Rivaroxaban and apixaban in orthopaedics: is there a difference in their plasma concentrations and anticoagulant effects?

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The aim of this study was to improve knowledge of what happens in the coagulation of orthopaedic patients under rivaroxaban and apixaban, in order to finalize and cross-validate effective measurement methods and to provide arguments for helping to reference one or the other drug in our central pharmacy. One hundred and two patients undergoing total hip or knee replacement were included. Half of them received rivaroxaban and the other half received apixaban. Blood samples ($n = 244$ with each drug) were taken at C_{max} preoperatively and twice a week, apart from the day of the patient's discharge, when C_{trough} concentration was targeted. Routine coagulation parameters, and functional and liquid chromatography tandem mass spectrometry assays for measurement of circulating concentrations were studied. The LC-MS/MS assay and the functional assays carried out in patients under routine conditions were highly correlated, apart from low concentrations (<30 ng/ml), which were affected by the variable individual potential to inhibit the exogenous bovine Xa used in the functional assays. After 1 week of treatment, the drugs differed: C_{max} and C_{trough} were closer when apixaban was taken twice daily (83 ± 39 and 58 ± 17 ng/ml) than with rivaroxaban taken once a day (113 ± 67 and 13 ± 20 ng/ml). Rivaroxaban had a greater influence on routine coagulation tests and reduced the maximum thrombin concentration more efficiently, as assessed by the thrombin generation test. Although rivaroxaban and apixaban present apparently similar constant rates, they exhibit significant differences in their concentrations and anticoagulant effects when studied *ex vivo* in orthopedic patients. *Blood Coagul Fibrinolysis* 26:925–933

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Introduction

Rivaroxaban and apixaban are two direct oral anticoagulants (DOACs) whose target specificity is factor Xa, which is why they are referred to here as Xabans. Although the use of these drugs is tending to be trivialized in clinical practice, and although DOACs are demonstrated to be associated with a lower rate of intracerebral haemorrhages relative to warfarin in spite of the availability of a VKA-antidote, their powerful anticoagulant capacity remains a concern, especially since no antidote is available. They are also a major concern in the laboratory, where they interfere with almost all coagulation results [1–12], as recently summarized in [13].

Although not required for most patients, knowledge of circulating drug concentrations can be crucial in rare circumstances, when it strongly influences patient management. It is surprising how few clinical studies undertaken independently by the drug industry have been published with circulating concentrations found in patients [14–18].

Our study was thus sparked by the following:

(1) The need to solve a practical question: Should our institution include both rivaroxaban and apixaban in the hospital formulary for patients undergoing major orthopaedic surgery?
(2) The need to establish the alterations they induce in coagulation parameters. As an increasing number of patients enter hospital while receiving chronic DOAC treatment, it is now a routine prerequisite for the correct interpretation of results to introduce DOAC-induced alterations into coagulation algorithms.
(3) The need to evaluate routine methods in order to determine the circulating concentrations of rivaroxaban and apixaban. Our recent experience in dramatic clinical situations demonstrates how useful rapid information on circulating concentrations can be. A recent study indicating that the individual benefit–risk might be improved by tailoring the dose of the factor IIa inhibitor, dabigatran [19], is expected

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to modify the initial provider's communication. We implemented rapid 24 h/24 available functional methods for each DOAC and a mass spectrometry method able to measure all locally available DOACs (rivaroxaban, apixaban, but did so dabigatran) in a single run, so that we could cross-validate both approaches.

Patients and methods

Patients

This observational, nonrandomized, one-period comparison study was conducted on behalf of the Committee for Health Products and Therapeutic Innovation (CoPSIT) of the University Hospital of Bordeaux before referencing the new DOACs in the hospital formulary. Half of the 102 patients included received rivaroxaban (Xarelto from Bayer) and half of them received apixaban (Eliquis; Bristol-Myers Squibb). Pseudo-randomization was performed by geographic localization of the patients. The orthopaedic ward covers two floors in our hospital and a single team of anaesthetists manages the patients. The nurses from the one floor had a provision of rivaroxaban and the nurses on the other one a provision of apixaban.

Rivaroxaban was given at 10 mg dosing in all patients whatever their age, provided that creatinine clearance was higher than 30 ml/min and that they were not being treated by azole antifungals or by the protease inhibitor ritonavir. Apixaban was given at 2.5 mg twice daily. The first apixaban dose was given the morning after the day of surgery, thus 16–22 h after surgery according to the end of the operating time.

According to the recommendations of use, the first dose of rivaroxaban has to be given 'at least' 6–8 h after surgery, 'provided that hemostasis has been established'. As drains generally need a 24 h period to stop leaking, it was decided to align the first dose intake time of rivaroxaban on that of apixaban. Thus, all the patients included in our study received their first Xaban tablet the day after surgery at eight o'clock, 16–22 h after surgery. The patients were routinely sampled after introduction of the Xabans and gave their written informed consent to participate in the study, which was approved by the local ethical committee and in accordance with the Helsinki II declaration. An independent group of 50 samples from untreated patients was also recruited from 7 days of our daily routine to evaluate the rivaroxaban and apixaban concentrations found in untreated patients when using anti-Xa functional assays.

