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Effects of the oral, direct factor Xa inhibitor rivaroxaban on commonly used coagulation assays.

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Running title

Effects of rivaroxaban on coagulation assays.

Keywords

Rivaroxaban, direct Xa inhibitor, anticoagulants, coagulation assays

Summary

Introduction: Rivaroxaban is an oral direct factor Xa inhibitor developed for prophylaxis and treatment of thromboembolic disorders. Laboratory monitoring is not necessary but the dose dependent effects on common reagents and assay procedures are largely unknown.

Objectives: To investigate the effect of rivaroxaban on commonly used coagulation assays.

Material and Methods: Rivaroxaban was added to plasma from healthy subjects in the concentration range 0 -1000 µg/L and analyzed using different reagents for activated partial thromboplastin time (APTT), prothrombin time (PT), antithrombin, fibrinogen and activated protein C (APC) resistance assays.

Results: At an expected peak concentration of rivaroxaban in clinical use, the APTTs were almost invariably prolonged but at lower concentrations the effect was weak. The concentration needed to double the APTT varied between 389 ± 106 µg/L to 617 ± 149 µg/L for different reagents. The PT assays showed a marked degree of difference. In general, the Quick PT type assays were more sensitive compared to the Owren type PT assays. The results from antithrombin assays were dependent on the type of reagent with the Xa-based assay being sensitive for rivaroxaban with an estimated increase of 0.09 IU/mL per 100 µg/L rivaroxaban. There were only minor effects on fibrinogen assays based on thrombin reagents. The APTT-based assay for APC resistance is affected in a dose dependent manner whereas an assay based on the activation of coagulation at the prothrombinase level was unaffected.

Conclusions: Different assays, and even different reagents within an assay group, display variable effects by therapeutic concentrations of rivaroxaban.

Introduction

Treatment and thromboprophylaxis have been traditionally performed with a limited set of anticoagulant drugs, of which the vitamin K antagonists (VKA) and heparinoids (unfractionated heparin and low molecular weight heparin variants) are the most important ones. During the last decades, more specific anticoagulant drugs have been developed, such as indirect factor Xa inhibitors (fondaparinux) and direct thrombin inhibitors (argatroban, lepirudin, bivalirudin and desirudin). For various reasons, these drugs have not yet gained a wide usage and have not been able to replace the VKA drugs for many common indications. However, experiences from recent large clinical trials, both with direct Xa and thrombin inhibitors indicate that this may change in the near future. Rivaroxaban (Xarelto[®]), a new direct Xa inhibitor from Bayer AG (Leverkusen, Germany), has passed the first threshold and is now an approved anticoagulant for the prevention of venous thromboembolism (VTE) following major elective orthopaedic surgery. Several phase III clinical trials are also underway where rivaroxaban is evaluated in patients with atrial fibrillation, acute VTE and acute coronary syndrome (1). The new anticoagulants have the advantage over VKAs that the effect does not need to be monitored with laboratory tests, at least not in the majority of patients. However, as the drugs are at least in part, eliminated by the kidneys usage in patients with renal insufficiency might need monitoring. Thus, it would be valuable to have access to a test that can indicate if rivaroxaban (and other anticoagulants) has accumulated in the circulation. Intended or unintended overdosing is another possible indication for testing. Furthermore, in bleeding patients on rivaroxaban treatment, it would be helpful to know if the drug is present or not in cases where it may be considered to try to use reversal agents, although there are not yet any established antidotes. There are some reports on the pharmacokinetic and pharmacodynamic profiles of rivaroxaban, including the general effects on a few coagulation assays (2-12). However, a more thorough knowledge of how rivaroxaban, at therapeutic and supratherapeutic doses, will affect commonly used coagulation assays is currently lacking. In this study, we have investigated the anticoagulant activity of the direct Xa inhibitor rivaroxaban using different reagents for coagulation assays that are common in clinical laboratories.

Material and Methods

Materials

Rivaroxaban was provided from the manufacturer Bayer Schering Pharma AG, Dimethyl sulfoxide (DMSO) was from Merck (Darmstadt, Germany). Lyophilized normal plasma (Unicalibrator) was purchased from Stago (Asnières sur Seine, France). Evacuated blood collection tube system was from BD (Franklin Lakes, NJ, USA).

Plasma samples

Human blood from 10 adult healthy volunteers, both men and women, was collected in evacuated plastic tubes containing 1:10 volume of 0.129 mol/L tri-sodium citrate as anticoagulant. Ten tubes, in total 50 mL citrated blood, were obtained from each individual. The tubes were centrifuged at 2000 x g for 20 min and the platelet poor plasma was pooled and stored frozen at -70°C. The plasma was thawed in 37 °C waterbath for 15 min and divided into 20 aliquots of 1.0 mL each and “spiked” with rivaroxaban to final concentrations ranging from 0.0 to 1000 µg/L and then stored at -70°C until analysis. The rivaroxaban was dissolved with 100 % DMSO and all dilutions in plasma was 1:100, resulting in a final DMSO concentration of 1 % (v/v) in all samples, irrespective of the rivaroxaban concentrations.

Coagulation assays

For this investigation we have mainly chosen assays and reagents used in laboratories that are taking part in the external quality programme of laboratory medicine in Sweden (EQUALIS).

