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ORIGINAL ARTICLE

A single test to assay warfarin, dabigatran, rivaroxaban, apixaban, unfractionated heparin, and enoxaparin in plasma

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Essentials

- Simple and fast assaying of different anticoagulants (ACs) is useful in emergent situations.
- We used highly diluted prothrombin time (dPT) or highly diluted Fiix-PT (dFiix-PT) to assay ACs.
- Both tests could quantify target specific anticoagulants and warfarin anticoagulation.
- Improved results were consistently observed with the dFiix-PT compared with the dPT.

Summary. *Background:* Assaying anticoagulants is useful in emergency situations or before surgery. Different specific assays are currently needed depending on the anticoagulant. *Objectives:* We hypothesized that levels of warfarin, dabigatran, rivaroxaban, apixaban, and heparins could be measured with use of the diluted prothrombin time (dPT) and diluted Fiix-PT (dFiix-PT), using highly diluted thromboplastin (TP). The latter test is affected only by reduced levels of active factors II and X but corrects test plasma for other deficiencies *Methods:* Increasing TP dilutions were used to identify suitable dilutions to measure dabigatran, rivaroxaban, apixaban, unfractionated heparin (UFH), and enoxaparin. Calibrators containing known amounts of direct oral anticoagulants (DOACs) were used to make standard curves. Citrated plasma samples were obtained from patients taking warfarin or DOACs with known drug concentrations as determined by specific assays. *Results:* The dFiix-PT at a TP dilution of 1:1156 could be used to measure all of the drugs tested at therapeutic concentrations except for fondaparinux. The dPT achieved the same but required two TP dilutions (1:750 and 1:300). The warfarin effect could be assessed by using dFiix-PT at 1:1156 with a PT ratio identical to the international normalized ratio. Six different TPs yielded similar results, but two were less sensitive. Dabigatran, rivaroxaban, and apixaban could be accurately measured in patient samples using both dilute PT assays, but a better correlation was consistently observed between the dFiix-PT and specific assays than with the dPT. *Conclusion:* The dFiix-PT using a single dilution of TP may be suitable to assess the anticoagulant effects of warfarin, dabigatran, rivaroxaban, apixaban, heparin, and enoxaparin.

Keywords: apixaban; assay; dabigatran; heparin; rivaroxaban.

Introduction

An advantage of the newer direct oral anticoagulants (DOACs) is that they do not require routine anticoagulant monitoring [1–4], but measuring their anticoagulant effect in certain circumstances may be useful, such as when assessing presence of effect or reversal, before invasive procedures or during emergencies, and in patients with renal impairment or major bleeding. Currently, different methods are used to measure levels of these various anticoagulant drugs. Standard coagulation assays—activated partial thromboplastin (TP) time (APTT) and prothrombin time (PT)—are not suitable for measuring the effect of the DOACs because they lack sensitivity to the anticoagulant effect of DOACs and yield markedly discrepant results based on the reagents used [5,6]. There-

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fore, dabigatran may currently be assayed best using the diluted thrombin time (dTT) or the ecarin clotting time [7–9] and the anti–factor Xa (FXa) oral agents using chromogenic anti-FXa assays [10], using drug-specific anti-FIIa or anti-FXa standards [5,6]. However, most medical laboratories cannot perform these tests on a rapid-turnaround 24-h basis.

It would be useful if a single assay could be used to measure levels of these as well as other anticoagulants using routinely available coagulation equipment. Based on the known sensitivity of the tissue TP inhibition test, also known as diluted PT (dPT), to heparin and thrombin inhibitors [11], we hypothesized that diluted TP could also be used to increase the sensitivity of the PT to the anticoagulant effect of DOACs and heparins while still maintaining the ability to assess warfarin. Because the new DOACs target FIIa or FXa, we also explored the potential of the Fiix-PT (pronounced "Fix PT"), a new variant of the PT sensitive only to reduction in FII and FX [12–14].

Methods

Study conduct

The use of patient samples in this study was approved by the National Bioethics Committee of the State of Iceland, permit VSN 15-065.

Plasma

Blood from 20 healthy volunteer donors (10 men and 10 women) was used to prepare platelet-poor pooled normal plasma (PNP). The blood was drawn using a minimal stasis technique through a 21-gauge needle into 1:10 vol:vol 3.2% (0.109 mol L^{-1}) buffered sodium citrate Vacuette® tubes (Greiner, Kremsmünster, Austria) and spun at 2,500×*g* for 15 min in a refrigerated centrifuge at 4 °C to obtain platelet-poor plasma (PPP). Both PNP and patient plasmas were stored in 0.5-mL aliquots at −80 °C until tested. Frozen PPP samples from patients taking dabigatran, rivaroxaban, and apixaban with known drug concentration by standardized functional assays were obtained from the Landspitali in Reykjavik, Iceland; Davis Medical Center, Sacramento, CA, USA; Danderyd Hospital, Stockholm, Sweden; and University of North Carolina Hospital, Chapel Hill, NC, USA. Plasma samples from patients taking warfarin were obtained at the Landspitali National University Hospital, Reykjavik, Iceland. PPP immunodepleted of both FII and FX (Fiix-depleted plasma) was specially made at Haematologic Technologies Inc. (Essex Junction, VT, USA).

