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Effects of thromboprophylactic doses of apixaban and rivaroxaban on coagulation and thrombin generation in association with total hip replacement

Tuukka A. Helin[1] · Lauri Virtanen[2] · Mikko Manninen[3] · Jarkko Leskinen[4] · Juhana Leppilahti[5] · Lotta Joutsi-Korhonen[1] · Riitta Lassila[2,6]

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Abstract Factor Xa inhibitors (FXaI) apixaban and rivaroxaban are used for thromboprophylaxis after major elective orthopaedic surgery. Because few patient sample studies exist, we postoperatively assessed patients undergoing unilateral total hip arthroplasty, including 22 treated with apixaban (2.5 mg BID) and 20 treated with rivaroxaban (10 mg OD). We collected blood samples before and 3 h after drug intake at 4 time points, preoperatively, as well as on day 1, week 1 (day 2–8) and day 28 post-operation. APTT and PT were immediately analysed. Calibrated anti-FXa activity, Russel's Viper Venom Time (RVVT) and thrombin generation (TG; Calibrated Automated Thrombogram®) captured the effects of FXaI on coagulation and TG. APTT and PT remained within the reference interval throughout, and did not correlate with FXaI levels (PT $R^2 = 0.44$, APTT $R^2 = 0.07$). Mean apixaban concentration at the peak varied by eightfold (19–153 ng/mL), but rivaroxaban only by 1.5-fold (111–183 ng/mL). Rivaroxaban, but not apixaban prolonged RVVT at peak levels. Both FXaIs had a prolonged lag time of TG ($p < 0.001$). Rivaroxaban decreased ETP peak at all time points and reached a minimum at day 28 (540 nM/min at rivaroxaban 184 ng/mL, $p < 0.001$), while rivaroxaban trough levels were low and ETP values normal. However, with apixaban, after an initial decrease, ETP did not differ between peak and trough levels until decreasing on day 28 at peak (990 nM/min at apixaban 112 ng/mL, $p = 0.005$). In conclusion, due to different dosing and pharmacology rivaroxaban and apixaban distinctly inhibited TG under postoperative conditions.

Keywords Anticoagulants · Apixaban · Blood coagulation tests · Drug monitoring · Rivaroxaban · Thrombin generation

Introduction

The direct FXa inhibitors (FXaI) apixaban and rivaroxaban are used for preventing deep venous thrombosis (DVT) after total hip arthroplasty (THA). The dosages for this indication are 2.5 mg BID for apixaban and 10 mg OD for rivaroxaban, and they have been reported to be superior to enoxaparin for thromboprophylaxis after THA [1, 2]. However, the clinical trials of these anticoagulants relied on fixed dosages without laboratory monitoring. Variable clinical circumstances, i.e., stable versus unstable conditions, the postoperative phase and bleeding complications, all added to the concentration ranges in the studies. Laboratory monitoring becomes vital during acute bleeding or thrombosis, upon anticoagulation reversal, prior to emergency surgery, or during kidney or liver failure. Additionally, in certain patients, especially the elderly, FXaI monitoring is beneficial [3, 4]. It is also important to assess the anticoagulation effects when considering reversal with an

✉ Riitta Lassila
riitta.lassila@hus.fi

1 Coagulation Disorders Unit, Clinical Chemistry, HUSLAB Laboratory Services, Helsinki University Hospital, Helsinki, Finland

2 Coagulation Disorders Unit, Hematology and Clinical Chemistry (HUSLAB Laboratory Services), Helsinki University Hospital, and University of Helsinki, POB 340, 00029 Helsinki, Finland

3 Orton Orthopaedic Hospital, Helsinki, Finland

4 Peijas Hospital, Helsinki University Hospital, Vantaa, Finland

5 Oulu University Hospital, Oulu, Finland

6 CSO of Aplagon Ltd, Helsinki, Finland

anti-Xa inhibitor antidote, currently in clinical trials [5]. Finally, the emergency interpretation is often complicated, when the individual baseline values are unknown.

Several laboratory assays may mirror anti-Xa effects. The minor impact of FXaIs on the coagulation screening assays of INR, PT, and APTT renders these measures inadequate for the drug detection [6, 7]. In contrast, the anti-Xa assay accurately measured apixaban and rivaroxaban with appropriate calibration [6, 8, 9]. Dilute Russel's Viper Venom Time (RVVT), commonly used as part of lupus anticoagulant testing, has the potential to detect and monitor FXaIs [10, 11]. Fluorogenic thrombin generation (TG) assay readily detects FXaI effects. In TG, FXaIs lengthen the lag time (time to initiation of thrombin formation) and diminish both the peak of thrombin generation and endogenous thrombin potential (ETP; total thrombin generated) [12, 13]. To assess thrombin formation more comprehensively, prothrombin fragment release using F1+ 2 assay can be used, which correlates with thrombin formation in vivo.

Our patient sample study examined the effects of thromboprophylactic doses of apixaban and rivaroxaban using a large panel of general and specific coagulation assays among unilateral THA patients. Although RVVT is thought to be less sensitive, it is widely available in routine in most laboratories so we aimed to evaluate it as a potential screening test. TG measures the overall net effect of anticoagulant drugs and native pro- and anticoagulants on the capacity to generate thrombin. It is a parameter increasingly linked to the development of VTE and has the potential to overcome the shortcomings of coagulation assays PT and APTT [14–16]. Anticoagulant effects were measured over a broad time period with 30-day follow-up. The aim was to gather data on the functional drug concentrations, and the effects of the postoperative condition on the drug levels, both screening and specific coagulation tests, and TG.