The activated partial thromboplastin time (APTT) was assayed using 5 different reagents: Actin FSL from Siemens Healthcare Diagnostics (Deerfield, IL, USA); PTT-Automate from Stago; APTT-SP IL test liquid from Instrumentation Laboratory SpA (Milano, Italy); TriniCLOT aPTT HS from Trinity Biotech (Bray, Ireland); and APTT-DG from Grifols (Barcelona, Spain). The APTT assays were performed on a BCS-XP instrument from Siemens Healthcare Diagnostics according to the manufacturers instructions.

The prothrombin time (PT) was performed with both plain (Quick type) and combined (Owren type) PT reagents. The main differences between Quick and Owren PT

assays are the final sample dilution, 1:3 in Quick type and 1:21 in Owren type PT assays, and that the Owren PT reagent contains adsorbed bovine plasma that provides factor V and fibrinogen but are devoid of the vitamin K-dependent coagulation factors. Thus, the Owren type PT assay is specific for the vitamin K-dependent factors II, VII and X whereas the Quick PT assay is also influenced by the level of factor V and fibrinogen in the sample. For this investigation we tested five common Quick type PT assays: Dade Innovin and Thromborel S (Siemens Healthcare Diagnostics); STA-Neoplastine CI Plus (Stago); RecombiPlastTin 2G (Instrumentation Laboratory; Technoplastin HIS (Technoclone GmbH, Vienna, Austria). Four different Owren type PT assays were also investigated: Stago prothrombin complex assay, SPA+, from Stago; Nycotest PT from Axis-Shield plc (Dundee, Scotland); PT Owren from MediRox AB (Nyköping, Sweden); and Simple Simon PT from Zafena AB (Borensberg, Sweden). The Quick PT-assays were calibrated using local determination of ISI and mean normal PT for each reagent. The Owren PT assays were all calibrated using the national INR calibrators certified by the Swedish external quality assessment organization EQUALIS (Uppsala, Sweden) (13). The PT assays were performed on an ACLTop (Instrumentation Laboratory SpA) coagulation instrument according to standard procedures.

Three different chromogenic reagents for determination of antithrombin activity were evaluated. Two were based on the inhibition of thrombin, Berichrom ATIII from Siemens Healthcare Diagnostics, which utilizes bovine thrombin and Stachrom ATIII from Stago that contains human thrombin. One reagent was based on inhibition of factor Xa, Coamatic LR from Chromogenix (Milano, Italy). The Stachrom ATIII test was run on the STA-R analyzer from Stago whereas the two other antithrombin assays were performed on the BCS-XP instrument (Siemens) according to the manufacturer's instructions.

Fibrinogen was evaluated using the following four Clauss type clotting assays: Fibrin-Prest from Stago; Fibrinogen-C from Instrumentation Laboratory SpA, Dade thrombin and Multifibren U from Siemens Healthcare Diagnostics. All fibrinogen tests were performed on the Sysmex CA-1500 instrument from Siemens Healthcare Diagnostics according to the manufacturer's instructions.

Coagulation based test for the determination of activated protein C (APC) resistance was performed with the following two methods: Coatest APC Resistance V from Chromogenix and Pefakit APC resistance Factor V Leiden from Pentapharm (Basel, Switzerland). The tests were performed on the STA-R analyzer from Stago according to the manufacturer's instructions.

Statistics

Results are presented as mean \pm standard deviation (SD). Concentrations required to double the PT or APTT (CT_2) were calculated using Excel 97 software (Microsoft®). For PT assays the CT_2 values were calculated by linear regression analysis and for the APTT assays a 2nd order polynomial function was chosen.

Results

Five different APTT-reagents were used in the study and despite differences in their composition of activating substances and phospholipids, their response to the drug were quite similar. The dose-responses were non-linear (Fig. 1) and at an expected trough concentration of the drug (here chosen as 25 µg/L) only one sample resulted in an APTT above 40 seconds with four of the five APTT-reagents tested. With the fifth APTT-reagent, two samples resulted in coagulation times above 40 seconds. The corresponding APTTs at an expected maximum (peak) drug concentration (at 20 mg dose) in plasma (here chosen at 200 µg/L) were always above the 40 seconds limit for four of the reagents. However, with one reagent (APTT-DG) two of the ten samples were still below the limit of 40 seconds. Mean APTT and ranges at baseline, 25 µg/L and 200 µg/L, are shown in table 1. The effect on the APTT, expressed as the concentration required to double the APTT (CT₂), was calculated to be between 389 - 617 µg/L for the five different reagents (table 1), which is a relative difference of 1.6-fold.