Instruments, reagents and drugs

All analyses were performed using the automated STA-R Evolution coagulation analyzer (Diagnostica Stago, Asnières-sur-Seine, France) that uses mechanical clot detection. Unless otherwise noted, experiments were done using Neoplastine CI plus ISI 1.35 (Diagnostica Stago) but confirmatory experiments were also done using different TPs [i.e. Innovin and Thromborel S (Siemens, Erlangen, Germany), Triniclot PT HTF and Triniclot PT Excel (Tcoag Ireland Limited, Wicklow, Ireland), and Recombi-Plus Tin 2G, PT-Fibrinogen HS Plus and PT-Fibrinogen (Instrumentation Laboratory, Bedford, MA, USA)]. The following drug calibrators and anticoagulant drugs were used for spiking experiments: Dabigatran plasma calibrators (Aniara, West Chester, OH, USA), rivaroxaban calibrators (Diagnostica Stago), apixaban calibrators (Technoclone, Vienna, Austria), UFH (Heparin Leo®, Leo Pharma, Ballerup, Denmark), enoxaparin (Clexane®/Klexane®, Sanofi, Paris, France), and fondaparinux (Arixtra™, GlaxoSmithKline, Brentford Middlesex, UK).

Calibrated assays for DOACs

The concentration of dabigatran was assessed using the dTT (calibrated Hemoclot Thrombin-Inhibitor reagent from Hyphen BioMed, France), while the concentrations of rivaroxaban and apixaban were assessed using chromogenic liquid anti-FXa assay (Diagnostica Stago), specially calibrated for each agent. The results of our calibrated functional assays of dabigatran and rivaroxaban compared well to liquid chromatography–tandem mass spectrometry (LC-MS/MS) in samples obtained from 21 patients taking each drug [i.e. R^2= 0.94, $y = 1.03x - 8.5$ for dabigatran and $R^2 = 0.95$, $y = 0.70x + 1.2$ for rivaroxaban].

PT and Fiix-PT assays

The PT and dPT was measured using 1 volume of undiluted TP or a dilution of TP in HEPES buffer (HEPES 20 mmol L^{-1}, NaCl 150 mmol L^{-1}, pH 7.4), 1 volume of $CaCl_2$ 0.025 mol L^{-1}, and 1 volume of test plasma (calibrators, PNP, or patient plasma). In the dFiix-PT assays, FII- and FX-depleted plasma (Fiix-depleted plasma) was mixed into the test plasma to correct for any factor deficiency other than FII or FX. The test components and final dilution (f.d.) of TP in each experiment are shown in Table S1. DOAC calibrators were used to make standard curves, and different dilutions of TP were tested to choose the best dilution to demonstrate the effect of the respective drug. In the dFiix-PT, 25 μL of Fiix-depleted plasma was added to 80 μL of prediluted test plasma (prediluted 1:7 with Owren buffer) and incubated for 240 s at 37 °C. Then, 80 μL of diluted TP was added with 40 μL of $CaCl_2$. The clotting time was recorded in seconds.

In samples from patients on warfarin, the Owren PT was used to calculate the international normalized ratio (INR). The Owren PT was done with diluted plasma (1/7

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in buffer) using the STA-SPA 50 reagent with international sensitivity index (ISI) of 1.01 (Diagnostica Stago). The Owren PT reagent is similar to the PT reagent but also contains adsorbed bovine plasma as a source of coagulation FV and fibrinogen and, thus, is only affected by reduced activity of coagulation FII, FVII, and FX in test plasma.

Statistics

Results were processed and expressed using GraphPad Prism 6 (GraphPad, Software, Inc. La Jolla, CA, USA). We used linear and nonlinear regression and calculated the Pearson correlation coefficient and the coefficient of variation as the fraction (percent) of the standard deviation to the mean.

Results

Effect of DOACs

Clotting times of calibrator plasmas containing various concentrations of DOACs were measured using both the dPT and dFiix-PT. All experiments were done using TP undiluted and at various dilutions. Figure 1 shows that the sensitivity of both the diluted dPT and the dFiix-PT to DOACs in plasma increases at higher TP dilutions. With TP dilutions (final concentrations) of 1:300–1:1500, the dPT was linearly sensitive to dabigatran at concentrations of 32–475 ng mL^{-1}, rivaroxaban up to 457 ng mL^{-1}, and apixaban to 572 ng mL^{-1}. With TP dilutions from 1:231 to 1:2313, the dFiix-PT was linearly sensitive to dabigatran 32–475 ng mL^{-1}, rivaroxaban up to 457 ng mL^{-1}, and apixaban to 572 ng mL^{-1}.

Effects of UFH, enoxaparin, and fondaparinux

The clotting times of spiked plasma with various concentrations of UFH, enoxaparin, or fondaparinux were also measured with increasing dilutions of TP using the dPT and dFiix-PT (Fig. 2). Higher TP dilutions increased assay sensitivity to UFH, enoxaparin, and DOACs (Fig. 1). Heparin at therapeutic concentrations could be measured at TP dilutions of 1:150 and 1:300 with the dPT and at 1:1156 with the dFiix-PT. Higher TP dilution of 1:750 or 1:1500 was required to measure enoxaparin with the dPT, whereas dilutions of 1:1156 and 1:2313 gave linear responses with the dFiix-PT. Neither the dPT nor the dFiix-PT was sensitive to fondaparinux at therapeutic concentrations using a range of TP dilutions.