Patients and methods

We included patients undergoing THA (using the direct posterior surgery technique) with a planned 30-day thromboprophylaxis with either apixaban (n = 22) or rivaroxaban (n = 20). Patients using apixaban were recruited from the Kymenlaakso Central Hospital (Kotka, Finland, by MM), and patients using rivaroxaban were recruited from the Helsinki University Hospital Peijas (Vantaa, Finland, by JL). The ethical permit from the Oulu University Hospital mandated a nationwide study. Written informed consent was obtained from all individual participants who were included in the study. Apixaban was started on postoperative day 1, at 24 h after the operation, and no thromboprophylaxis was given on the day of the operation according to the summary of product characteristics. Rivaroxaban prophylaxis was also started on day 1, at 24 h after the operation. A single 40 mg dose of enoxaparin preceded rivaroxaban and was administered at 6–10 h after the surgery, which is the routine praxis in the orthopaedic ward to secure early anticoagulant exposure in case the patient experiences postoperative nausea. The doses were 2.5 mg BID for apixaban and 10 mg OD for rivaroxaban.

The exclusion criteria consisted active malignancy, persistent anticoagulation or antithrombotic medication (e.g., warfarin, LMWH, fondaparinux, clopidogrel, dipyridamole, prasugrel, ticagrelor, or any other antithrombotic medication except 100 mg aspirin OD), inflammatory bowel disease or rheumatic disease, phospholipid antibody syndrome or lupus anticoagulant, bleeding tendency, severe renal insufficiency (i.e., a glomerular filtration rate [GFR] <30 mL/min), liver disease, pregnancy, breastfeeding, a contraindication for apixaban or rivaroxaban and age less than 18 years. Patient medications were recorded during recruitment and follow-up. None of the patients used CYP3A4 or P-glycoprotein inhibiting drugs. A single-dose iv tranexamic acid 1 g was used during the operation to achieve perioperative hemostasis. All the patients used either acetaminophen or an NSAID post-operation.

Blood samples

Sample collection occurred at 7 time points: (A) pre-operation; (B) postoperative day 1 before and (C) 3 h after drug intake; (D) postoperative days 2–8 (week 1) before and (E) 3 h after drug intake; and (F) 1 month (day 28) post-operation before and (G) 3 h after drug intake. The samples were collected using a large-bore needle (20–21 G) and BD Vacutainer® blood tubes. Coagulation samples were collected in sodium citrate tubes (3.2%, 109 nM) according to the local hospital procedures. Blood cell count, C reactive protein (CRP), alanine aminotransferase (ALT), creatinine and GFR (MDRD formula), albumin, PT/INR, APTT, fibrinogen, antithrombin and D dimer were analysed immediately using the local hospital protocol. Additionally, citrated plasma separated by centrifugation (15 min, 2000 g) within 2 h of collection was stored at −40 °C for further analyses, including an anti-Xa assay (Berichrom®, Siemens Healthcare Diagnostics) calibrated with apixaban (Anti-Xa Apix, Aniara®) or rivaroxaban (Anti-Xa Riva, Aniara®), a FVIII clotting assay (FVIII:C, Pathromtin SL®, Siemens Healthcare Diagnostics), dilute RVVT (DVVTest 10®, Sekisui Diagnostics), prothrombin fragments (F1 + 2, Siemens) and thrombin generation with tissue factor (5 pM) without corn trypsin inhibitor using the Calibrated Automated Thrombogram (CAT®, Thrombinoscope, Netherlands) method. All 7 plasma samples from the same patient were run on a single TG plate to eliminate inter-experiment variability. Additionally, we analysed normal pooled plasma

controls at each TG run: The average lag time was 3.7 min (range 2.8–6.7); the endogenous thrombin potential (ETP) was 1295 nM/min (871–3095) and peak 167 nM (98–310).

Statistical analysis

For data analysis, the IBM SPSS® program (Version 24) was used. We compared parameter means between the FXaI groups using Mann–Whitney's U test because the normality of the data in this small sample size may be skewed. For longitudinal changes, pair-wise differences at time points were compared with the Wilcoxon test. Pearson's correlations were explored with a 2-tailed test for significance.

Results

Patient characteristics

The average age of the apixaban group was 68 years (range 52–82). Among the subjects, 12/22 subjects (55%) were women, and the average BMI was 27.2 kg/m^2 (20.0–40.0). The average age of the rivaroxaban group was 67 years (44–82). Among those subjects 15/20 (75%) were women, and they had an average BMI of 28.8 kg/m^2 (21.0–39.0). Overall, 4/22 patients in the apixaban group used ASA, and 5/20 in the rivaroxaban group used ASA. In the apixaban group, 3/22 and 4/22 patients used antidiabetic and statin medications, respectively, and in the rivaroxaban group, the corresponding patient numbers were 1/20 and 5/20. None of the patients had a history of VTE or experienced VTE during the study, nor were there postoperative bleeds during the 30-day follow-up.

Basic laboratory tests

Haemoglobin and albumin decreased post-operation in all subjects ($p < 0.001$), the lowest average values being 109 g/L (78–132) and 29 g/L (24–37); at week 1 in both men and women. These values were below the lower reference. Leukocytes and CRP initially increased in all patients, and then decreased to or towards normal at day 28 ($p < 0.001$). Platelet count remained within the reference intervals throughout the study. ALT and creatinine also stayed stable while remaining within the reference interval (Table 1). GFR was normal in most patients. A single patient using rivaroxaban had a decreased GFR (40 ml/min), which stayed stable.