Laboratory variables

Blood samples were taken the day before surgery (T0), then 2 h ± 10 min after drug ingestion under a nurse's supervision (C_{max}) on the day after surgery (T1), on day 4 ± 1 (T2), and day 7 ± 1 (T3) after surgery. A final withdrawal was carried out the day of the patient's discharge. In order to obtain a residual concentration (C_{trough}) after a treatment period of about a week (day 8 ± 1, T4), withdrawal was performed just before the morning drug ingestion. T0–T4 are referred to as 'times of withdrawals' hereafter.

The number of samples obtained was 244 in patients receiving rivaroxaban (T0 : 51/T1 : 50/T2 : 50/T3 : 45/T4 : 48) and 244 in patients receiving apixaban (T0 : 51/T1 : 49/T2 : 50/T3 : 49/T4 : 45).

Coagulation parameters were performed on an ACL Top from Werfen/Instrumentation Laboratory (IL, Bedford, Massachusetts, USA). Activated partial thromboplastin clotting time (aPTT, in seconds) was measured using HemosIL APTT-SP from IL, prothrombin time (PT, in seconds) using HemosIL RecombiPlasTin 2G from IL, and antithrombin was measured using HemosIL Liquid Antithrombin IL. D-dimers (ng/ml) were measured using the VIDAS D-Dimer Exclusion test (bioMérieux SA, Marcy l'Etoile, France). Thrombin generation test (TGT) was carried out on a Thrombinoscope system (Diagnostica Stago, Asnieres-sur-Seine, France).

Serum creatinine was measured using the Beckman Coulter reagent on an Olympus AU 5400 (Beckman Coulter, Brea, California, USA), and creatinine clearance was calculated using the Cockcroft–Gault formula (ml/min).

Drug-circulating concentrations were functionally measured by an anti-Xa test carried out in the daily routine at the same time as the other coagulation tests. Our methods have been available in the core laboratory 24 h/24 since 2009 for rivaroxaban, and since the end of 2012 for apixaban. Mass spectrometry measurements were carried out in six series after the end of patients' inclusion from samples frozen at −80°C for a 3–9-month period.

The rivaroxaban anti-Xa test used commercially available standards and controls (Rivaroxaban Plasma Calibrator and Rivaroxaban Control Plasma from Hyphen Biomed, Neuville-sur-Oise, France) and Chromogenix Coamatic Heparin (IL) as previously described [14].

As commercialized apixaban calibrators became available only in early 2014 after the end of inclusion, the apixaban anti-Xa test was carried out using home-made calibrators obtained by spiking a normal plasma pool (CRYOcheck pooled normal plasma from Cryopep, Montpellier, France) with appropriate concentrations of apixaban obtained free of charge from BMS (Princeton, New Jersey, USA).

Plasma concentrations of rivaroxaban and apixaban were also determined using liquid chromatography tandem mass spectrometry (LC-MS/MS) validated according to the US Food and Drug Administration (FDA) guidelines [20]. Rivaroxaban, [$^{13}C_6$]-rivaroxaban, apixaban, and [^{13}C, $^{2}H_7$]-apixaban were purchased from Alsachim

(Strasburg, France). Sample preparations consisted of mixing 300 µl methanol containing internal standards and 100 µl of plasma sample in Captiva ND ^{lipids} 96-well plates (Agilent Technologies, Santa Clara, California, USA). After centrifugation, 10 µl of this mix was injected into the LC-MS/MS system. Chromatographic separation of the analytes was achieved on an Atlantis dc18 5 µm 150 mm column (Waters) using a gradient run with ammonium formate (4 mmol/l, pH 4.2) and acetonitrile. The analytes were detected using a Waters Quattro Micro mass spectrophotometer operating in positive electrospray ionization (ESI) utilizing multiple reaction monitoring (MRM). The calibration curves for rivaroxaban and apixaban in plasma were linear over the range 5–500 ng/ml, and the lower limit of quantification (LLOQ) was estimated at 5 ng/ml. Validation experiments with three levels of control samples (15, 250, and 350 ng/ml) on three different runs of five determinations showed an interassay precision between 8.1 and 14.3%, and an interassay accuracy within the confidence range (±20%).

Statistics

Owing to the non-normality found for half of the parameters when using the formula from Tabachnick and Fidell [21], the Mann–Whitney *U* test was used for comparison of independent parameters (patients receiving rivaroxaban versus patients receiving apixaban) and the Wilcoxon signed-rank test for repeated measures in rivaroxaban or apixaban-receiving patients. Table 1 shows the median, first, and third quartile of the parameters. The strength of the relationship between the two parameters was studied by a linear regression model fitted using the least square approach.

Results

Fifty-one patients received rivaroxaban (sex ratio 0.82 M/F, mean age 69 ± 11 years) and 51 patients received apixaban (sex ratio 0.54, mean age 65 ± 12 years). Their mean hospital stay was 8 ± 1 days for total hip replacement and 8 ± 2 days for total knee replacement. None of the parameters studied was different between the two drugs before treatment at T0. Clinically, the groups were similar in terms of sex, age, BMI and renal function as reflected by their creatinine clearance (Table 1). Interestingly, renal function improved significantly both rapidly and steadily after hospitalization (at T1, and after), probably owing to better patient hydration. No thrombotic event or bleeding occurred during the study. Two patients were not included during the study period (20 December 2012 to 6 June 2013), owing to inadequate venous access for blood withdrawal.