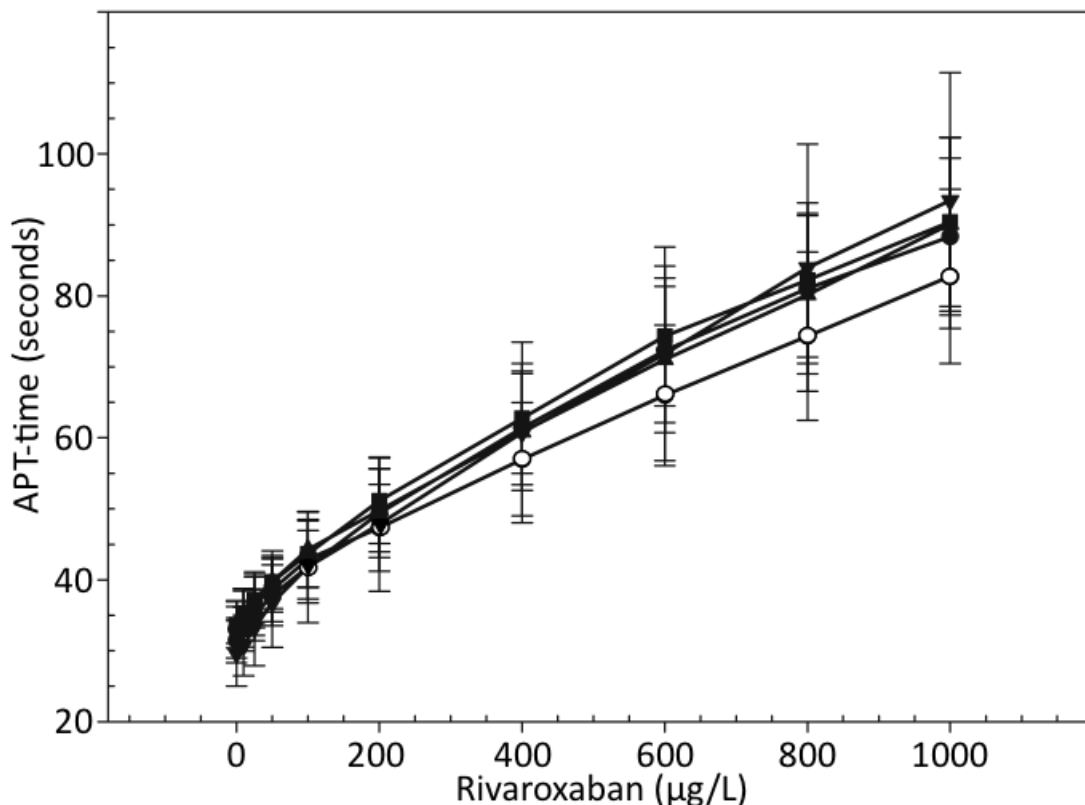


Figure 1. Effect of rivaroxaban on the APTT. The obtained APTT using five different reagents were plotted against the rivaroxaban concentration in plasma. Triniclot aPTT HS (○), Actin FSL (●), PTT-Automate (■), APTT-SP (▲) and DG-APTT (▼). Results are mean of 10 different healthy donors ±SD.

The PT-assays showed quite dissimilar results (Fig. 2) with a wide variation in sensitivities. The Quick PT-assays were the most sensitive tests with INR ranging between 1.24 and 1.63 at an expected peak concentration of 200 µg/L (Table 2). Of the four Owren type PT-assays, the SPA+ from Stago was the least sensitive assay. With this assay, none of the samples had an INR above the upper reference limit, which was set at 1.20, at a rivaroxaban concentration of 200 µg/L. From the calculations of the concentrations needed to double the clotting time the PT-assays could be divided in three groups with different sensitivities. One group comprised four Quick type PT-assays (Dade Innovin, Neoplastine CI Plus, RecombiPlasTin 2G and Technoplastin) that were the most sensitive with CT₂-values between 498 and 591 µg/L. Two Owren type reagents (Nycotest PT and SPA+) were the most insensitive with corresponding values of 1300 and 1375 µg/L, respectively. One Quick and two Owren PT reagent (Thromborel S, Owren's PT and Simple Simon) had an intermediate sensitivity with CT₂-values between 871 and 891 µg/L (Table 2).

The antithrombin activity assay based on factor Xa-inhibition increased with the rivaroxaban concentration whereas the other assays, based on thrombin inhibition, were not affected (Fig. 3). Linear regression analysis gave in a slope that corresponded to an increase of 0.09 IU/mL per 100 µg/L rivaroxaban.

The fibrinogen content, determined with four different Clauss type fibrinogen assays, were almost unaffected by rivaroxaban in plasma (not shown). The differences between no drug (0 µg/L) and the expected peak concentration of 200 µg/L were between 0.02 g/L (Multifibren U) to -0.17 g/L (Fibrinogen-C). At the highest rivaroxaban concentration all assays gave fibrinogen levels that were approx. 10 % lower than the basal level.

The APC resistance assays were also different in their sensitivity towards rivaroxaban (Fig. 4 A and B). The APTT-based assay (Coatest APC Resistance V) resulted in higher ratios with increasing rivaroxaban concentration. The increase was caused by a raise in the underlying APTT determinations where the APTT with APC increased more than the basal APTT (not shown). The other assay, based on prothrombinase activation with snake venom enzymes, was not affected, at least not at the expected therapeutic concentrations of the drug.