Drug concentration analysis

Peak and trough levels of apixaban and rivaroxaban varied significantly in individual patients, and the mean values resembled earlier trial reports and summary of product characteristics. The apixaban peak was only 19 ng/mL (0–73) after the first dose. The peak at steady state reached a maximum of 135 ng/mL (17–301) at week 1, with a minimum trough of 46 ng/mL (0–103) at day 28. One patient had undetectable trough level on day 28. Rivaroxaban peaked at 111 ng/mL (7–267) after only the first dose; however, the single preceding enoxaparin dose could have potentiated this anti-Xa activity. The peaks at steady state reached a maximum of 184 ng/mL (81–355) at day 28, with a minimum trough of 16 ng/ml (0–48) at day 28 (Fig. 1), and 3 patients had unmeasurable levels. The drug concentrations differed between apixaban and rivaroxaban at all time points ($p < 0.001$). BMI, GFR and the use of ASA did not correlate with drug levels.

Table 1 Basic laboratory tests during the study period with mean values, 95% confidence intervals and local reference ranges

	Preop	Day 1	Week 1	Day 28	Ref. range
Hgb men g/L	142 (135–148)	120 (115–125)	117 (111–122)	131 (123–138)	134–167
Hgb women g/L	131 (127–135)	105 (101–110)	98 (94–103)	120 (115–124)	117–155
Leukocytes × 10E6/mL	6.1 (5.6–6.5)	8.2 (7.5–8.8)	8.0 (7.5–8.5)	6.1 (5.6–6.5)	3.4–8.2
Platelets × 10E6/mL	253 (235–270)	206 (192–220)	225 (209–242)	297 (274–320)	150–450
Albumin men g/L	39.7 (38.1–41.2)	31.9 (30.6–33.3)	31.9 (30.3–33.6)	39.0 (37.4–40.6)	34–45
Albumin women g/L	38.2 (36.9–39.5)	28.8 (27.8–29.8)	27.5 (26.4–28.5)	36.9 (35.6–38.1)	34–45
CRP mg/L	4 (1–7)	50 (42–58)	126 (106–147)	5 (2–8)	<3
ALT U/L	25 (20–30)	21 (17–24)	24 (21–28)	22(16–28)	<35
Creatinine µM	68 (64–73)	63 (59–68)	64 (60–69)	67 (62–72)	60–90

Haemoglobin and albumin reached their nadirs at days 2–8, whereas inflammatory markers including leukocytes and CRP increased. ALT and creatinine values did not change

ALT alanine aminotransferase, *CI* confidence interval, *CRP* C-reactive protein, *Hgb* haemoglobin, *Preop* preoperative, Day 1, postoperative day 1; Week 1, postoperative day 2–8; Day 28, postoperative day 28; *Ref. range* reference range

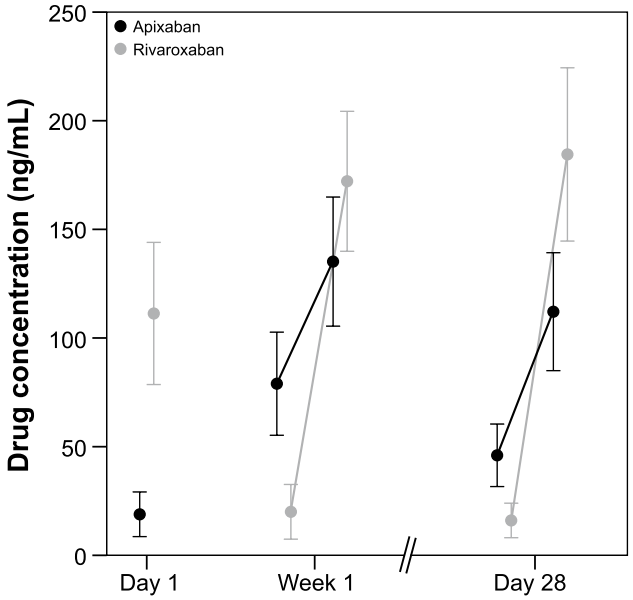

Fig. 1 Factor Xa inhibitor (FXaI) concentrations at different time points at trough and peak levels. The concentrations significantly differed, with apixaban reaching higher trough levels (pre-drug intake samples), whereas rivaroxaban showed higher peak levels (post-drug intake samples; $p < 0.05$). Mean values (*dots*) and the 95% confidence intervals (*bars*) of FXaI concentrations are shown

Thrombin generation

Apixaban and rivaroxaban distinctly modified thrombin generation. With rivaroxaban, the ETP and peak diminished after drug intake more compared to apixaban ($p < 0.05$). Both drugs prolonged the lag time, even at low concentrations ($p < 0.01$; Fig. 2a). On day 28, ETP and peak remained decreased at apixaban trough levels (sample before drug intake). In contrast to patients using rivaroxaban, at 28 days ETP, the peak apixaban values had become normal (Fig. 2b, c). The single dose LMWH effect on coagulation was negligible, as baseline TG activity in samples before drug intake were similar for rivaroxaban and apixaban.