Rivaroxaban and apixaban concentration results in orthopaedic patients according to time of withdrawal (T0 to T4)

Figure 1 shows rivaroxaban and apixaban plasma concentrations measured by using LC-MS/MS according to withdrawal time. The C_{trough} summarized at T4 (Fig. 1a and b) include data from D6 to D10. Residual concentrations were very low in all patients receiving rivaroxaban, except in one who was not an outlier at the other sampling times during his hospital stay (see the dotted line). On the contrary, residual apixaban concentrations were closer to the peak concentrations than those of rivaroxaban. The peak concentrations from T1 to T3 were highly variable among the patients and also in a given patient. The mean peak concentration at T1 was lower than the peak concentrations in the following periods, so steady state was not attained before day 4.

Similar graphs were obtained by using the functional anti-Xa assay (data not shown). Figure 2a illustrates the good correlation between mass spectrometry and anti-Xa results, with *r* values higher than 0.900. Results were very similar with both methods, although the paired Student's test showed a significant difference between functional and physical assays for apixaban at T1 and T4, in which case the mean values were 20 versus 30 and 48 versus 56 ng/ml, respectively – a difference which is not clinically relevant.

At T0, mass spectrometry data were always lower than the detection limit (5 ng/ml), which was transformed into a zero value for all the quantitative tests. On the contrary, mean concentrations observed with the anti-Xa functional tests at T0 (before starting the treatment) were 4.2 ± 7.6 ng/ml (range 0–25 ng/ml), with rivaroxaban and 6 ± 7.5 ng/ml (range 0–24 ng/ml) with apixaban. The results observed in an independent additional group of 50 patients not receiving any drug with any anti-Xa effect (neither rivaroxaban, apixaban, or any medication in the heparin family) were within a range of 0–31 ng/ml for rivaroxaban and 0–27 ng/ml for apixaban.

At T1, after the first intake of the drug, rivaroxaban induced higher early concentrations, a finding consistent with the higher dose intake provided by the once-a-day prescription scheme. The difference in C_{max} values persisted thereafter, although to a lesser extent (except for anti-Xa results at T2). On the contrary, the C_{trough} measured at T4 was notably higher in patients receiving apixaban twice daily, thus attesting to the smaller difference between peak and trough concentrations. Moreover, the SDs from T1 to T3 were 30–50% higher in rivaroxaban-treated patients, as shown in Fig. 1c and 2d. Mean concentrations became steady from day 4, although this was not the case at an individual level (see Fig. 1a and b).

Interactions of rivaroxaban and apixaban with routine coagulation tests in orthopaedic patients

Functional antithrombin measurement is another assay based on exogenous factor Xa inactivation. Table 1 shows that, after an expected modest postsurgery decrease at T1, antithrombin levels increased in the presence of the

Table 1 **Median, 25th percentile, and 75th percentile of clinical and laboratory parameters**

	Rivaroxaban		Apixaban
Age			
	70 [63 – 75]	NS	67 [55 – 75]
BMI			
	27 [24 – 30]	NS	27 [23 – 30]
ClCr			
T0	82 [67 – 104] ***	NS	87 [71 – 110] ***
T1	86 [72 – 116] *	NS	104 [84 – 124] NS
T2	99 [78 – 116] NS	NS	112 [85 – 142] NS
T3	97 [81 – 140] *	NS	97 [84 – 126] NS
T4	96 [79 – 131]	NS	107 [91 – 138]
PT. sec (Norm = 111±15. RtR CV<4%)			
T0	10.5 [9.9 – 10.9] ***	NS	10.2 [10.1 – 11] ***
T1	15.2 [14 – 16.7] NS	***	12.8 [11.4 – 13.7] NS
T2	16.8 [14.3 – 18.1] NS	***	13.2 [12.7 – 14.3] NS
T3	14.8 [13.2 – 17.5] ***	***	13.1 [12.6 – 13.7] ***
T4	12 [11.7 – 12.9]	NS	12.4 [12.1 – 13.2]
aPTT. sec (Norm = 31±3.3. RtR CV<3%)			
T0	30 [28 – 32] ***	NS	29 [27 – 32] **
T1	38 [34 – 41] ***	***	32 [30 – 36] ***
T2	45 [38 – 51] NS	***	35 [32 – 39] NS
T3	43 [37 – 48] ***	***	35 [32 – 38] NS
T4	34 [33 – 39]	NS	34 [31 – 38]
FgC. g/l (Norm = 2.8±0.6. RtR CV<5%)			
T0	3 [2.7 – 3.6] ***	NS	3.1 [2.6 – 4] *
T1	4 [3.7 – 4.6] ***	NS	3.7 [3.5 – 4.2] ***
T2	7 [5.9 – 8] NS	NS	6.8 [6.3 – 8.1] NS
T3	7.7 [6.8 – 8.2] NS	NS	6.9 [6.3 – 7.9] NS
T4	7 [6.1 – 7.5]	NS	6.4 [6 – 8.1]
D–Dimer ng/ml (Norm = 395±256. RtR CV<7%)			
T0	736 [523 – 1276] ***	NS	669 [500 – 1258] ***
T1	6012 [3757 – 11111] ***	NS	4994 [2615 – 11111] ***
T2	3107 [2124 – 3886] ***	*	2348 [1575 – 3289] ***
T3	4084 [2979 – 4959] NS	*	2992 [2198 – 4312] **
T4	3919 [2787 – 5291]	NS	3276 [2215 – 4252]
AT. % (Norm = 97±12. RtR CV<5%)			
T0	105 [98 – 114] ***	NS	109 [101 – 119] ***
T1	94 [88 – 101] ***	NS	94 [85 – 98] ***
T2	108 [99 – 120] ***	NS	112 [100 – 121] ***
T3	122 [113 – 129] ***	NS	122 [114 – 134] *
T4	111 [103 – 118]	*	120 [111 – 125]