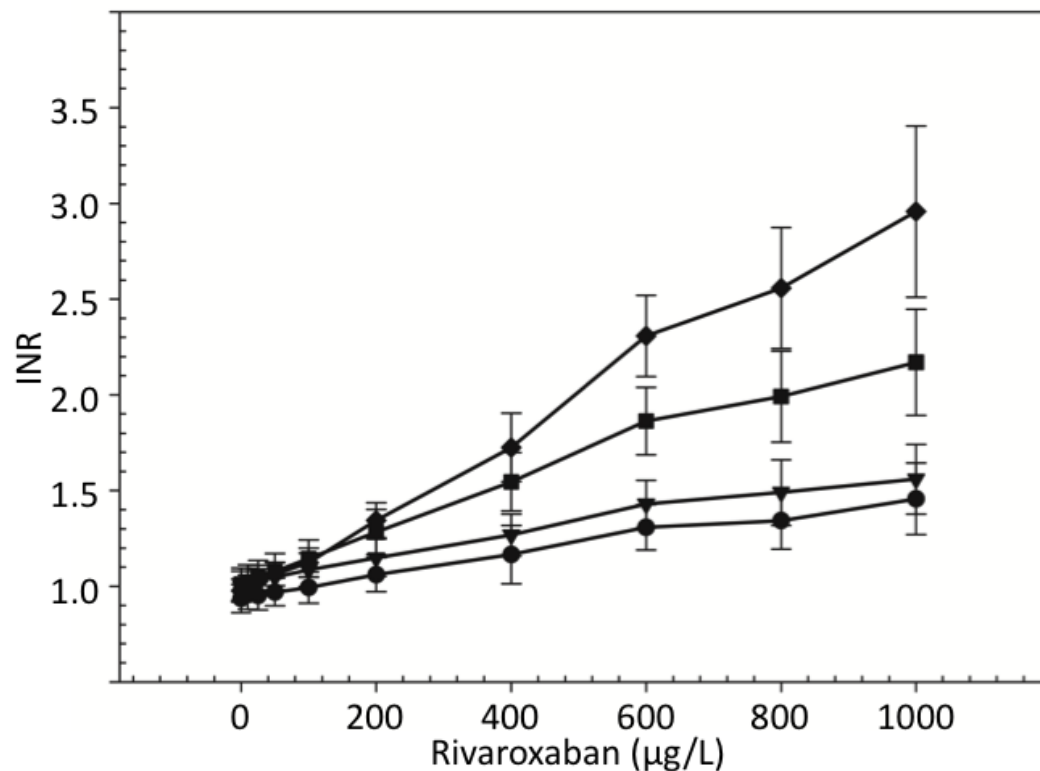
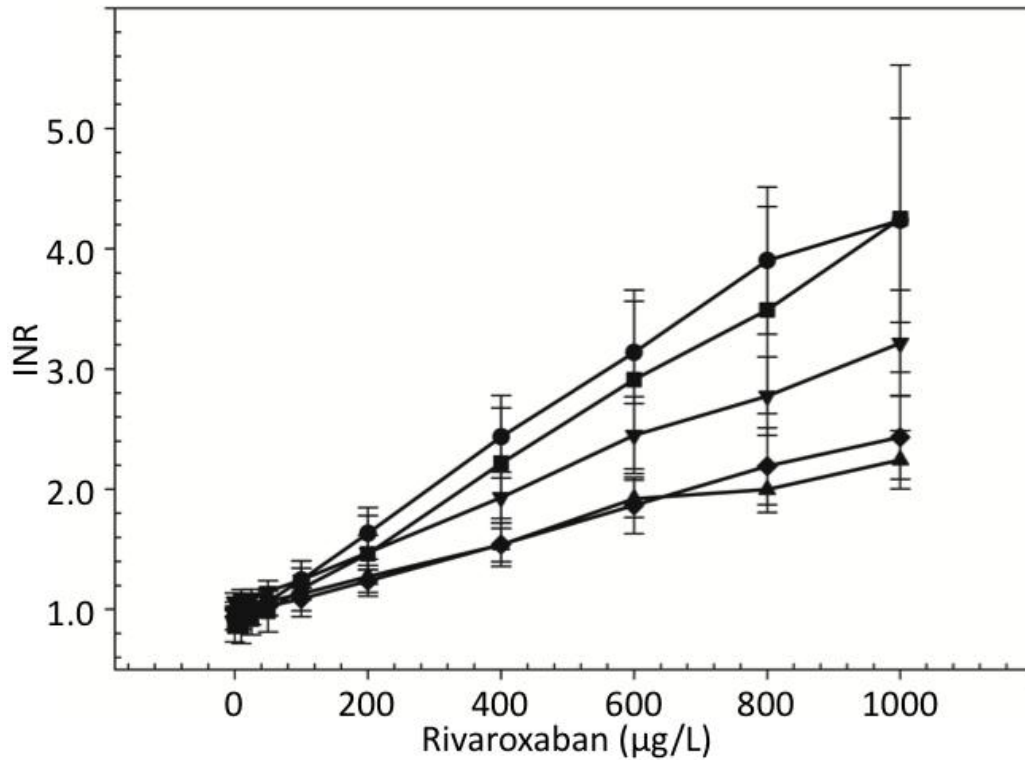


Figure 2. Effect of rivaroxaban on the PT assay expressed as INR. A) Analysis of five Quick PT assays based on plain thromboplastin reagents: STA-Neoplastine (●), RecombiPlastTin 2G (▼), Technoplastin HIS (■), Thromborel S (◆), and Dade Innovin (▲). B) Analysis of four Owren type PT assays, based on rabbit thromboplastin reagents: SPA+ (●), Nycotest PT (▼), Owren's PT (■), and Simple Simon (◆). Results are shown as the mean INR of 10 different healthy donors \pm SD.