As shown, apixaban and rivaroxaban generally diminished the ETP and the peak while prolonging lag time. ETP and peak correlated with one another ($R^2 = 0.54$) Apixaban showed a very large inter-individual range on postoperative days 2–8, however. In one 68-year-old patient at postoperative week 1, while apixaban levels increased from 50 to 115 ng/mL (delta 65, 130%), ETP strikingly also increased from 1251 to 2036 nM/min (delta 786, 62%). Accordingly, the peak also increased with delta 210 nM (106%), and the lag time was shortened (delta 30 s, 8%). In contrast, in one 73-year-old patient at the same time point, with a similar apixaban level change of 59 ng/mL (increase from 86 to 156, 67%), ETP diminished from 1543 to 1140 nM/min (delta 401, 26%). The peak TG also decreased by 95 nM (24%), and the lag time prolonged by 80 s (27%). However, on day 28, apixaban exhibited similar changes in TG as rivaroxaban on weeks 1 and day 28, i.e., decreasing the ETP and peak and prolonging the lag time. Overall, ETP decreased with increasing drug concentration ($p < 0.001$).

Coagulation tests

Coagulation screening test PT (the Owren method) modestly correlated with rivaroxaban concentration ($R^2 = 0.18$, $p < 0.001$) but not with apixaban concentration. Despite the correlation, the mean PT remained within the reference interval at all time points. Additionally, APTT remained within the references for rivaroxaban and apixaban at all time points. APTT correlated poorly with apixaban ($R^2 = 0.09$, $p = 0.001$) levels but instead had a better correlation with rivaroxaban levels ($R^2 = 0.43$, $p < 0.001$). In contrast to apixaban, rivaroxaban peak levels clearly prolonged RVVT (Fig. 2d).

Fibrinogen and FVIII:C activity peaked at week 1, consistent with the postoperative inflammatory state (Fig. 3a, b), and they were higher in the apixaban group ($p < 0.01$). D dimer levels were very high at all time points and peaked both on postoperative day 1 and again at day 28 (Fig. 3c), despite the quite constant F1 + 2 throughout (Fig. 3d). The mean antithrombin decreased during the acute phase (week 1), but not below the lower reference limit at any time in either group (data not shown).

Discussion

The most important findings of our study were the wide individual variations of effective drug concentrations of rivaroxaban and apixaban, and their distinct effects on TG ex vivo using CAT®, but not in vivo, using F1 + 2 assay. More precisely, the twice-daily versus once-daily dosing of apixaban and rivaroxaban, respectively, resulted in significant differences of their peak and trough concentrations. The TG response in ETP and peak varied among the two FXaIs, as well as among individual patients, whereas the in vivo TG in form of circulating biomarker F1 + 2 was relatively stable. Yet, D dimer, a marker of fibrin turnover also varied individually, increasing towards the end of follow-up. Finally, the widely available RVVT assay was sensitive to rivaroxaban at peak, but not to its trough levels, nor to any of the measured apixaban levels. This may imply the suitability of diluted RVVT as a screening test for high levels of FXaIs.

The concentrations of apixaban and rivaroxaban differed significantly, apixaban reaching higher trough levels, whereas rivaroxaban exhibited higher peak levels in line