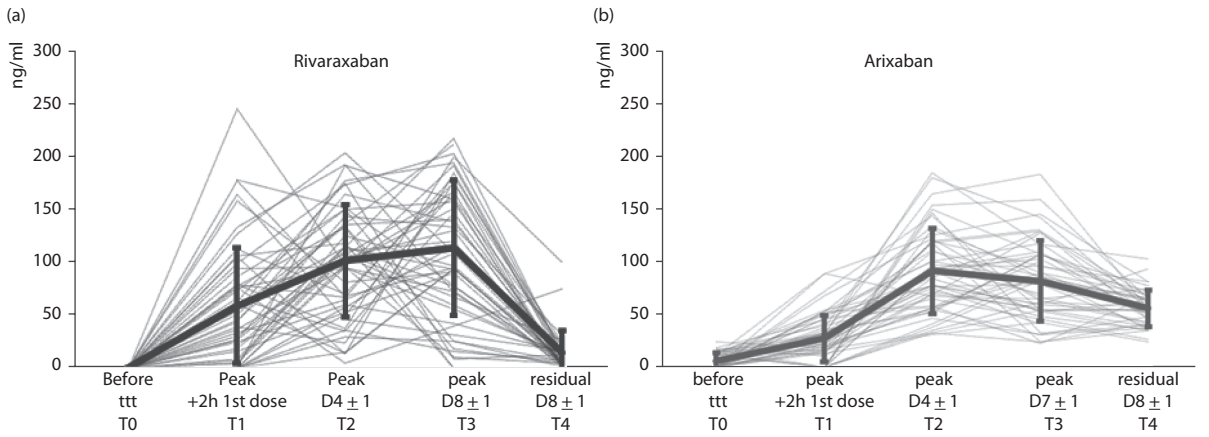
Tn → Wilcoxon → Tn+1 [R]; Tn → Wilcoxon → Tn+1 [A]; [R] ← Mann–whitney → [A]

* $p < 0.025$
** $p < 0.001$
*** $p < 0.0001$

	Rivaroxaban		Apixaban
LC–MS/MS. ng/ml (see text for performances)			
T0	0 [0 – 0] ***	NS	0 [0 – 0] ***
T1	53 [17 – 80] **	***	11 [0 – 31] ***
T2	102 [58 – 132] NS	***	85 [70 – 107] NS
T3	113 [71 – 162] ***	***	76 [52 – 107] ***
T4	7 [4 – 14]	***	45 [35 – 57]
antiXa. ng/ml (see text for performances)			
T0	0 [0 – 8] ***	NS	0 [0 – 13] ***
T1	48 [20 – 80] **	***	23 [13 – 40] **
T2	92 [49 – 134] NS	NS	90 [58 – 121] NS
T3	113 [67 – 159] ***	**	83 [54 – 109] ***
T4	11 [0 – 20]	***	56 [42 – 67]
TGT: Lag Time sec (Norm = 2.7±0.4. RtR CV<7%)			
T0	3 [2.7 – 3.3] ***	NS	3 [2.8 – 3.4] *
T1	5.3 [4.3 – 6.9] ***	***	3.2 [2.9 – 4] ***
T2	7.2 [6.2 – 7.9] ***	***	5.2 [4.3 – 6.2] *
T3	9.1 [7.7 – 10.9] ***	***	5.7 [4.8 – 6.3] **
T4	5.2 [4.2 – 6.3]	NS	5.1 [4.6 – 6]
TGT: ETP nM×min (Norm = 1455±218. RtR CV<12%)			
T0	1631 [1379 – 1834] ***	NS	1737 [1512 – 1884] ***
T1	1309 [1110 – 1475] NS	**	1515 [1326 – 1679] NS
T2	1333 [1144 – 1521] NS	**	1516 [1375 – 1791] *
T3	1291 [1060 – 1588] ***	**	1599 [1370 – 1942] NS
T4	1645 [1444 – 1944]	NS	1641 [1324 – 1899]
TGT: Cmax nM (Norm = 207±55. RtR CV<15%)			
T0	290 [250 – 345] ***	NS	317 [278 – 371] ***
T1	162 [100 – 224] NS	***	296 [264 – 319] ***
T2	118 [91 – 181] NS	***	219 [194 – 276] ***
T3	105 [74 – 142] ***	***	266 [172 – 338] NS
T4	321 [235 – 378]	NS	294 [224 – 336]
TGT: velocity nM/min (Norm = 140±37. RtR CV<12%)			
T0	111 [82 – 143] ***	NS	126 [93 – 157] NS
T1	48 [21 – 78] NS	***	131 [103 – 147] ***
T2	35 [23 – 59] NS	**	89 [65 – 108] ***
T3	31 [19 – 43] ***	**	111 [68 – 137] NS
T4	135 [79 – 174]	NS	126 [77 – 148]