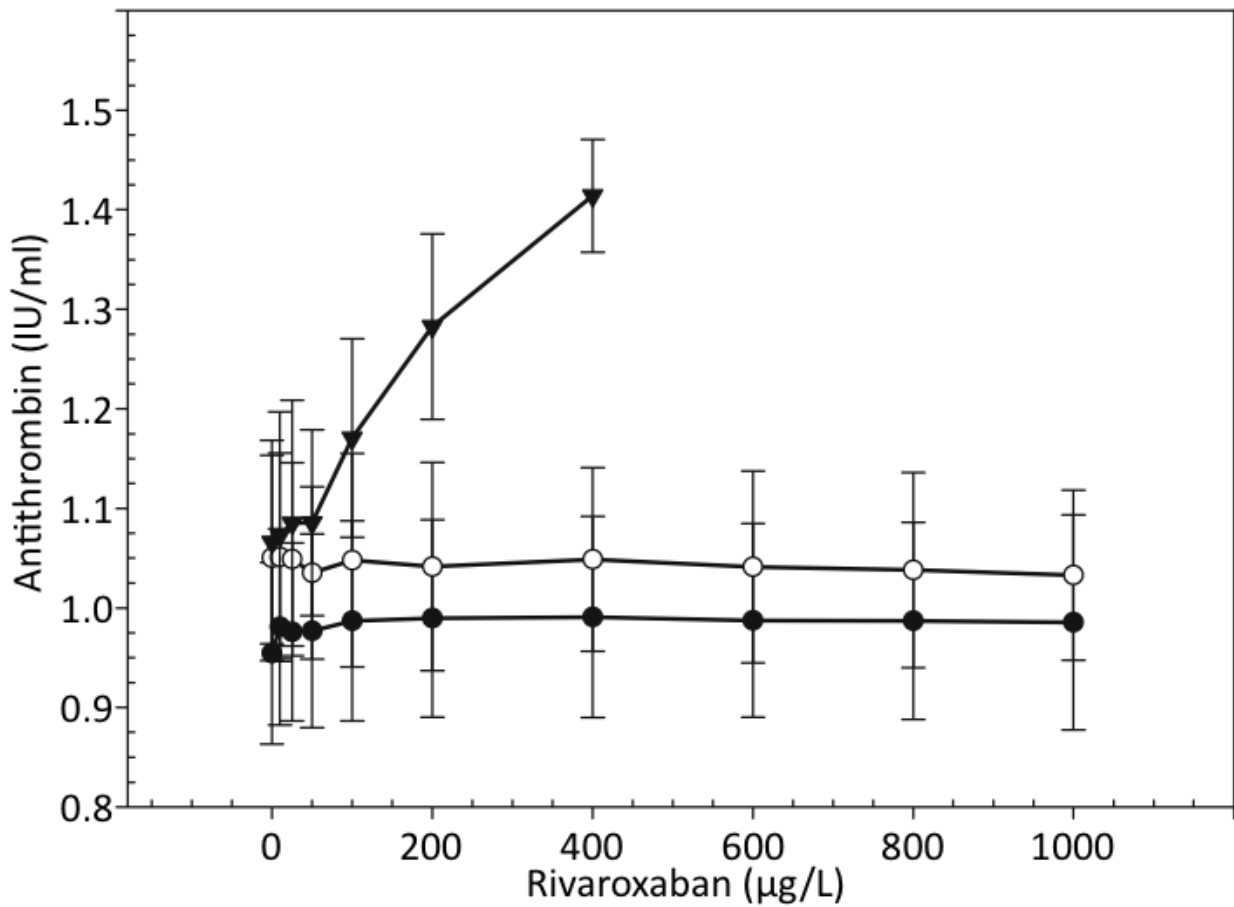


Figure 3. Antithrombin activity and the effect of rivaroxaban. Three different assays based on chromogenic substrates were analyzed using plasma spiked with rivaroxaban, Berichrom ATIII (○), Stachrom ATIII (●), and Coamatic LR (▼). Results are shown as the mean activity (IU/mL) of 10 different healthy donors \pm SD.

Discussion

Rivaroxaban is an oral, direct FXa inhibitor that not only has the capacity to inactivate free FXa but also clot-associated FXa activity and the FXa that is part of the prothrombinase complex (3). Thus, rivaroxaban has the potential to down-regulate the coagulation processes very efficiently and therefore may also interfere in many coagulation assays in which FXa activity has an impact on the results obtained with the assay system. This has also been shown for some global clotting tests such as the Quick PT, APTT and the prothrombinase-induced clotting time (PiCT) assays (3-7, 9, 10) and for more specialized assays such as FXa activity and endogenous thrombin potential (5, 6, 14).

In this investigation we have focused on commonly used coagulation assays and included several different reagents in order to get a picture of the differences in sensitivities within each assay group. An *in vitro* approach was chosen because of its relative simplicity and the fact that rivaroxaban is not a prodrug and display high specificity and predictable pharmacokinetic properties that make it possible to mimic a real life situation. The selected plasma concentration interval was based on the expected concentrations obtained from published dose-ranging studies where once-daily or twice-daily dosing of different amounts of rivaroxaban have been described (2, 3, 5, 6, 8-10, 12, 15). These studies show that with the same total daily dose, once-daily administration will increase the peak concentration and lower the trough concentrations compared to twice-daily dosing but the 90 % confidence intervals for the two dosing regimens overlap (11). A once-daily dose of 10 mg rivaroxaban has been proposed as therapeutic strategy for VTE prophylaxis in patients undergoing elective hip- and knee-replacement surgery, which results in a peak concentration of 125 µg/L (91/195 µg/L; 5th/95th percentile). The expected trough levels with 10 mg once daily dosing is 9 µg/L (1/38 µg/L; 5th/95th percentile) (10). A slightly different regimen has been chosen in a large phase III study aiming at stroke prevention in patients with atrial fibrillation where a 20 mg once-daily dosing is used with a reduction to 15 mg in patients with moderate renal impairment. Yet another dosing variant is used in a phase III study that involve patients with deep vein thrombosis and/or pulmonary embolism where 15 mg of the drug was given twice-daily during the first 3 weeks and then reduced to 20 mg once-daily. Experiences from the use of 20 mg once-daily in a dose-escalation study in healthy male subjects resulted in