Effect of warfarin anticoagulation

The results with DOAC (Fig. 1) and heparin (Fig. 2) indicated that the best dilution of TP was 1:750 for the dPT and 1:1156 for the dFiix-PT. Therefore, we used these dilutions for assaying plasma samples from 48 patients taking warfarin with INRs ranging from 0.97 to 4.65 (Fig. 3). Both the dPT and the dFiix-PT showed a linear increase at higher INR values over the entire range. The dFiix-PT ratio (clotting time of anticoagulated plasma/clotting time of normal plasma), but not the dPT ratio, corresponded closely to the INR.

Intra-assay and interassay variations of measurements of DOACs

We selected TP dilutions of 1:750 for the dPT and 1:1156 for the dFiix-PT to evaluate assay reproducibility for DOACs. The intraassay variability was obtained using nine measurements of each drug calibrator concentration in the same run and was about 2% with the dPT and about 4–6% with the dFiix-PT at mid-range therapeutic concentrations of rivaroxaban and dabigatran**.** The interassay variation was similarly obtained by measuring each calibrator daily for 9 consecutive days using nine fresh preparations of the same lot of TP and was ~4–7.5% with dPT and ~5–6.3% at mid-range therapeutic concentrations, respectively. With both dPT 1:750 and dFiix-PT 1:1156, both intra-assay and interassay variability varied more at lower drug concentrations (Table 1) and tended to vary more with the dFiix-PT, which uses a diluted test sample.

Influence of different TP reagents

We also examined the use of several TP reagents on the dPT and the dFiix-PT for measuring plasma levels of the DOACs. (Fig. 4). A linear relationship was observed with all TPs in measurement of dabigatran (Figs. 4A and 4B). A linear relationship was also observed for rivaroxaban (Figs. 4C and D) and apixaban (Figs. 4E and F), but the recombinant TPs were less sensitive, particularly for measurement of rivaroxaban (Figs. 4C and D). The influence of different TPs was similar for both the dPT and the dFiix-PT.

DOAC concentrations in patient samples

The dFiix-PT at a 1:1156 TP dilution and dPT at a TP dilution of 1:750 were used to measure the concentrations of dabigatran and rivaroxaban over the range of 20–500 ng mL^{-1} and apixaban at 20–600 ng mL^{-1} in samples obtained from patients (Fig. 5). The results were compared with results obtained with the calibrated diluted sample thrombin time for dabigatran and the calibrated chromogenic anti-FXa assay for rivaroxaban and apixaban. In 63 dabigatran patient samples, a linear relationship was observed when correlating the dFiix-PT with the dTT ($y = 0.94 \times x + 27.6$, $R^2 = 0.77$). A linear relationship was also obtained compared with dTT using the dPT to measure dabigatran, but there was a steeper slope,

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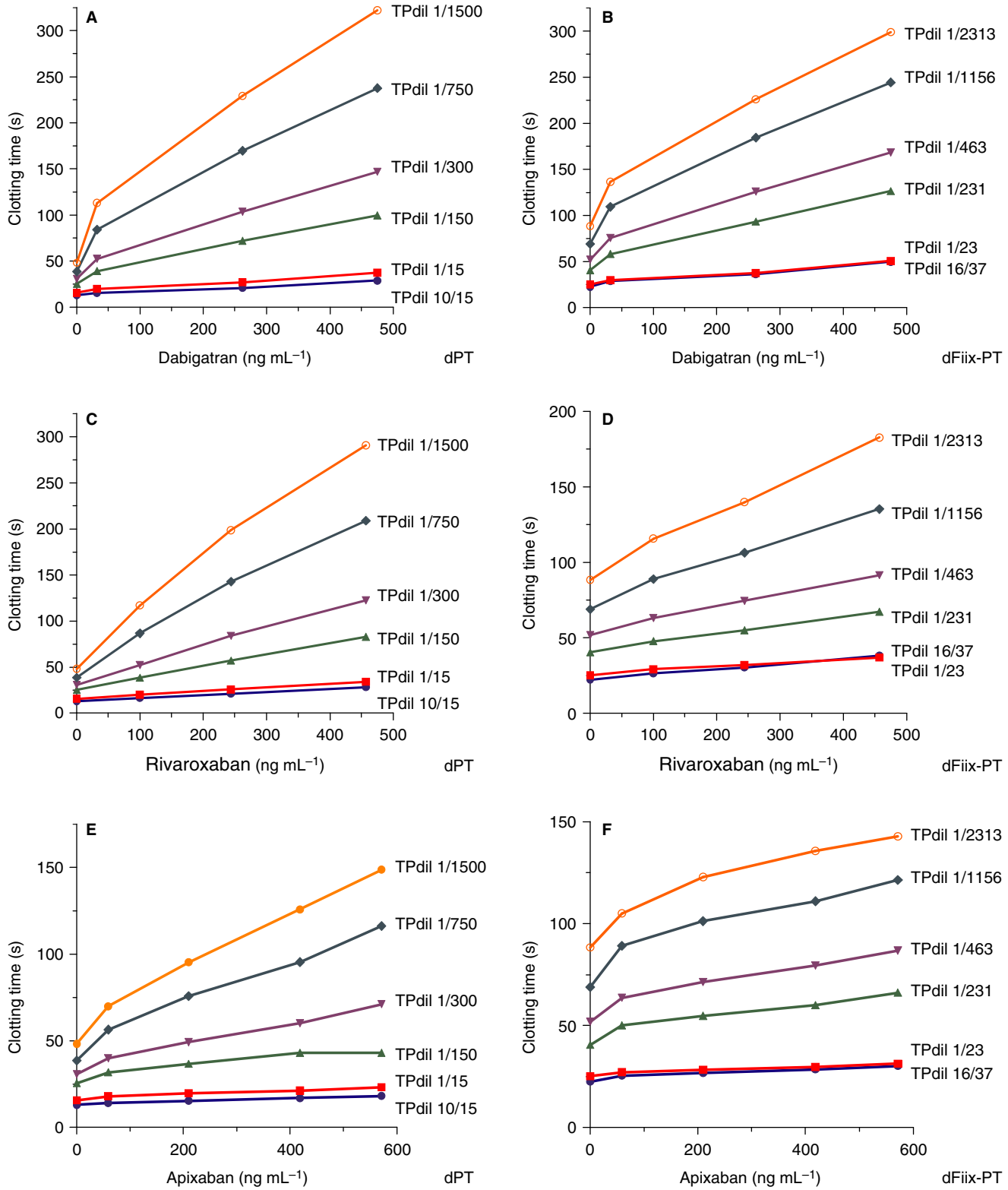