The Wilcoxon signed-rank test was used for repeated measures (Tn versus Tn + 1 in rivaroxaban or apixaban-treated patients), and the Mann–Whitney *U* test was used for independent parameters, that is, for comparison between patients receiving rivaroxaban or receiving apixaban (rivaroxaban versus apixaban at each Tn). Tn represents one of the five periods of time from T0 to T4, Tn + 1 represents time the next to Tn (e.g. Tn = T0, Tn + 1 = T1). The normal values obtained from 50 healthy volunteers, 25 men and 25 women, are given to the right of each laboratory parameter name (except for D-dimers, where samples from 20 volunteers were assessed). The coefficient of variation of the run-to run precision (RtR CV) is also given in order to reflect the real performance of the tests when run routinely. As two machines ensure the routine parameters, the higher coefficient of variation was chosen when different.

Fig. 1

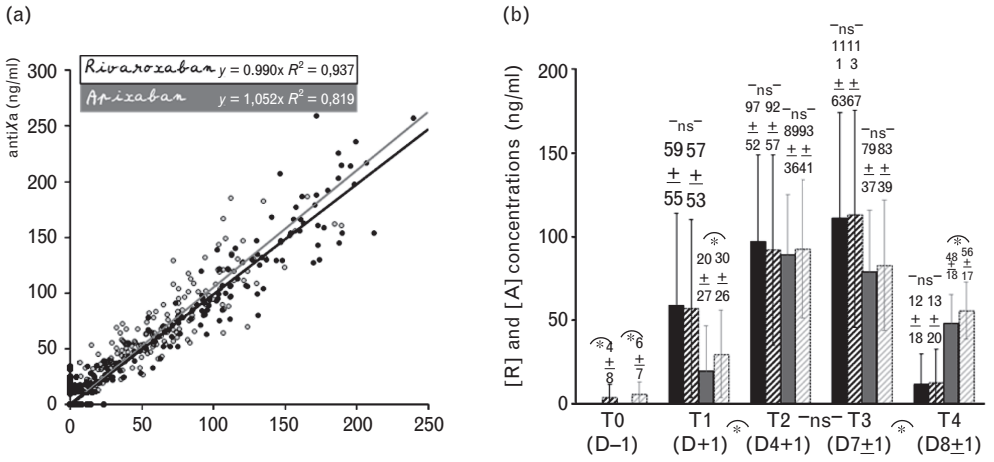


Rivaroxaban and apixaban plasma concentrations as measured by using mass spectrometry according to withdrawal time grouped into five periods of time from T0 to T4. The bold lines represent the mean values and SDs.

Xabans. The 53 samples from T1 to T4 exhibiting rivaroxaban plasma concentrations higher than 100 ng/ml testify to a mean antithrombin increase of 24% when compared to the 51 pretreatment T0 samples ($P = 0.007$), whereas the 39 samples exhibiting apixaban plasma concentrations higher than 100 ng/ml demonstrate a mean antithrombin increase of 18% ($P = 0.007$ and 0.0002, respectively, unpaired *t*-test).

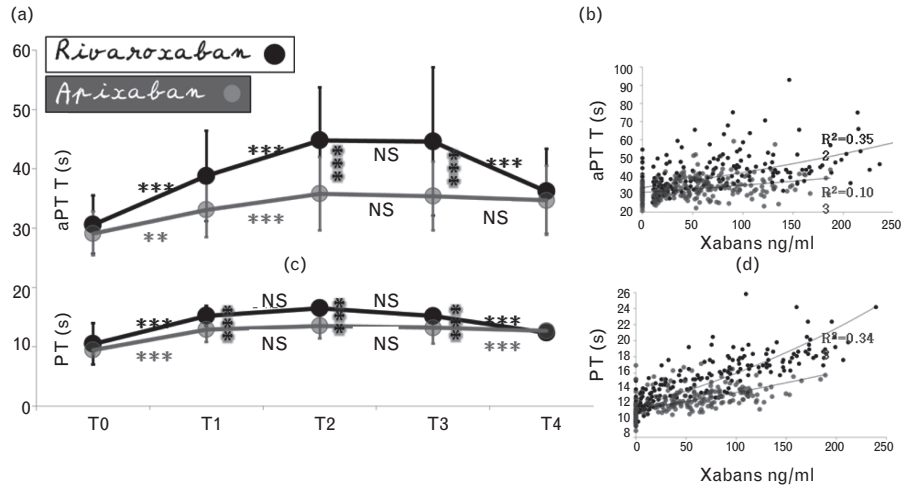
Figure 3 illustrates the relative sensitivity of PT and aPTT to both drugs according to the time of withdrawal. Correlations between aPTT and Xabans concentrations were very poor (Fig. 3b). Significance at *P* value less than 0.01 was reached only with rivaroxaban ($r^2 = 0.352$). However, Table 1 and Fig. 3 demonstrate a significant increase between T0 and T1, and then between T1 and T2, under both rivaroxaban and apixaban. At peak concentration and at steady state (T2–T3), the mean increase in aPTT was 6 s in patients receiving apixaban, whereas rivaroxaban induced a larger mean increase of 15 s. Although modest, the aPTT increase remained highly significant when comparing T0 and