mean peak levels of 173 $\mu\text{g/L}$ (3) whereas the same dose used in a dose-ranging study for treatment of patients with acute deep venous thrombosis resulted in a median peak level of rivaroxaban in plasma of 244 $\mu\text{g/L}$ (15). The corresponding median trough level in the venous thrombosis study was 32 $\mu\text{g/L}$ (15). Based on these data we decided to highlight our results from the APTT and PT analyses with a maximal concentration of 200 $\mu\text{g/L}$ and a trough concentration at 25 $\mu\text{g/L}$ as indicated in Tables 1 and 2. In our study, we included up to 4-5-fold higher concentrations (1000 $\mu\text{g/L}$) than the expected peak level, which may be found if the drug has accumulated in the circulation for some reason (e.g., kidney failure) or if too much of the drug has been ingested.

We observed that the APTT is more sensitive to rivaroxaban than the Owren type PT assays. This is in contrast to earlier findings based on the Quick PT assays (3-6) that concluded that the PT is in general more sensitive than the APTT. In the paper by Perzborn et al. (4) the assay concentration CT_2 values were indicated which, correspond to plasma CT_2 value for the PT assay of 0.69 μM (approx. 300 $\mu\text{g/L}$) and 2.07 μM (approx. 900 $\mu\text{g/L}$) for the APTT. We calculated lower CT_2 values for the APTT reagents used in this investigation (1.5 – 2.3 times lower) whereas the CT_2 values for the PT assays were higher (Table 2), especially for the Owren type PT assays. However, in a later study by Mueck et al. it was shown that different Quick PT reagents differ considerably in rivaroxaban sensitivity (10). In their study they showed that the correlation between rivaroxaban concentration in plasma and PT followed a simple linear intercept model but one PT reagent gave a slope of 0.8 seconds/(100 $\mu\text{g/L}$) and the other a slope of 3.2 seconds/(100 $\mu\text{g/L}$). In our study, the corresponding slopes varied between 1.5 to 3.6 seconds/(100 $\mu\text{g/L}$), which further illustrates the variability between reagents. With this observation one should be cautious to suggest that the conventional PT assay can be an appropriate coagulation test to assess the plasma concentration of rivaroxaban (10). A recent study by Samama, with the aim to measure the rivaroxaban pharmacodynamics, showed that the PT reagent variations could not be reduced by INR calibration (16). However, as the PT assay response was linear over a broad concentration range it was suggested that the effect of rivaroxaban could be expressed as plasma concentration of rivaroxaban if plasma probes with rivaroxaban were used to calibrate the assay (16). In this study we also showed that the Owren PT assay is, in

general, less sensitive to rivaroxaban compared to the Quick PT type, which is also illustrated by the high CT_2 values (table 2) for the four Owren type PT reagents used in this study. The reason for the observed differences is probably partly explained by the higher final dilution of the plasma sample, 1:21 in Owren PT compared to 1:3 in Quick PT, which makes the Owren PT type less sensitive for interfering substances as has been shown previously for other anticoagulant drugs (17). However, other less obvious factors may also be of importance as there are PT reagents of both Quick and Owren type with intermediate sensitivities.

The 5 different APTT reagents that were investigated in this study represented a variety of commonly used activators and phospholipids but nevertheless the dose response to rivaroxaban was quite similar. The calculated CT_2 values differed only 1.6 fold between the two reagents with the highest and lowest values, respectively (Table 1). In fact, the inter-individual differences between the 10 plasma samples at peak concentrations using the same reagent could differ to the same extent. At trough level (25 $\mu\text{g/L}$), only few samples had prolonged APTT. Interestingly, the most prolonged APTT at this low concentration for all five different reagents was from the same donor, indicating that the inter-individual differences observed may be explained by properties of the sample rather than the reagent. For the clinician, it is important to note that rivaroxaban may be the only explanation for a prolonged APTT if the sample is drawn a few hours after ingestion of the drug. If a repeat test just before the next rivaroxaban dose shows a considerable shortening of the APTT no further testing is needed, at least not in the non-bleeding patient.

The results from the antithrombin testing (Fig.3) were not surprising and clearly illustrated that this assay is dependent on the type of enzyme used in the reagent. While the reagents based on bovine or human thrombin were left unaffected, the amidolytic activity of the factor Xa-based assay was inhibited in a dose-dependent manner by rivaroxaban. A crude estimate by linear regression analysis indicated that the antithrombin activity was increased by 0.09 IU/mL per 100 $\mu\text{g/L}$ rivaroxaban in plasma. This means, that an inherited antithrombin deficiency associated with thromboembolic disease, with antithrombin levels around 0.60 IU/mL, could be missed if a thrombophilia investigation is performed during rivaroxaban therapy.

Not unexpectedly, the fibrinogen assays tested were only affected to a minor extent. As the Clauss type of fibrinogen assays are based on thrombin as a fibrinogen to fibrin converting enzyme it was not anticipated that the Xa-inhibitor would affect this type of assay principle. However, the observed changes were all indicating a small dose dependency and at the maximum rivaroxaban concentration (1000 µg/L) all four reagents gave approx. 10 % lower values compared to the basal fibrinogen levels (not shown). The changes within the expected therapeutic concentration range were considered clinically insignificant but the reason for the lowered fibrinogen with increasing rivaroxaban is unclear.