Fig. 1. Effect of direct oral anticoagulants on dilute thromboplastin assays. Clotting times obtained using prothrombin time (PT) and diluted PT (dPT) (left panels) and Fiix-PT and dFiix-PT (right panels) were measured in dabigatran (A, B), rivaroxaban (C, D), and apixaban (E,F) calibrator plasmas. Baseline unspiked (0 ng/mL) normal pooled plasma values are shown. Progressive thromboplastin dilutions are shown in each graph, and the bottom curves in each panel show results obtained with the unmodified PT (TP 10/15) and Fiix-PT (TP 16/37).

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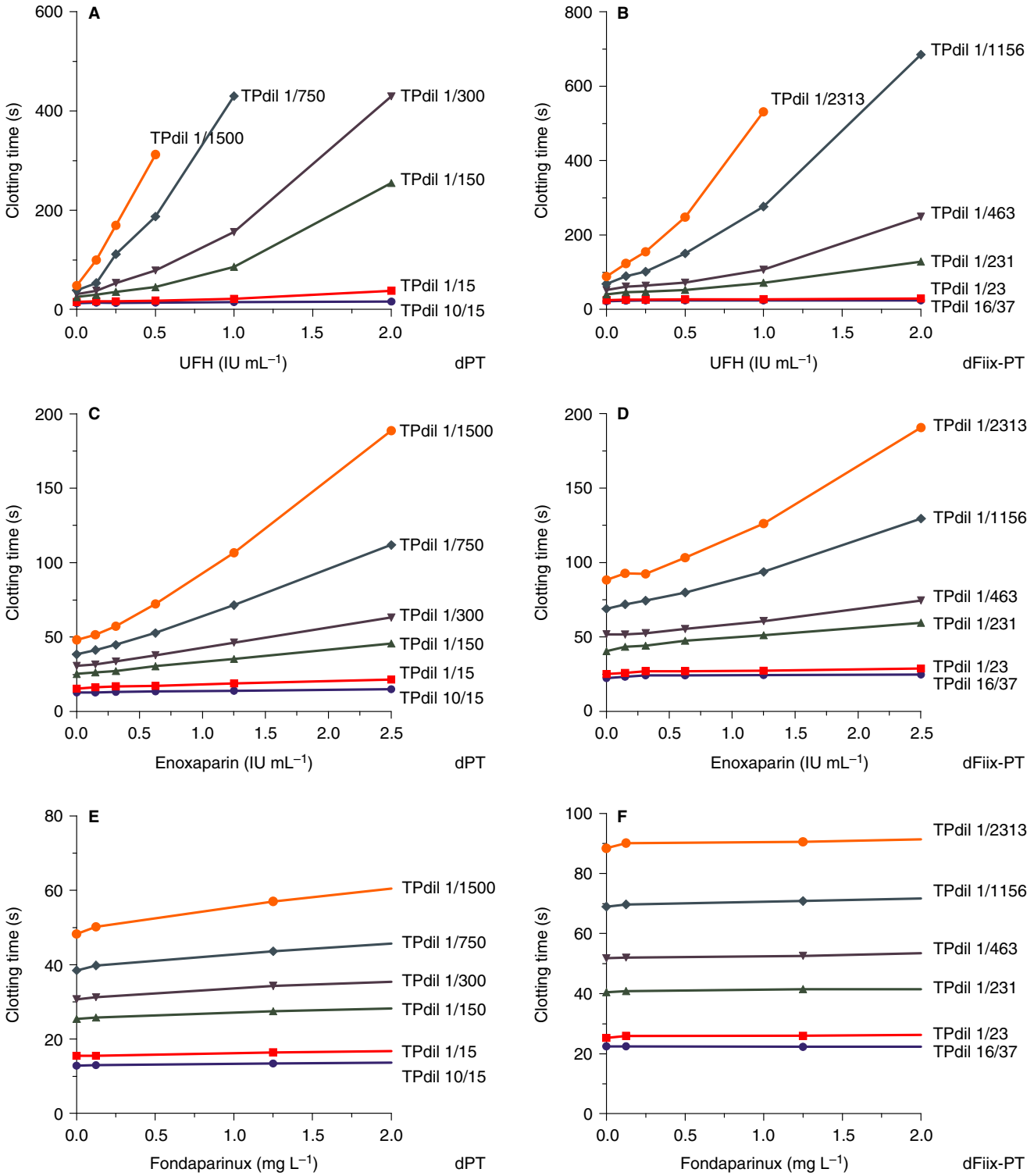