Fig. 2



(a) On the left, a linear regression model fitted using the least square approach shows the relationship between the two methods for Xabans concentrations (black circles for rivaroxaban and gray circles for apixaban): mass spectrometry (LC-MS/MS) on the X-axis and the functional anti-Xa tests on the Y-axis. (b) On the right, the histograms represent the arithmetic mean values and SDs of concentrations obtained with both drugs by mass spectrometry and by the anti-Xa functional assays at the different periods. Black solid bars (■ LC-MS/MS) and black hatched (▨ anti-Xa) bars represent the results from rivaroxaban-treated patients. Gray solid (■ LC-MS/MS) and gray hatched (▨ anti-Xa) bars represent the results from apixaban-treated patients. * when significant (Student's *t*-test $P < 0.05$).

Fig. 3



(a) Activated partial thromboplastin time (aPTT) and prothrombin time (PT) evolution (mean and SD) according to the time of withdrawal in rivaroxaban (black circles) and apixaban (gray circles)-treated patients. Wilcoxon test significance (changes between two successive times in rivaroxaban or apixaban-treated patients) is represented by horizontal stars (**$P<0.001$, ***$P<0.0001$), whereas Mann–Whitney test significance (rivaroxaban versus apixaban treatment at each time) is represented by shaded vertical stars (⋮ $P<0.0001$). On the right, aPTT (b) and PT (d) correlations with rivaroxaban (black circles) and apixaban (grey circles) as measured by mass spectrometry. Best fitting results from a power function ($y = a \times x^b$), that is, a linear function between the log-transformed PT or aPTT values and the untransformed Xabans concentrations. *<0.025, **≤0.001, *** < 0.0001.

T4 at residual concentrations ($P<0.0001$ for both drugs).

Prothrombin time correlated relatively well with rivaroxaban concentrations ($r^2=0.724$, Fig. 3c), but very poorly with apixaban concentrations ($r^2=0.343$, Fig. 3c). Normal PT ratios (<1.30) were found in patients with concentrations as high as 111 ng/ml for rivaroxaban (PTr 1.19, Quick's value 79%) and 153 ng/ml for apixaban (PTr 1.21, Quick's value 77%). The lower increase in PT values in apixaban-receiving patients is illustrated in Fig. 3a and 4c and in Table 1.

As expected, fibrinogen increased in the postoperative setting, but there was no difference between the increase observed with the two Xabans. Interestingly, D-dimers also increased postoperatively as expected, but the levels observed at T2, T3, and T4 were lower in patients receiving apixaban.

Interactions of rivaroxaban and apixaban with the thrombin generation test in orthopaedic patients and *in vitro*