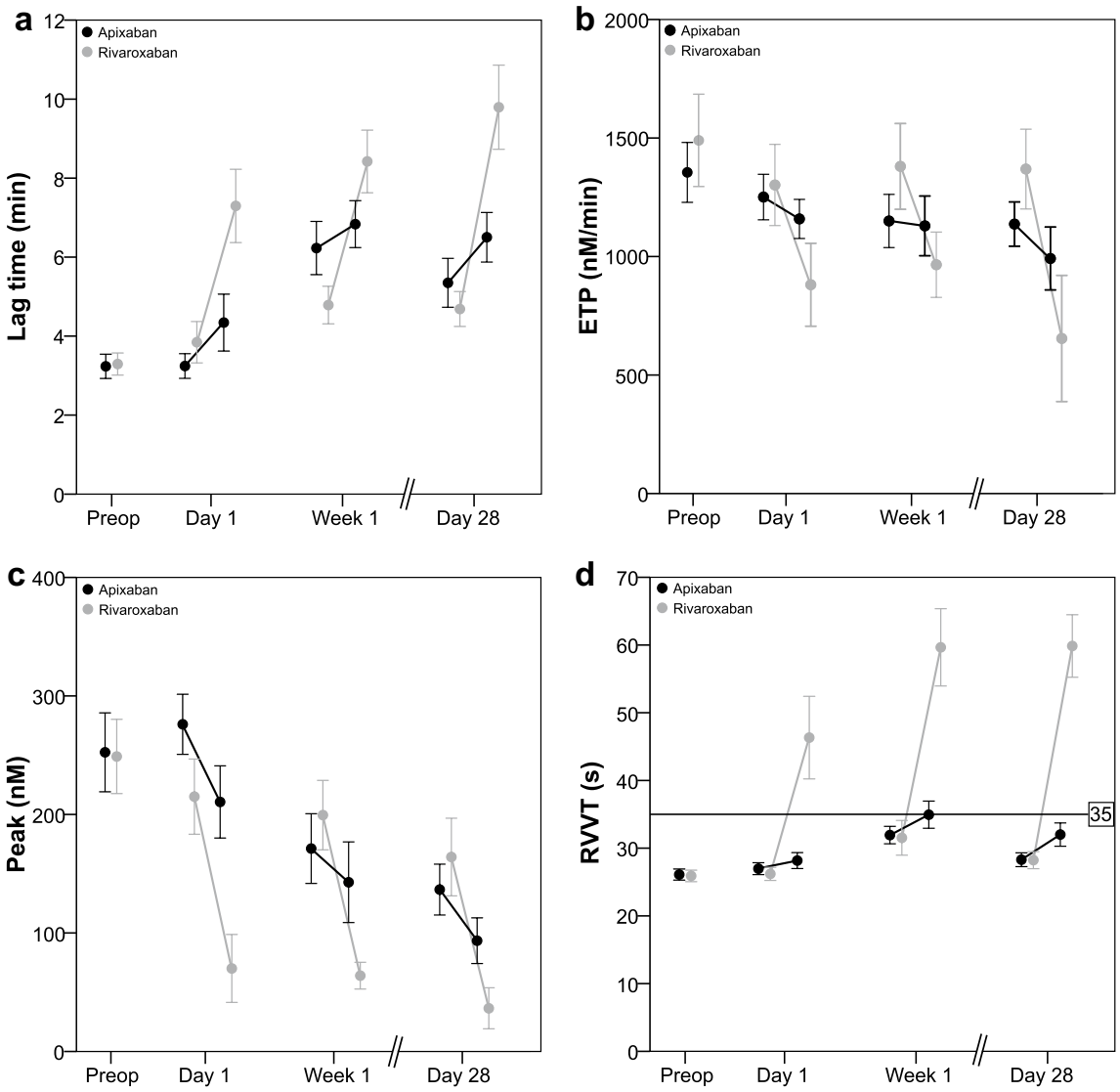

Fig. 2 Thrombin generation and RVVT response under apixaban and rivaroxaban management. The lag time was sensitive to both apixaban and rivaroxaban by increasing by 23–90%, respectively, after only the first drug dose (mean lag time 3.5 min in normal pooled plasma, **a**). The ETP and thrombin peak were inhibited more by rivaroxaban than by apixaban at all time points (**b, c**). Apixaban had only a small effect on the ETP and peak until day 28, when they declined. RVVT was sensitive to rivaroxaban, whereas it did not respond to apixaban (**d**). Mean values (*dots*) and 95% confidence intervals (*bars*) are shown, and for RVVT, the upper limit of the local reference interval is shown

with their BID and OD dosing. The peak levels at steady state were 135 ng/mL for apixaban and 184 ng/mL for rivaroxaban, with minimum trough levels of on average 46 ng/mL for apixaban but only 16 ng/mL for rivaroxaban. The peak levels are slightly higher and trough levels similar than those observed in phase III studies in this indication, with an apixaban peak at 77 ng/mL as well as a rivaroxaban peak at 101 ng/mL at these studies [17, 18]. Mean apixaban concentration at our study at the peak level varied by eightfold, and rivaroxaban only by 1.5-fold, while in the phase III studies, a fourfold peak for apixaban and a 40-fold peak for rivaroxaban were observed. Our findings in orthopaedic patient population confirm the previous comparison of apixaban and rivaroxaban [19]; both markedly affected thrombin-generation variables in vitro, while F1 + 2 were stable in vivo. As ETP and peak correlated, and are both known to associate with VTE [20], we decided to focus on the ETP results. The initiation of thrombin generation (lag time) was prolonged and, as expected, thrombin formation diminished upon challenge with tissue factor. Rivaroxaban inhibited ex vivo thrombin generation more than apixaban. Thrombin generation diminished well below the preoperative levels at peak concentrations of rivaroxaban. In contrast, for the rivaroxaban trough levels, the absolute effect