Fig. 2. Effect of unfractionated heparin, enoxaparin, and fondaparinux on dilute thromboplastin assays. Clotting times obtained using prothrombin time (PT) and diluted PT (dPT) (left panels) and Fiix-PT and dFiix-PT (right panels) with progressive thromboplastin (TP) dilutions were measured in PNP (platelet poor pooled normal plasma) (0 ng/mL) and PNP spiked with unfractionated heparin, enoxaparin, and fondaparinux. The bottom curves in each panel show results obtained with the unmodified PT (TP 10/15) and Fiix-PT (TP 16/37).

higher intercept, more scattering, and lower correlation coefficient ($y = 1.09 \times x + 73.0$, $R^2 = 0.59$). Similarly, a linear relationship was obtained when comparing dFiix-PT with the anti-FXa assay ($y = 0.80 \times x + 18.8$, $R^2 = 0.81$) in 86 rivaroxaban samples. Comparing dPT with the anti-FXa assay, a linear relationship was also

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observed but with a higher intercept and lower correlation coefficient ($y = 0.78 \times x + 66.2$, $R^2 = 0.72$). For apixaban, apparent linear relationships were also observed with both dFiix-PT and dPT in relation to the anti-FXa assay: $y = 1.03 \times x + 30.7$, $R^2 = 0.91$ and $y = 0.74 + 46.2$, $R^2 = 0.69$, respectively. However, only 10 apixaban patient samples were available for the analysis, so results are inconclusive.

Discussion

The results indicate that the dFiix-PT using a single high TP final dilution of ~1:1200 of the particular TP used in our experiments can be used to determine the INR and the concentrations of dabigatran, rivaroxaban, apixaban, UFH, and enoxaparin but not of fondaparinux in plasma samples. The same anticoagulants could also be measured with the dPT, but two TP dilutions were needed, there was more deviation from linearity, and the dPT ratio did not correspond to the INR in warfarin-treated patients. These results differ from the dRVVT, which has recently been shown to be usable to assess dabigatran and rivaroxaban but, contrary to the dFiix-PT, was not usable to assess warfarin [15].

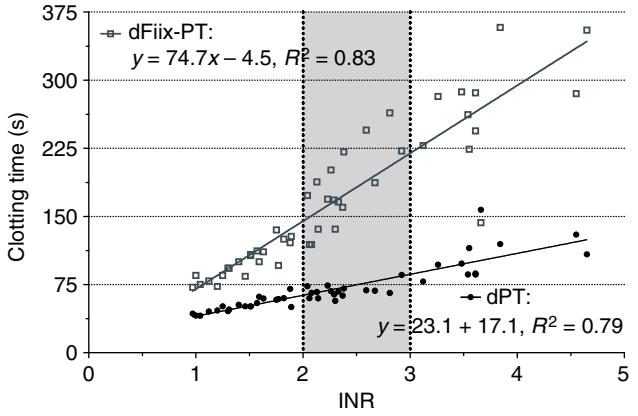

Fig. 3. Diluted thromboplastin assays in samples from patients treated with warfarin. Relationship between international normalized ratio and diluted prothrombin time (dPT) at a TP dilution of 1:750 and diluted Fiix prothrombin time (dFiix-PT) at a dilution of 1:1156 in samples obtained from patients on warfarin.

It is known that standard undiluted PT assays are not very sensitive to the plasma concentrations of dabigatran, UFH, or low molecular weight heparin [7,8], and this was confirmed in our experiments. Our results show, however, that increasing sensitivity to these agents develops with higher dilutions of TP, although the dilution factor will probably vary depending on the particular TP used. Additionally, the dFiix-PT has several advantages over the dPT in these assays. First, a single TP dilution can be used for the dFiix-PT, whereas two dilutions are required for the dPT. Second, the dPT is influenced by levels of fibrinogen, FV, and FVII in the test sample, whereas this is eliminated in the dFiix-PT by adding plasma depleted in FII and FX, making the dFiix-PT specific for only reduced FII and FX or to the influence of inhibitors such as anti-FIIa and anti-FXa agents. This reduces potential confounding effects of reduced FI, FV, or FVII. Using the dFiix-PT, a single TP dilution therefore allows screening for oral anticoagulants and heparins with the exception of fondaparinux. Third, the dFiix-PT correlated better with the standard functional assays, and the slope of the correlation curves was closer to 1 than with the dPT. Fourth, there was a larger spread of the dPT results, albeit still considerable, with the dFiix-PT for both dabigatran and rivaroxaban in Fig. 5. The spread is larger than that reported reported when comparing the dTT and LC in dabigatran patient samples [16] and chromogenic anti-FXa to LC in rivaroxaban patient samples [17]. Less spread with dFiix-PT than with dPT is likely explained