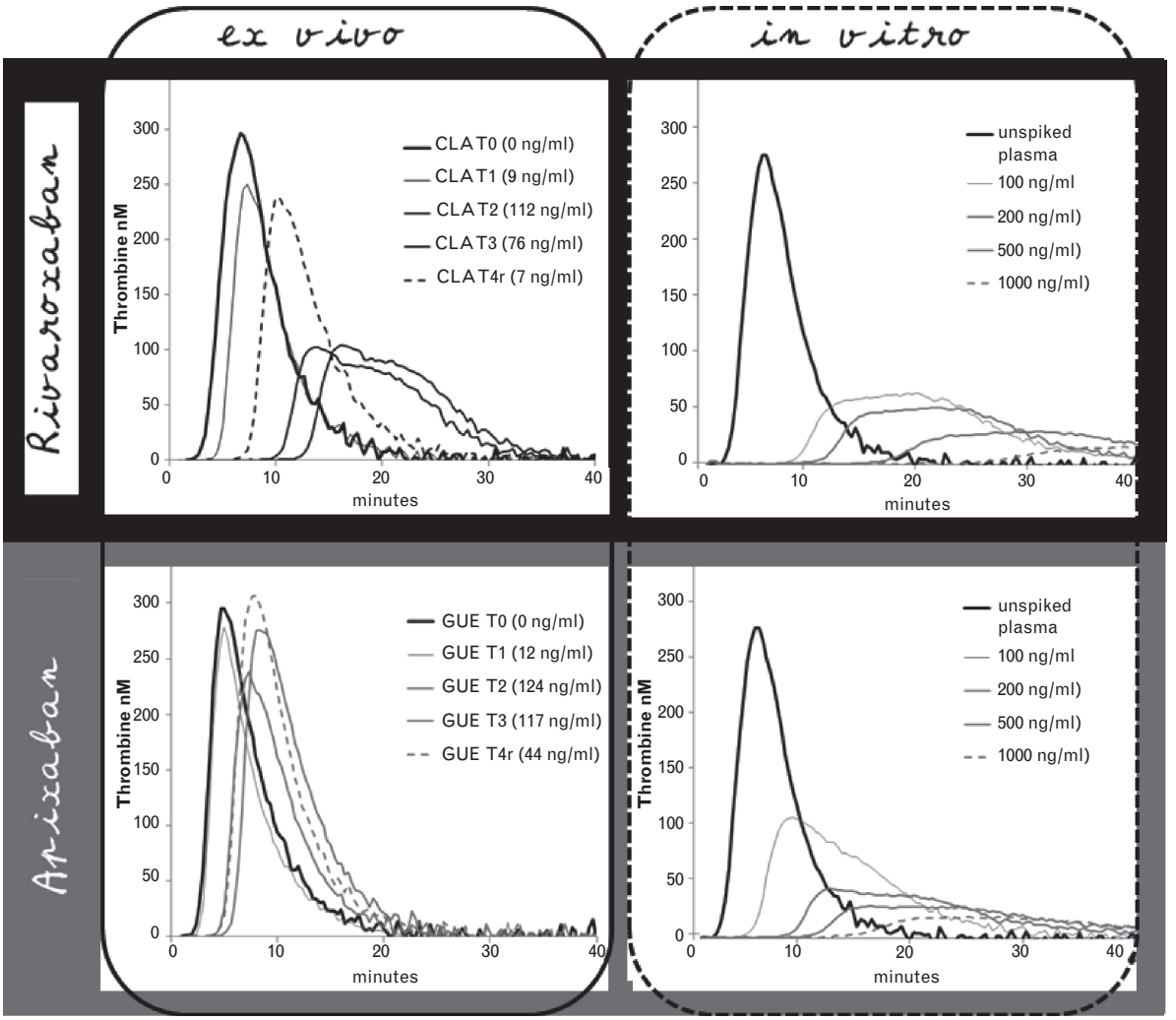
Effects on kinetic parameters (lag time and peak time) and C_{max} resulted in a dramatically altered velocity in all samples from rivaroxaban-treated patients, whereas it was moderately yet significantly affected by apixaban from T2 only (Table 1). Figure 4 shows the TGT follow-up of two representative patients: patient C.L.A. receiving rivaroxaban and patient G.U.E. receiving apixaban, both selected for TGT values near the median values of their treatment group, as shown in Table 1. Whereas the thrombin maximum concentration (C_{max}, Table 1 and example in Fig. 4) was dramatically decreased at T2 and T3 with rivaroxaban treatment, it remained almost unchanged in patients receiving apixaban. On the contrary, both drugs delayed thrombin generation, although to a lesser extent for apixaban. Surprisingly, the TGT observed in the ex-vivo samples from patients receiving apixaban only partly reflect the in-vitro alterations observed in plasmas spiked with increasing concentrations of apixaban (from zero = control to 1000 ng/ml), which are more closely in line with those observed with rivaroxaban, both *in vitro* and *ex vivo*.

Discussion

Functional drug measurements of the Xabans have been available in our laboratory since 2009, when rivaroxaban and dabigatran became available. Indeed, we were convinced from the outset that a concentration measurement should be available 24 h/24 in our hospital laboratory to address rare but difficult clinical situations in DOAC-treated patients, for example, acute hemorrhage or emergency surgery, and the reality confirmed our initial concerns. Other indications may arise in the future [22].

The LC-MS/MS measurements made it possible to validate our functional assays for rivaroxaban and apixaban measurements. The correlation of the functional anti-Xa results with the mass spectrometry measurements was very satisfactory and in line with those published by

Fig. 4



Thrombin generation test curves showing the concentration of thrombin (in nmol/l, Y-axis) according to the time (min, X-axis) after stimulation by a low concentration of tissue factor in the presence of phospholipids. The two upper graphs represent rivaroxaban-treated samples and the two lower graphs represent apixaban-treated samples. The two left-hand graphs represent the TGT curves obtained from a representative rivaroxaban-treated patient (top left graph) and from a representative apixaban-treated patient (bottom left graph) at the different times of their laboratory follow-up (T0 to T4, the corresponding drug concentrations are indicated), whereas the two right-hand graphs represent the TGT curves obtained with the same drugs, but after in-vitro spiking.

others [17,23,24]. However, the rivaroxaban and apixaban results observed in the study patients at T0, and in 50 other untreated patients, were spread over a 0–30 ng/ml range. That means that, whereas our anti-Xa [R] and apixaban determinations are very reliable in the 30–600 ng/ml range, they cannot be trusted to recognize a physiological versus a therapeutic anti-Xa effect in the 0–30 ng/ml zone, in which case LC-MS/MS is indisputably more reliable. In the clinical setting, the anti-Xa test remains the only one that can be used for patient management, provided that the 0–30 ng/ml is accepted to be a 'gray zone', where no information can be drawn from the result concerning the presence of the drug.

Thus, our results strengthen our confidence in the anti-Xa functional approach, which is rapid (5 min from the time the plasma is available), simple, and inexpensive by using a reagent already present on our machines for heparin measurement. The extra costs are limited to the calibrations and control kits.