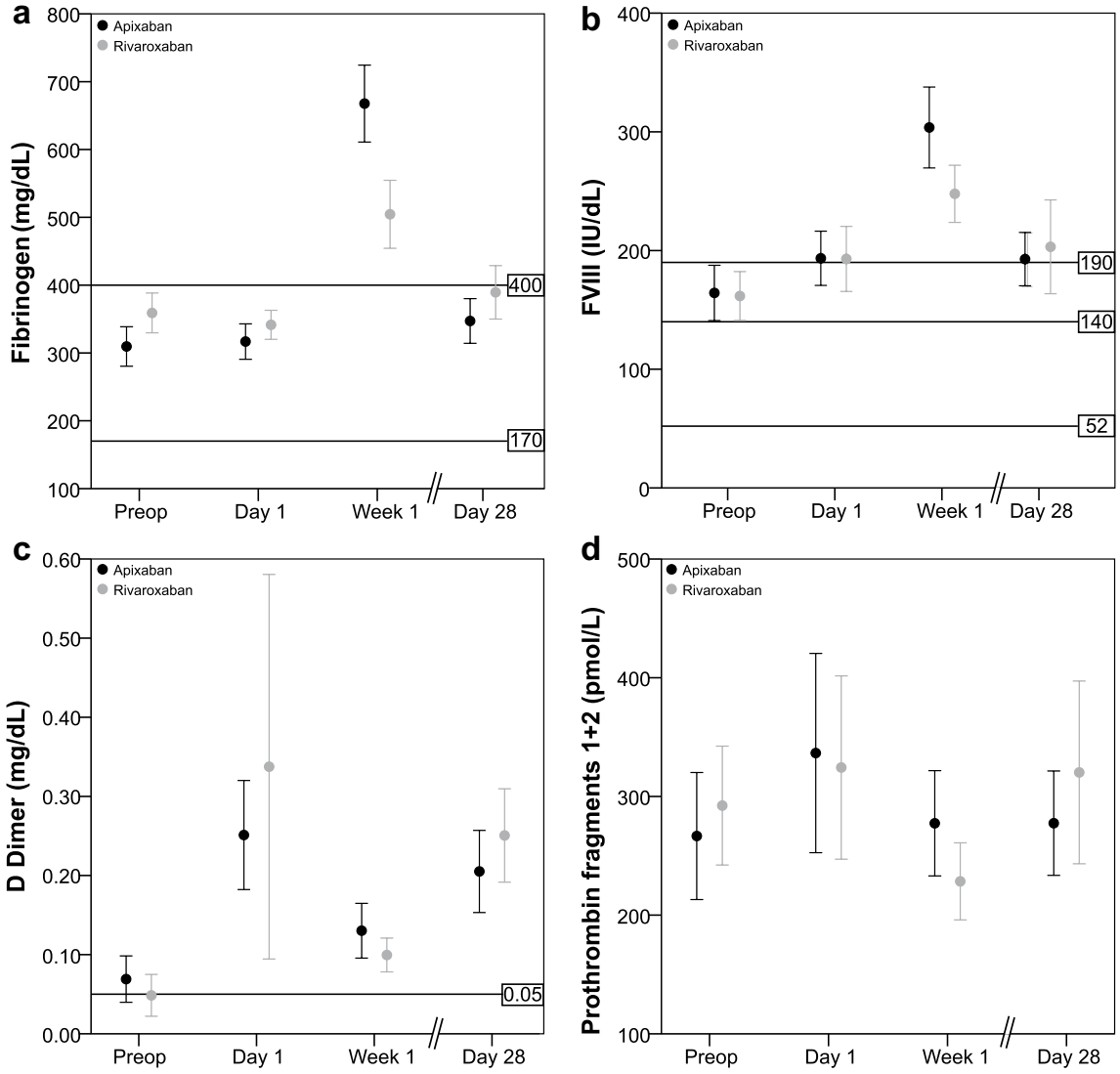

Fig. 3 Fibrinogen and FVIII:C during the course of the study. Fibrinogen and FVIII both peaked at week 1, with FVIII exceeding 190 IU/dL, and higher peaks occurred with apixaban ($p < 0.01$; **a, b**). In contrast, D dimer levels were equally high throughout the study, aside from a dip at week 1 with both FXaIs (**c**). Mean prothrombin fragment F1 + 2 levels remained constant throughout the study (normal reference according to the manufacturer <1200 pM; **d**). Mean values (*dots*) and 95% confidence intervals (*bars*) and, where applicable, local reference intervals are shown. For factor FVIII:C, the reference point of 190 IU/dL is shown as well

on thrombin generation was small, and the trough level at day 28 returned to the preoperative baseline level. This result coincided with the low rivaroxaban levels, which were below 50 ng/mL, under the trough concentrations among all patients. Apixaban, however, initially hardly inhibited thrombin generation, and the difference between apixaban and rivaroxaban coincided with the higher peak level of rivaroxaban at that point (Fig. 1). Thrombin initiation (lag time) appeared to be the most sensitive to FXaIs, since it was clearly prolonged at all drug concentrations. The early use of single dose enoxaparin prior to administration of rivaroxaban as per hospital praxis, however, impacted the functional Xa-based concentration, precluding comparative conclusions on the absolute drug level. However, this one dose did not alter the activity in thrombin generation according to the preoperative baseline samples prior to LMWH and the first direct FXaI intake.

In the postoperative phase, very large inter-individual variation emerged in response to both drugs. The early postoperative setting likely induced individual responses to surgery and differences in the anticoagulant responses. At this time point, the inflammatory markers CRP and FVIII:C were highly increased, consisted with previous findings [21, 22]. FVIII:C peaked higher with apixaban than rivaroxaban at week 1. This might be due to a confounder of the small sample size. Rivaroxaban and

apixaban both bind to albumin (95 and 87%, respectively) [23]. The perioperative inflammatory response and loss of blood and protein associated with the nadir in haemoglobin and albumin, with low levels of albumin known to be associated with intracranial hemorrhage [22, 24, 25]. D dimer levels were high throughout the study in most patients. The D dimer reached its nadir at postoperative week 1 before increasing again at day 28. D dimer levels exhibit a sinusoidal pattern that follows THA, with levels increasing again on postoperative days 2–3 [21, 26]. The healing process, including fibrin formation and degradation, are still ongoing at 4 weeks, and these activities may mirror the continued VTE risk, even at 3 months after surgery [27, 28]. Increase in TG, best reflected with increasing ETP associates with higher risk for thrombosis [29].

In the RVVT, rivaroxaban showed a strong response, whereas apixaban was ineffective. This outcome contrasts with the results of a previous study that used Stago screening and confirmation tests, in which spiked apixaban prolonged RVVT at levels exceeding 20 ng/mL [30]. This result highlights the differences observed with various reagents and the clinical setting in actual patient samples. In addition to specific drug-related differences, the differences in dosing and subsequent differences at peak FXaI levels might explain this clinically relevant finding, since the RVVT may permit detection of rivaroxaban in patient plasma. In the APTT and PT, the correlations between drug concentrations were poor, in line with the previous findings from in vitro surveys [8, 31].

Study limitations included the use of 5 pM tissue factor in the TG assay, which promotes the extrinsic pathway, but is highly reproducible in the CAT® assay. Under these conditions, distinct activities of FV may have contributed since FV inhibits thrombin generation [32]. The role of the intrinsic activation system, relevant after orthopaedic surgery and the inflammatory response is undermined under high tissue factor conditions [33]. Another confounder was the early administration of a single dose LMWH before rivaroxaban, which deviates from the current summary of product characteristics, but is considered to impact the anticoagulant response only on day 1.