Table 1 Intra-assay and interassay variations of dPT and dFiix-PT for measurement of direct oral anticoagulants

	dPT, ng mL^{-1}		dFiix-PT, ng mL^{-1}	
	Intra-assay	Interassay	Intra-assay	Interassay
Rivaroxaban				
100	107.1 ± 9 (8.4%)	109.6 ± 11.1 (10.2%)	116.5 ± 9.1 (7.8%)	108.2 ± 21.1 (19.5%)
244	258.4 ± 4.8 (1.8%)	260.6 ± 17.6 (6.8%)	246.6 ± 14.8 (6.0%)	247.2 ± 12.5 (5.1%)%)
457	447.8 ± 13,8 (3.1%)	436.4 ± 32.6 (7.5%)	456.6 ± 21.0 (4.6%)	453.5 ± 22.9 (5.0%)
Dabigatran				
32	24.4 ± 2.7 (10.9%)	26.6 ± 5.3 (19.9%)	26.5 ± 4.5 (17.1%)	31.7 ± 10.5 (33.2%)
262	277.8 ± 6.3 (2.3%)	273.2 ± 11.2 (4.1%)	273.3 ± 12.0 (4.4%)	262.7 ± 15.0 (5.7%)
475	466.8 ± 9,3 (2.0%)	469.2 ± 21.0 (4.5%)	469.1 ± 19.2 (4.1%)	474.7 ± 29.9 (6.3%)
Apixaban				
139	119.7 ± 7.3 (6.1)	130 ± 7.0 (5.4)	119.3 ± 22.3 (18.7)	148.3 ± 13.3 (8.9)
210	202.4 ± 6.5 (3.2)	219.0 ± 4.5 (4.1)	225.4 ± 22.4 (9.9)	241.5 ± 10.6 (4.4)
419	378.4 ± 11.8 (3.1)	402.5 ± 6.7 (1.7)	392.6 ± 27.9 (7.1)	415.7 ± 13.0 (3.1)

Clotting times were measured repeatedly with dabigatran, rivaroxaban, and apixaban calibrator plasmas using dilute PT with a 1:750 final dilution TP and dFiix-PT with 1:1156 final TP dilution.
Results are expressed as: mean in ng mL−1 ± SD (CV%).

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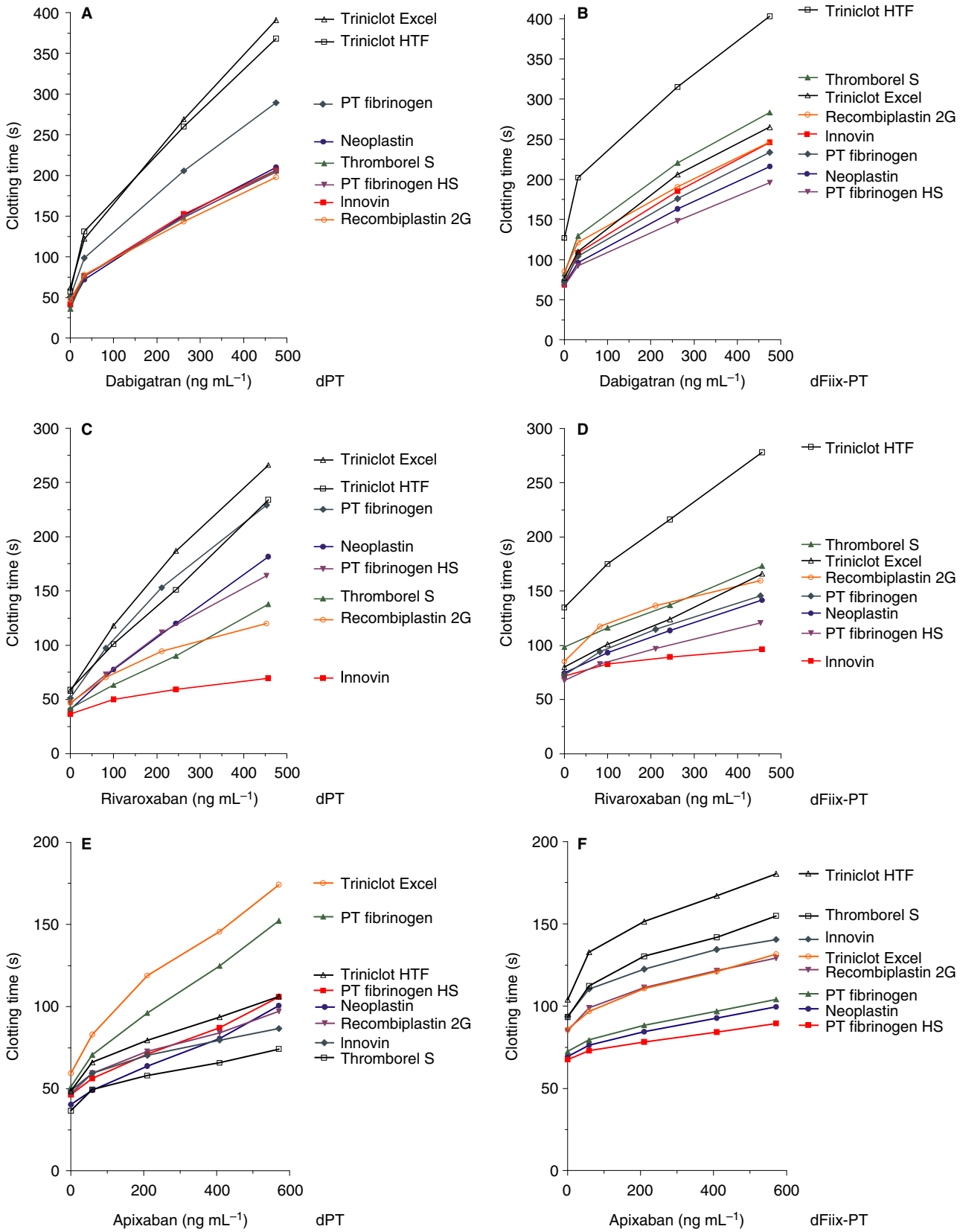

