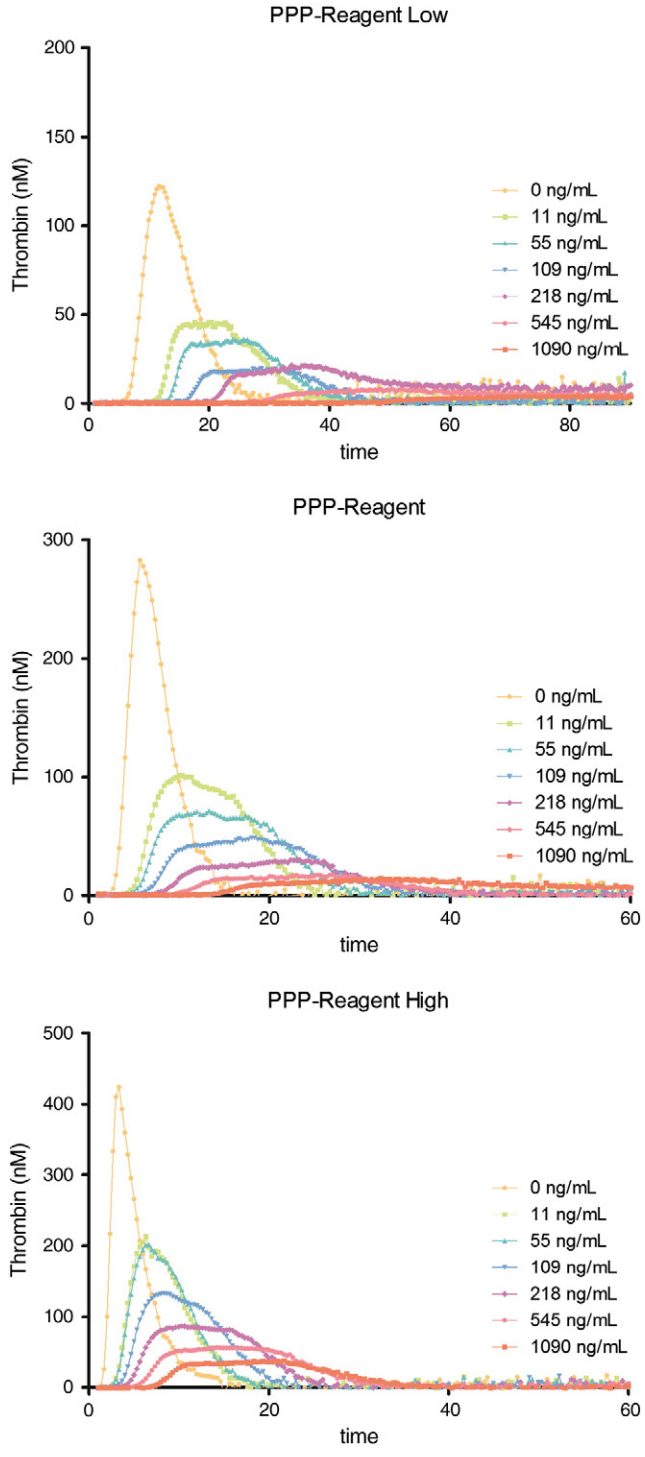

Fig. 4. Influence of rivaroxaban on Calibrated Automated Thrombogram (CAT) using different inductors. The most sensitive parameters are the mVRI and the Peak whatever the reagent that is used. PPP-Reagent Low is too sensitive and for higher concentration in rivaroxaban, the curve may be confounded with background noise. Thus, PPP-Reagent and PPP-Reagent High are more suitable to evaluate CAT parameters of rivaroxaban.