In summary, apixaban and rivaroxaban distinctly control thrombin activity after total hip replacement with stronger responses at peak levels with rivaroxaban. In contrast, with apixaban, the trough levels are higher, consistent with the once- vs twice-daily dosing. The widely used RVVT is impacted with high concentrations of rivaroxaban, but not with peak concentrations of apixaban. Our data on covering the impact of FXaI in the laboratory measures after THA may provide a helpful backup, in case bleeding or thrombosis would occur among these patients during their recovery. Yet, as shown, the effects of FXaIs on TG are variable and a holistic approach to coagulation, rather than any single assay, is required for patient management.

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Compliance with ethical standards

Ethical approval All procedures performed in studies involving human participants were conducted in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration as well as its later amendments or comparable ethical standards.

References

1. Eriksson BI, Borris LC, Friedman RJ, Haas S, Huisman MV, Kakkar AK, Bandel TJ, Beckmann H, Muehlhofer E, Misselwitz F, Geerts W, RECORD1 Study Group (2008) Rivaroxaban versus enoxaparin for thromboprophylaxis after hip arthroplasty. N Engl J Med 358:2765–2775. doi:10.1056/NEJMoa0800374
2. Lassen MR, Gallus A, Raskob GE, Pineo G, Chen D, Ramirez LM, ADVANCE-3 Investigators (2010) Apixaban versus enoxaparin for thromboprophylaxis after hip replacement. N Engl J Med 363:2487–2498. doi:10.1056/NEJMoa1006885
3. Salmela B, Joutsi-Korhonen L, Armstrong E, Lassila R (2012) Active online assessment of patients using new oral anticoagulants: bleeding risk, compliance, and coagulation analysis. Semin Thromb Hemost 38:23–30. doi:10.1055/s-0031-1300948
4. Cuker A (2016) Laboratory measurement of the non-vitamin K antagonist oral anticoagulants: selecting the optimal assay based on drug, assay availability, and clinical indication. J Thromb Thrombolysis 41:241–247. doi:10.1007/s11239-015-1282-7
5. Siegal DM, Curnutte JT, Connolly SJ, Lu G, Conley PB, Wiens BL, Mathur VS, Castillo J, Bronson MD, Leeds JM, Mar FA, Gold A, Crowther MA (2015) Andexanet Alfa for the reversal of factor Xa inhibitor activity. N Engl J Med 373:2413–2424. doi:10.1056/NEJMoa1510991
6. Hillarp A, Baghaei F, Fagerberg Blixter I, Gustafsson KM, Stigendal L, Sten-Linder M, Strandberg K, Lindahl TL (2011) Effects of the oral, direct factor Xa inhibitor rivaroxaban on commonly used coagulation assays. J Thromb Haemost 9:133–139. doi:10.1111/j.1538-7836.2010.04098.x
7. Douxfils J, Chatelain C, Chatelain B, Dogne JM, Mullier F (2013) Impact of apixaban on routine and specific coagulation assays: a practical laboratory guide. Thromb Haemost 110:283–294. doi:10.1160/TH12-12-0898
8. Helin TA, Pakkanen A, Lassila R, Joutsi-Korhonen L (2013) Laboratory assessment of novel oral anticoagulants: method suitability and variability between coagulation laboratories. Clin Chem 59:807–814. doi:10.1373/clinchem.2012.198788
9. Hillarp A, Gustafsson KM, Faxalv L, Strandberg K, Baghaei F, Fagerberg Blixter I, Berndtsson M, Lindahl TL (2014) Effects of the oral, direct factor Xa inhibitor apixaban on routine coagulation assays and anti-FXa assays. J Thromb Haemost 12:1545–1553. doi:10.1111/jth.12649