The rivaroxaban concentrations found in our study are in line with the previously published data [14,25,26]. These values are about two-fold higher than the C_{max} reached at the first drug intake, the day after surgery, a situation when the drug pharmacokinetics seems to be altered. As we systematically carried out the withdrawals 2 h after the

drug intake, we hypothesize that we sometimes missed from the 'true' C_{max}, although Douxfils *et al.* recently demonstrated in patients treated with rivaroxaban for atrial fibrillation or venous thrombosis that concentrations are similar, irrespective of whether the blood is taken 2 or 3 h after intake [27]. However, an altered postoperative drug absorption may participate in the very high inter-individual variation in the C_{max}, which culminates at T1 (coefficient of variation around 100%), but remains high at T2 and T3 (coefficient of variation around 50%).

For apixaban, a single dose of 2.5 mg oral solution produced a geometric mean C_{max} of 52.5 ± 35 ng/ml in male volunteers [29], which is higher than the arithmetic mean obtained in our study at T1 in the postsurgery context (Fig. 2). However, the C_{max} observed at later times (T2–T3 C_{max} pooled data, geometric mean 75 ± 37 ng/ml; T4 C_{trough} data, geometric mean 54 ± 17 ng/ml) are in line with those modeled [28] from the data in a phase II study in orthopedics [30]. Recently, a study in healthy Japanese individuals demonstrated that a C_{max} of 51 ± 25 ng/ml was observed after a first intake of 2.5 mg apixaban, whereas the C_{max} was 84 ng/ml after 7 days of a 2.5-mg twice-daily treatment, indicating the need for a minimum period of 3 treatment days before reaching the steady state [31].

Knowing the circulating concentrations found in the patients throughout the study made it possible to relate them to the other coagulation parameters, as summarized in Table 1 and illustrated in Figs. 3 and 4. We recognized early on that routine coagulation tests were poor predictors of circulating concentrations of rivaroxaban [14], and this notion is confirmed here. Numerous in-vitro studies have already shown that Xabans-induced alterations of coagulation parameters are highly dependent, not only on the reagents used [2,3,5–7,10,32,33] but also on the Xaban itself. The present ex-vivo data in orthopaedic surgery patients show that apixaban is a poorer routine coagulation test modifier than rivaroxaban, as already shown *in vitro* [7,10] and *ex vivo* in normal volunteers [28,31].

Rivaroxaban and apixaban affected the TGT very differently (Table 1 and Fig. 4). In a nutshell, ex-vivo apixaban-induced alterations of the TGT look more like dabigatran-induced alterations than rivaroxaban-induced alterations. What is puzzling is that in-vitro spiking of plasmas with apixaban resulted in a profile similar to that obtained from in-vitro-spiked plasmas with rivaroxaban. Although both drugs present similar affinity, association, and dissociation rate constants for free factor Xa, they differ in their pharmacodynamics, as described *in vitro* by Perzborn *et al.* [34]. Free factor Xa does not cleave prothrombin at a rate that is sufficient to support hemostasis [35], and the major target for the DOACs is the Xa embedded in the prothrombinase complex. Sinha *et al.* [36] demonstrated that anti-Xa drugs with similar Ki towards free factor Xa can differ significantly for prothrombinase complex inhibition, but Perzborn *et al.*'s results also suggest a high similarity in the rate constants for prothrombinase-bound factor Xa. The mystery of the mechanisms underlying the highly different pharmacodynamics results in response to drugs exhibiting apparently comparable constant rates remains, but our ex-vivo results confirm the difference in the effect of both drugs on coagulation.

There were some limitations in our study. While the anti-Xa dosages were carried out as the samples arrived over 117 different days in the middle of routine samples tested by more than 20 technicians using a series of at least 12 different reagents, the mass spectrometry measurements were carried out *a posteriori* from $-80\,^{\circ}C$ frozen samples by a single operator and in only six series. Correlations between the two approaches would probably have been better if we had carried out the anti-Xa measurements in a few series from frozen samples. However, our study design reflects real-life conditions and is the best estimate of expected performances. Edoxaban was not included in our study because it is not yet available in France. The questions addressed by our study concerning rivaroxaban and apixaban will be the same and will require a similar examination. This observational, one-period comparative study was nonrandomized. However, only extra-medical circumstances affected assignment to the rivaroxaban or apixaban patient groups. Thus, this limitation is not expected to influence the overall outcome of our study.

Our study allowed us to throw light on some questions, if not to answer all of them, which are listed as follows:

(1) We precisely defined which alterations in our routine coagulation parameters are expected in orthopaedic patients receiving rivaroxaban and apixaban Xabans, and those alterations are now included in our decision trees for patient management.
(2) This study was a great opportunity to validate our functional methods for measuring Xabans concentrations from clinical samples – thanks to the comparison with the results of the physical LC-MS/MS approach. The results are excellent and make it possible to easily carry out rivaroxaban and apixaban measurement in the daily practice. The prescription must, however, remain restricted to the real needs, which are scarce.
(3) We dashed our pharmacists' hopes of reducing their medication list from potentially redundant drugs by demonstrating that rivaroxaban and apixaban concentrations and coagulation effects differ.

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Conflicts of interest

There are no conflicts of interest.

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