The APC resistance assay was also investigated and the influence was also dependent of the type of assay. Coatest APC Resistance V® and the HemosIL APC Resistance V®, two aPTT-based assay, are influenced by rivaroxaban while Pefakit APC resistance Factor V Leiden® is a specific test using Russell Viper Venom from *Daboia Russelli* to activate prothrombinase complex showing no interference with rivaroxaban [21]. In case of thrombophilia testing, rivaroxaban also interfere with the one-stage and chromogenic factor VIII:C assays [40] but do not influence the Coamatic® Protein-C assay. The measurement of free Protein-S using latex ligand immunoassay (Hemosil® Free Protein S) was not influenced by rivaroxaban (Supplementary material: Table 3) in comparison with the chronometric method (Staclot® Protein S) where an over-estimation of approximately 15% per 100 ng/mL of rivaroxaban was found (Supplementary material: Table 3). In addition, a study showed that the presence of rivaroxaban in plasma samples at pharmacological concentrations (±250 ng/mL) can change the results of lupus anticoagulant (LAC) determinations as measured with the officially recommended assays for the detection of LAC: the aPTT and the dRVVT [41]. Therefore the use of Taipan snake venom time and Ecarin clotting time are useful to determine the presence of LAC in patients treated by rivaroxaban [41]. In addition, clinically relevant concentrations of rivaroxaban, interfere with PT- and aPTT- based assays for the measurement of clotting factor activity in plasma [42]. These results are confirmed by our data (Supplementary material: Table 3) but in our study there is a lower influence of the presence of rivaroxaban for the factors in the extrinsic pathway. This may be explained by the fact that the reagent used in this study (Innovin®) is less sensitive to rivaroxaban than Thomborel S® which was used in a previous study [22,42]. Nevertheless, when higher dilution of the plasma sample is performed a normalisation of the factor activity may be observed. Thus we recommend to perform a wash out period of at least 24 hours (preferred 48 hours) before testing, to use the less sensitive PT- and aPTT-reagents and to increase the sample dilution for clotting factor assays.

Delay between the drug intake and the blood sampling

Another point to consider is the delay between the last drug intake and the time of blood collection since assays are influenced by rivaroxaban plasma concentration that depends on the pharmacokinetic properties [38]. Indeed, C_{max} is reached after 2 to 4 hours [11] and it seems to be preferable to collect sample at C_{trough} to avoid misinterpretation due to a prolonged or shortened delay in the absorption phase where the variability in concentration is higher. Nevertheless, C_{trough} is inappropriate to evaluate a lack of efficacy in case of recurrent thrombosis with PT reagents due to lower sensitivity and thus, more sensitive assays like anti-FXa chromogenic assays should be used. However, in case of bleedings, C_{trough} could be assessed with PT reagents since drug levels are higher in this

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Table 3
Baseline, mean or median C_{trough} and C_{max}, and cut-offs associated with a risk of bleeding or with sub-therapeutic level for PT (Recombiplastin®, Neoplastin CI + ®, Neoplastin R®, Innovin®, Triniclot PT HTF®, Triniclot PT Excel®, Triniclot PT Excel S®), Biophen Direct FactorXa Inhibitor (DiXaI) and Liquid Anti-Xa (LAX) in the orthopaedic indication (A) and in AF (B). The results are expressed in seconds and/or ratio of the clotting time of a NPP spiked with rivaroxaban divided by the clotting time of NPP without spiking. (NPP: Normal Pooled Plasma; AF: Atrial Fibrillation; N.D.: Not Determined).

A. In Major Orthopaedic Surgery: rivaroxaban 10 mg qd.

Reagent	Baseline time	Clotting time corresponding to a sub-therapeutic level at Ctrough (i.e 1 ng/mL) †		Clotting time corresponding to median Ctrough (i.e 9 ng/mL)		Clotting time corresponding to a risk of bleedings at Ctrough (i.e 38 ng/mL) ‡		Clotting time corresponding to a sub-therapeutic level at Cmax (i.e 91 ng/mL) †		Clotting time corresponding to median Cmax (i.e 125 ng/mL)		Clotting time corresponding to a risk of bleedings at Cmax (i.e 196 ng/mL) ‡	
	Sec ± SD	Sec	Ratio	Sec	Ratio	Sec	Ratio	Sec	Ratio	Sec	Ratio	Sec	Ratio
Recombiplastin®	12.1 ± 0.2	12.2	1.01	12.6	1.04	14.2	1.17	17.1	1.42	19.1	1.58	22.9	1.89
Neoplastin CI®	15.7 ± 0.5	15.8	1.00	16.1	1.02	17.2	1.09	19.3	1.23	20.6	1.31	23.3	1.48
Neoplastin R®	15.1 ± 0.4	15.1	1.00	15.4	1.02	16.5	1.10	18.6	1.24	19.9	1.32	22.7	1.50
Innovin®	12.1 ± 0.1	10.4	1.00	10.5	1.01	10.9	1.05	10.7	1.12	12.1	1.16	13.1	1.25
Triniclot PT HTF®	14.3 ± 0.2	14.3	1.00	14.5	1.02	15.2	1.07	16.7	1.17	17.5	1.23	19.4	1.36
Triniclot PT Excel®	12.5 ± 0.2	12.5	1.00	12.7	1.02	13.5	1.08	14.9	1.19	15.7	1.26	17.5	1.40
Triniclot PT Excel S®	16.2 ± 0.7	16.3	1.01	16.9	1.05	19.2	1.19	23.6	1.46	26.3	1.63	32.1	1.98
	OD/min ± SD	OD/min	Ratio	OD/min	Ratio	OD/min	Ratio	OD/min	Ratio	OD/min	Ratio	OD/min	Ratio
Biophen DiXaI®	2.543 ± 0.009	2.539	1.00	2.514	0.99	2.423	0.95	2.252	0.89	2.147	0.84	1.922	0.76
LAX®	1.013 ± 0.012	1.007	0.99	0.970	0.96	0.846	0.83	0.654	0.65	0.553	0.55	0.399	0.39