10. Triplett DA (2000) Use of the dilute Russell viper venom time (dRVVT): its importance and pitfalls. J Autoimmun 15:173–178. doi:10.1006/jaut.2000.0414
11. Favaloro EJ, Lippi G, Koutts J (2011) Laboratory testing of anticoagulants: the present and the future. Pathology 43:682–692. doi:10.1097/PAT.0b013e32834bf5f4
12. Tripodi A, Padovan L, Veena C, Scalambrino E, Testa S, Peyvandi F (2015) How the direct oral anticoagulant apixaban affects thrombin generation parameters. Thromb Res 135:1186–1190. doi:10.1016/j.thromres.2015.03.032
13. Wan H, Yang Y, Zhu J, Wu S, Zhou Z, Huang B, Wang J, Shao X, Zhang H (2016) An in-vitro evaluation of direct thrombin inhibitor and factor Xa inhibitor on tissue factor-induced thrombin generation and platelet aggregation: a comparison of dabigatran and rivaroxaban. Blood Coagul Fibrinolysis. doi:10.1097/MBC.0000000000000509
14. Martin-Fernandez L, Ziyatdinov A, Carrasco M, Millon JA, Martinez-Perez A, Vilalta N, Brunel H, Font M, Hamsten A, Souto JC, Soria JM (2016) Genetic determinants of thrombin generation and their relation to venous thrombosis: results from the GAIT-2 Project. PLoS ONE 11:e0146922. doi:10.1371/journal.pone.0146922
15. Lutsey PL, Folsom AR, Heckbert SR, Cushman M (2009) Peak thrombin generation and subsequent venous thromboembolism: the Longitudinal Investigation of Thromboembolism Etiology (LITE) study. J Thromb Haemost 7:1639–1648. doi:10.1111/j.1538-7836.2009.03561.x
16. Ay C, Dunkler D, Simanek R, Thaler J, Koder S, Marosi C, Zielinski C, Pabinger I (2011) Prediction of venous thromboembolism in patients with cancer by measuring thrombin generation: results from the Vienna Cancer and Thrombosis Study. J Clin Oncol 29:2099–2103. doi:10.1200/JCO.2010.32.8294
17. EMA. Eliquis Summary of Product Characteristics. EMEA/H/C/002148-R/0034 2011
18. EMA. Xarelto Summary of Product Characteristics. EMEA/H/C/000944-IB/0040/G 2009
19. Freyburger G, Macouillard G, Khennoufa K, Labrouche S, Molimard M, Sztark F (2015) Rivaroxaban and apixaban in orthopaedics: is there a difference in their plasma concentrations and anticoagulant effects? Blood Coagul Fibrinolysis 26:925–933. doi:10.1097/MBC.0000000000000371
20. Falanga A, Marchetti M, Verzeroli C, Giaccherini C, Masci G, Santoro A, De Braud F, Celio L, Labianca R, Mandalà M, Merelli B, Tondini C, Gasparini G, Sarmiento R, Tartari C, Piras F, Gamba S, Rio F, Milesi V, Tessarolo S, Vignoli A, Russo L (2015) Measurement of thrombin generation is a positive predictive biomarker of v enous thromboembolism (VTE) in metastatic cancer patients enrolled in the Hypercan Study. Blood 126:654
21. Virtanen L, Salmela B, Leinonen J, Lemponen M, Huhtala J, Joutsi-Korhonen L, Lassila R (2014) Laboratory-monitored fondaparinux and coagulation activity in association with total hip replacement. Blood Coagul Fibrinolysis 25:597–603. doi:10.1097/MBC.0000000000000116
22. Mjaaland KE, Kivle K, Svenningsen S, Pripp AH, Nordsletten L (2015) Comparison of markers for muscle damage, inflammation, and pain using minimally invasive direct anterior versus direct lateral approach in total hip arthroplasty: a prospective, randomized, controlled trial. J Orthop Res 33:1305–1310. doi:10.1002/jor.22911
23. Mueck W, Schwers S, Stampfuss J (2013) Rivaroxaban and other novel oral anticoagulants: pharmacokinetics in healthy subjects, specific patient populations and relevance of coagulation monitoring. Thromb J 11:10-9560-11-10. doi:10.1186/1477-9560-11-10
24. Thordardottir S, Vikingsdottir T, Bjarnadottir H, Jonsson H Jr, Gudbjornsson B (2016) Activation of complement following total hip replacement. Scand J Immunol. doi:10.1111/sji.12411
25. Hankey GJ, Stevens SR, Piccini JP, Lokhnygina Y, Mahaffey KW, Halperin JL, Patel MR, Breithardt G, Singer DE, Becker RC, Berkowitz SD, Paolini JF, Nessel CC, Hacke W, Fox KA, Califf RM, ROCKET AF Steering Committee and Investigators (2014) Intracranial hemorrhage among patients with atrial fibrillation anticoagulated with warfarin or rivaroxaban: the rivaroxaban once daily, oral, direct factor Xa inhibition compared with vitamin K antagonism for prevention of stroke and embolism trial in atrial fibrillation. Stroke 45:1304–1312. doi:10.1161/STROKEAHA.113.004506
26. Bytniewski P, Machala W, Romanowski L, Wisniewski W, Kosowski K (2014) The dynamics of D-dimer level fluctuation in patients after the cemented and cementless total hip and total knee replacement. J Orthop Surg Res 9:89-014-0089-0. doi:10.1186/s13018-014-0089-0
27. Dahl OE, Harenberg J, Wexels F, Preissner KT (2015) Arterial and venous thrombosis following trauma and major orthopedic surgery: molecular mechanisms and strategies for intervention. Semin Thromb Hemost 41:141–145. doi:10.1055/s-0035-1544230
28. Warwick D, Rosencher N (2010) The ‘‘critical thrombosis period’’ in major orthopedic surgery: when to start and when to stop prophylaxis. Clin Appl Thromb Hemost 16:394–405. doi:10.1177/1076029609355151
29. Hemker HC, Al Dieri R, De Smedt E, Beguin S (2006) Thrombin generation, a function test of the haemostatic-thrombotic system. Thromb Haemost 96:553–561
30. Ratzinger F, Lang M, Belik S, Jilma-Stohlawetz P, Schmetterer KG, Haslacher H, Perkmann T, Quehenberger P (2016) Lupus-anticoagulant testing at NOAC trough levels. Thromb Haemost 116:235–240. doi:10.1160/TH16-02-0081
31. Helin TA, Pakkanen A, Lassila R, Joutsi-Korhonen L (2016) Effects of apixaban on prothrombin time, activated partial thromboplastin time and anti-Xa assays: a European survey. Clin Chem Lab Med. doi:10.1515/cclm-2016-0742
32. Al Dieri R, Bloemen S, Kelchtermans H, Wagenvoord R, Hemker HC (2013) A new regulatory function of activated factor V: inhibition of the activation by tissue factor/factor VII(a) of factor X. J Thromb Haemost 11:503–511. doi:10.1111/jth.12126
33. Long AT, Kenne E, Jung R, Fuchs TA, Renne T (2016) Contact system revisited: an interface between inflammation, coagulation, and innate immunity. J Thromb Haemost 14:427–437. doi:10.1111/jth.13235