Fig. 4. Comparison of diluted prothrombin time (dPT) and diluted Fiix-PT assays using different source thromboplastins. Influence of dabigatran (A, B), rivaroxaban (C, D), and apixaban (E, F) on dPT (left panels) and dFiix-PT (right panels) using various thromboplastin reagents. Final thromboplastin dilutions of 1:750 for measurement of dPT and 1:1156 for dFiix-PT were used in these experiments.

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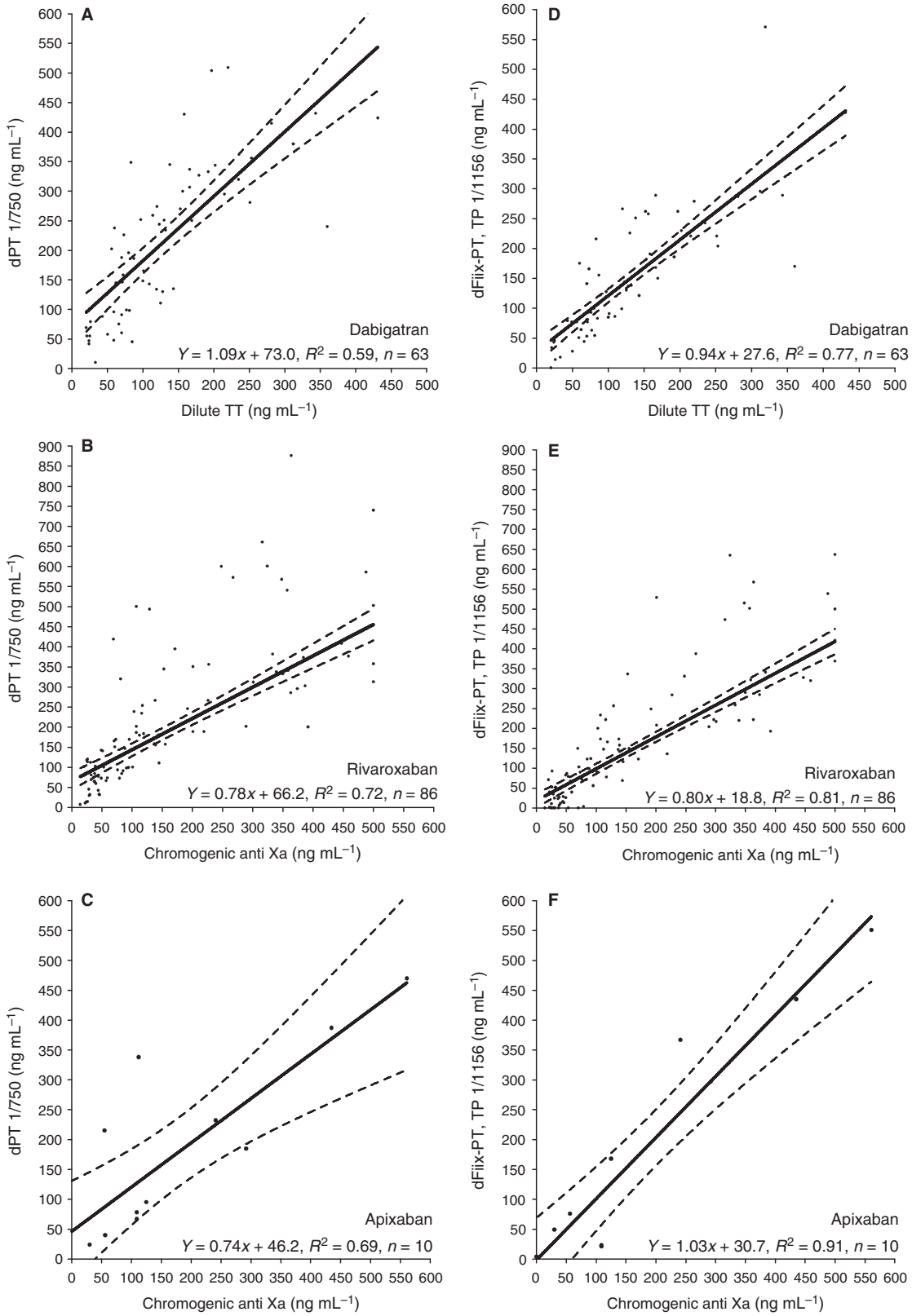

Fig. 5. Diluted prothrombin time (dPT) (left panels, A–C) and dilute Fiix-PT (right panels, D–F) for assessment of dabigatran, rivaroxaban, and apixaban in patient samples. The results were compared with calibrated standard functional assays (diluted thrombin time for dabigatran and chromogenic anti-FXa assay for rivaroxaban and apixaban).