† Sub-therapeutic level in the orthopaedic indication is defined as the lower 5th percentile at C_{trough} and C_{max}.
‡ Plasmatic rate in the orthopaedic indication associated with a risk of bleeding is defined as the upper 95th percentile at C_{trough} and C_{max}.

B. In Atrial Fibrillation or acute deep vein thrombosis: rivaroxaban 20 mg qd.

Reagent	Baseline time	Clotting time corresponding to a sub-therapeutic level at C_{trough} (i.e 5 ng/mL) †		Clotting time corresponding to mean C_{trough} (i.e 32 ng/mL)		Clotting time corresponding to a risk of bleedings at C_{trough} (i.e 155 ng/mL) ‡		Clotting time corresponding to a sub-therapeutic level at C_{max} (i.e 177 ng/mL) †		Clotting time corresponding to mean C_{max} (i.e 290 ng/mL)		Clotting time corresponding to a risk of bleedings at C_{max} (i.e 409 ng/mL) ‡	
	Sec ± SD	Sec	Ratio	Sec	Ratio	Sec	Ratio	Sec	Ratio	Sec	Ratio	Sec	Ratio
Recombiplastin®	12.1 ± 0.2	12.4	1.02	13.9	1.15	20,7	1,71	21,9	1,81	28.1	2.32	34,7	2,87
Neoplastin CI + ®	15.7 ± 0.5	15.9	1.01	17.0	1.08	21,8	1,38	22,6	1,44	27	1.72	31,6	2,01
Neoplastin R®	15.1 ± 0.4	15.4	1.02	17.0	1.13	24,3	1,61	25,6	1,70	32.4	2.15	39,5	2,62
Innovin®	12.1 ± 0.1	10.5	1.01	10.9	1.04	12,5	1,20	12,8	1,23	14.3	1.38	15,9	1,53
Triniclot PT HTF®	14.3 ± 0.2	14.4	1.01	15.1	1.06	18,3	1,29	18,9	1,33	21.9	1.54	25,0	1,76
Triniclot PT Excel®	12.5 ± 0.2	12.6	1.01	13.3	1.07	16,5	1,32	17,1	1,37	20.0	1.60	23,1	1,84
Triniclot PT Excel S®	16.2 ± 0.7	16.6	1.03	18.8	1.16	28,8	1,78	30,6	1,89	39.8	2.46	49,4	3,06
	OD/min ± SD	OD/min	Ratio	OD/min	Ratio	OD/min	Ratio	OD/min	Ratio	OD/min	Ratio	OD/min	Ratio
Biophen DiXaI®	2.543 ± 0.009	2.527	0.99	2.441	0.96	2.050	0.81	1.980	0.78	1.621	0.64	1.243	0.49
LAX®	1.013 ± 0.012	0.989	0.98	0.869	0.86	0.483	0.48	0,435	0.43	0.256	0.25	0.149	0.15

† Sub-therapeutic level is defined as the lower 5th percentile at C_{trough} and C_{max}.
‡ Plasmatic rate associated with a risk of bleeding is defined as the upper 95th percentile at C_{trough} and C_{max}.

situation. Thus, different sampling seems to be mandatory to have an efficient estimation of drug exposure. In addition, the marketing authorization holder should publish all relevant data on drug levels (i.e. pharmacokinetic curve based on the time of administration) so that it becomes clear what the approximate therapeutic ranges of laboratory tests outcome are [17].