The two types of assays for the determination of APC resistance were also differently affected by rivaroxaban. The APTT-based assay was moderately affected by rivaroxaban with an apparent linear increase of the APC resistance ratio (Fig. 4a). One of the donors expressed an APC-resistant phenotype with a ratio of 1.7 that is probably explained by heterozygosity for the factor V Leiden mutation. At an expected peak concentration of rivaroxaban (200 µg/L) this ratio had only increased by 0.1 to 1.8 and would probably not be misclassified as a normal phenotype. Nevertheless, with the knowledge that there may be a considerable inter-individual variation regarding the underlying APTT determination the risk of misclassification with this type of test must be further investigated using donors with known APC resistance genotype. The other type of APC resistance assay was not influenced to the same extent (Fig. 4b), probably explained by the different mode of activation that eliminates the influence of factor Xa. Despite this, there was a dose dependent prolongation of the underlying coagulation times. At very high concentrations the ratios were markedly increased in the samples from some donors. The reason for this effect is unknown and warrants further investigations before the assay can be recommended to be used during rivaroxaban therapy.

As rivaroxaban has several advantages compared to warfarin it is likely to believe that it will be used as an anticoagulant in a considerable number of patients in a near future (if approved for the same indications). The drug affects global coagulation assays such as the APTT and PT and will probably affect most tests that rely on the activity of factor Xa. However, considerable differences between reagents within the same assay group, combined with an inter-individual variability to the drug, will make

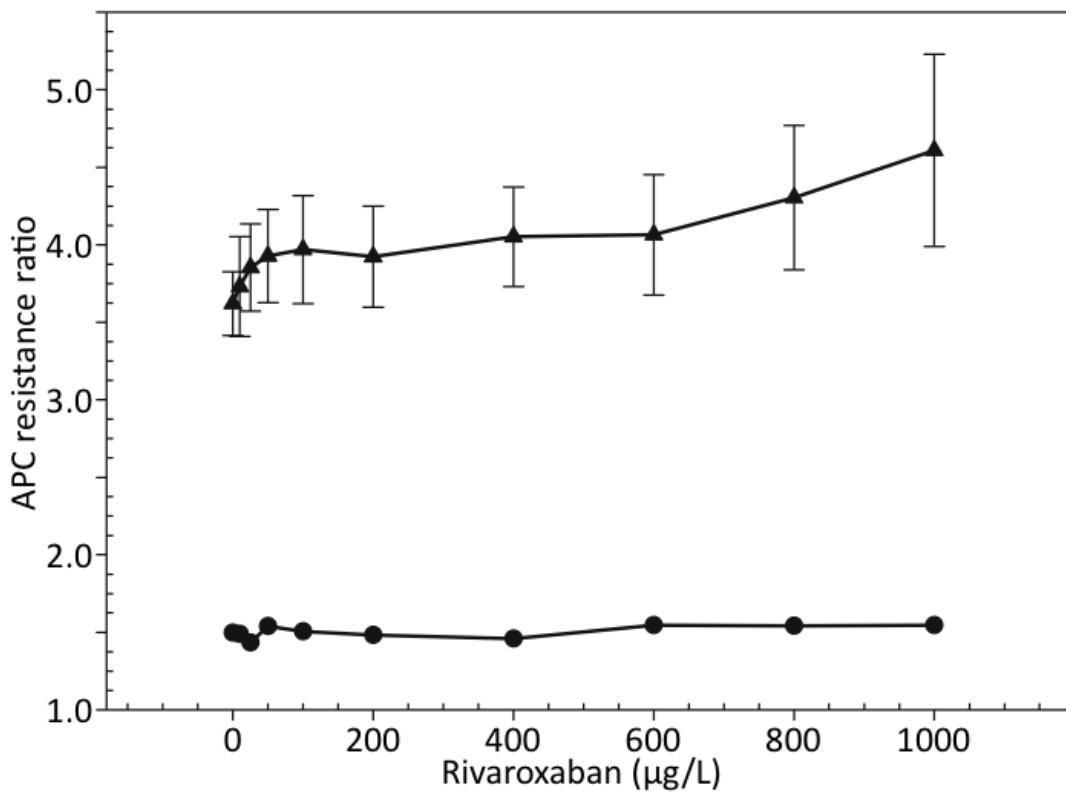
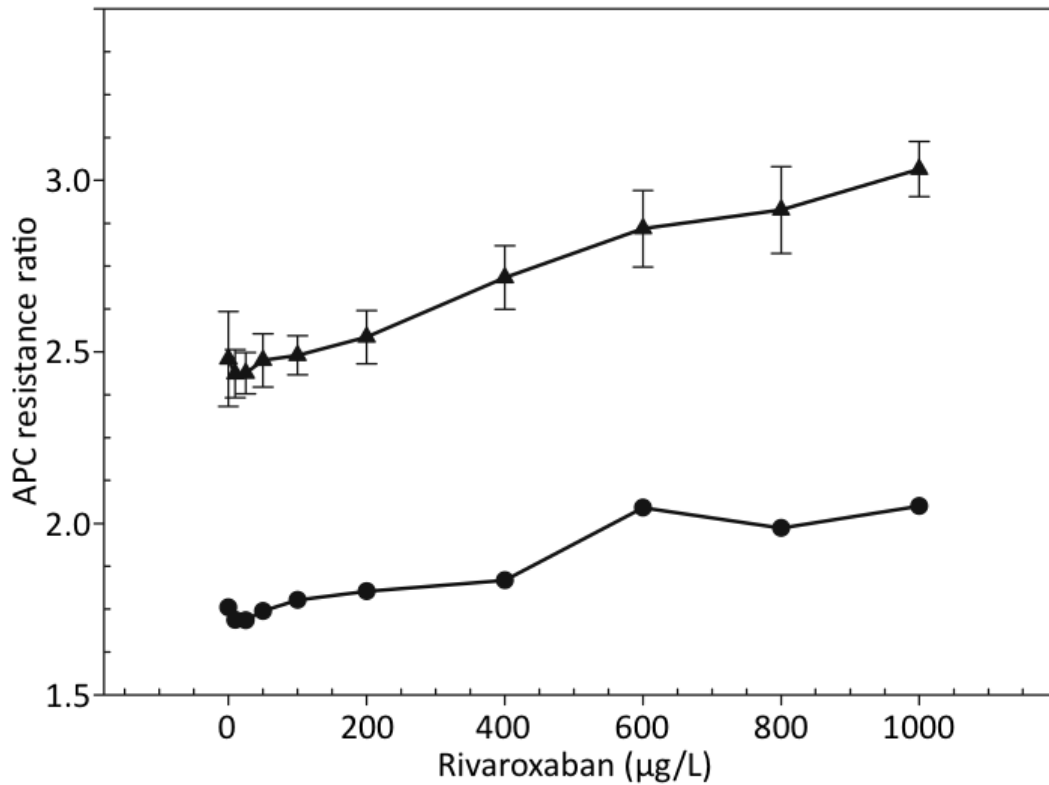