Recommendation for an accurate monitoring of patients on rivaroxaban

As specific cut-offs associated with a risk of bleeding are currently not available, we have used plasmatic range (5th-95th percentile) of the different pharmacokinetic studies as cut-offs. In a simulated AF population, rivaroxaban given 20 mg *qd* gave a mean C_{max} after 2 – 4 h of 290 ng/mL (5th – 95th percentile ≈ 177 – 409 ng/mL) and a mean C_{trough} after 24 h of 32 ng/mL (5th – 95th percentile ≈ 5 – 155 ng/mL) [11]. In patients undergoing total hip replacement receiving Xarelto® 10 mg *qd*, median C_{max} reaches 125 ng/mL (5th – 95th percentile: 91 – 196 ng/mL) and median C_{trough} is 9 ng/mL (5th – 95th percentile 1 – 38 ng/mL) [10]. Expected results in time or in OD/min were presented for PT with the different reagents, for LAX and for Biophen DiXaI (Table 3). Sensitivity of PT is dependent on the reagent; therefore the use of PT for the monitoring of rivaroxaban requires a calibration for each lot on each instrument and in each laboratory to define local cut-off values. One limitation of PT is the weak sensitivity in comparison with chromogenic assays and some pre-analytical variables such as inappropriate proportion between blood and anticoagulant or storage that influence the results [43,44]. Nevertheless, the intra-assay variability was<than 10% and the inter-assay variability was<than 15% whatever the reagent used in this study. Chromogenic anti-Xa assays are preferable because they are less sensitive than PT to sample collection conditions and variations in the amount of intrinsic pathway clotting factors among patients [20]. In addition, a recent study showed that anti-factor Xa chromogenic assay using rivaroxaban and controls, is suitable for the measurement of a wide range of rivaroxaban plasma concentration [35]. Moreover, Biophen DiXaI appears to be insensitive to the presence of antithrombin-dependent factor Xa inhibitor (i.e: fondaparinux and LMWH) [33]. Nevertheless, as PT is less expensive than chromogenic assays, we recommend performing calibrated PT as a screening test and if value exceeds specific cut-offs, calibrated chromogenic anti-Xa assays should be performed.

Limitation of the study

One limitation of this study is the fact that we used spiked plasma and that the study is mono-centric. These results should therefore be validated in patients receiving Xarelto®. Moreover, it is currently unknown how coagulation assays are predictive for the bleeding

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risk [45]. However, it is not ethically acceptable to expose patients to high-risk overdose of rivaroxaban to study the impact on coagulation tests. Freyburger et al. performed such analysis in patients undergoing THR or TKR and their results showed a correlation with those obtained *in vitro* [46] and Mani et al. stated in their papers that their *ex vivo* findings are in accordance with the *in vitro* data published by Hillarp et al. reinforcing the positive correlation between *in vitro* and *ex vivo* data. An inter-individual variability is also mentioned in these studies confirming the hypothesis that monitoring may be valuable to minimize the risk in high-risk population and to potentially improve the efficacy by searching for the optimal dose in particular patients.

Conclusion

In this study we showed that chromogenic anti-Xa assays and, to a lesser extent, PT are clearly the most appreciate assays to measure pharmacodynamic effects of rivaroxaban on the coagulation in routine practice. We therefore recommend performing calibrated PT as a screening test and if value exceeds specific cut-offs, calibrated anti-factor Xa chromogenic assays should be done. Rivaroxaban also influenced routine coagulation assays such as measurement of clotting factor, proteins C and S, antithrombin, activated protein-C resistance, as well as determination of lupus anticoagulant both depending on the reagent and the method that is used. The time between the drug intake and the sampling is primary to interpret correctly the results. Relevant data on drug levels associated with approximate therapeutic and harmful ranges have been proposed in this study but require confirmation from specific pharmacokinetic data in patients with recurrent thrombosis or bleedings to minimize the risk associated with rivaroxaban.

Conflict of interest disclosures

The authors declare no competing financial interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.thromres.2012.09.004.

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