Figure 4. Effect of rivaroxaban on coagulation-based APC resistance assays. A) APTT-based assay (Coatest APC Resistance V); B, assay based on activation at the prothrombinase level (Pefakit APC Resistance Factor V Leiden). The results from 9 individuals with normal phenotype (▲) are expressed as mean ratio \pm SD and one individual with a APC-resistant phenotype (●).

it difficult to create general information on the rivaroxaban effects on common laboratory assays. Our investigations indicate that at concentrations close to the expected peak concentration the APTT will be prolonged but the effect on the PT assays is weak. Thus, the APTT and PT are not useful to measure the plasma concentration of rivaroxaban and can merely be used to obtain a crude estimate. If the aim is to measure the pharmacodynamic effect of rivaroxaban more specific assays, preferably based on factor Xa-inhibition, should be used. Another conclusion is that a useful laboratory screening for thrombophilia cannot not be performed during rivaroxaban therapy without knowledge about how the drug affects the locally used assays. The results obtained in our study will facilitate for laboratories to adequately handle the effect of the drug on common coagulation assays.

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Table 1. Effect of rivaroxaban on different APTT-reagents.

Type of APTT	Baseline (0 µg/L) Mean (±SD) (range)	“Trough” (25µg/L) Mean (±SD) (range)	“Peak” (200 µg/L) Mean (±SD) (range)	CT ₂ (µg/L)
TriniCLOT	33.0 (±4.1) (27.9-37.5)	36.6 (±4.5) (32.5-48.0)	47.3 (±6.1) (40.3-60.1)	617 ±149
Actin FSL	31.5 (±3.1) (26.9-36.7)	35.1 (±3.6) (30.8-43.3)	49.4 (±6.3) (43.2-61.5)	435 ±113
APTT-DG	29.6 (±4.6) (23.3-38.7)	33.3 (±5.3) (27.9-46.7)	47.8 (±9.5) (37.7-70.8)	389 ±106
APTT-SP	33.7 (±3.3) (28.2-38.6)	37.0 (±3.7) (31.7-44.3)	49.8 (±5.9) (42.0-59.7)	530 ±103
PTT-A	33.7 (±2.6) (28.2-37.8)	37.2 (±3.2) (31.7-44.4)	51.1 (±6.0) (41.3-63.0)	491 ±105

Table 2. Mean INR at different concentrations of rivaroxaban and concentrations required to double the prothrombin time (CT₂).

PT-assay	Baseline (0 µg/L) Mean (±SD)	“Trough” (25µg/L) Mean (±SD)	“Peak” (200 µg/L) Mean (±SD)	CT₂ (µg/L)
<i>Quick type PT</i>				
Thromborel S	1.00 (±0.06)	0.99 (±0.06)	1.24 (±0.13)	885 ±202
Dade Innovin	1.02 (±0.06)	1.03 (±0.07)	1.46 (±0.10)	591 ±76
Technoplastin	0.86 (±0.13)	0.92 (±0.13)	1.46 (±0.32)	557 ±119
Neoplastine	0.92 (±0.09)	0.97 (±0.10)	1.63 (±0.21)	506 ±70
Recombiplastin	1.06 (±0.07)	1.09 (±0.07)	1.47 (±0.14)	498 ±54
<i>Owren type PT</i>				
SPA+	0.94 (±0.07)	0.95 (±0.07)	1.06 (±0.09)	1375 ±201
Nycotest PT	1.00 (±0.08)	1.03 (±0.08)	1.15(±0.10)	1300 ±130
Owren’s PT	1.01 (±0.09)	1.05 (±0.09)	1.28 (±0.12)	891 ±49
Simple Simon	0.98 (±0.06)	1.08 (±0.07)	1.35 (±0.09)	871 ±80