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by the correction of potential confounders by mixing in Fiix-deficient plasma, but the considerable spread suggests that dFiix-PT results should be looked on as being semi-quantitative.

DOAC levels in the range of 30–200 ng mL^{-1} are important as dabigatran trough levels >90–100 ng mL^{-1} have been prospectively shown to progressively increase the bleeding risk with dabigatran [18] and levels >200 ng mL^{-1} have been shown to increase bleeding with both dabigatran and rivaroxaban [19–21]. The dFiix-PT assay provided an estimation of drug concentrations in the range of 30–200 ng mL^{-1} for the three DOACs tested, but at higher concentrations sample dilutions with normal plasma are needed or results could be reported as > 200 ng mL^{-1}. The test can easily be automated using standard coagulation equipment available at most hospitals and clinics. Therefore, our findings suggest that the dFiix-PT could be applied in practice as a first screening test to estimate anticoagulant levels of several direct oral agents and the vitamin K antagonist effect. The results could be reported either based on calibration curves or as seconds. The main use likely would be in emergency situations, although a point-of-care device could possibly be developed for office use. As an example, the dFiix-PT could be used to assess the presence or absence of an anticoagulant effect during bleeding or with trauma or before surgery. Measuring a simultaneous INR, thrombin time (TT), and a repeat dFiix-PT after mixing the test sample 1:1 with normal plasma could help identify the class of anticoagulant drug present when no history is available. Thus, a prolonged TT would indicate thrombin inhibition and not anti-FXa inhibition and an uncorrected mixing study would identify the presence of a direct acting inhibitor as opposed to factor deficiency. In order to provide quantification, knowledge of what drug the patient was taking and separate calibration curves for dabigatran, apixaban, rivaroxaban, UFH, and enoxaparin could be generated to express the the drug concentrations in ng mL^{-1} or in IU mL^{-1}.

It is possible that the dFiix-PT clotting time itself actually indicates quantitatively similar anticoagulation in patients on warfarin, UFH, or dabigatran. In our experiments, at INR 1.0, 1.5, 2.0, 3.0, and 3.5, the dFiix-PT was 75, 113, 150, 225, and 263 s, respectively. At therapeutic UFH concentrations (0.3–0.7 U mL^{-1}) the dFiix-PT ranged from ~125 to ~225 s, similar to clotting times observed in warfarin samples with INR 1.5–3.0. At dabigatran median trough levels similar to those observed in plasma of patients after 110 mg and 150 mg twice/day of 65 and 93 ng mL^{-1} and median peak levels of 133 and 184 ng mL^{-1} [18], the corresponding dFiix-PT values in our samples were 115 and 125 and 140 s and 160 s, respectively, similar to that found in samples with INR 1.5–2.0 in patients taking warfarin. However, the therapeutic concentration of dabigatran (10th–90th percentiles) varies 5-fold in patients receiving the same dose [18]. Therefore, trough and peak concentrations could be as low as 28 ng mL^{-1} and as high as 383 ng mL^{-1}, respectively, during therapeutic anticoagulation with dabigatran. The corresponding dFiix-PT is 90 and 225 s, again similar to the clotting times observed with INR 1.5–3.0 in patients taking warfarin. In the Randomised Evaluation of Long-term Anticoagulation Therapy (RE-LY) trial arterial thromboembolism increased when trough levels were < 64 ng mL^{-1} (corresponding to dFiix-PT < 115 s in our experiments), and major bleeding increased progressively with increasing trough levels > 88 ng mL^{-1} (corresponding to dFiix-PT > 125 s) [18]. To our knowledge, correlations of drug levels with drug efficacy and safety have not yet been published for rivaroxaban or apixaban. Taken together, the expected therapeutic range of dFiix-PT 1:1156 for warfarin, UFH, and dabigatran may be in the 112–250 s range using our experimental conditions, although this will vary depending on the TP used. Experiments are under way for assessing how thrombin generation and clot formation correspond to similar clotting times observed with the different agents.

It is interesting that fondaparinux, an antithrombin-dependent specific anti-FXa agent, did not influence the dFiix-PT or dPT and that direct anti-FXa agents (rivaroxaban, apixaban) appeared to influence the clotting times less than the anti-FIIa agent dabigatran, UFH, and warfarin. The difference in results between heparins and fondaparinux might be because heparins (UFH and enoxaparin) in complex with antithrombin inhibit IIa by themselves and FIIa generation via FXa formation, while fondaparinux associated with antithrombin only inhibits FXa. It has been suggested that if FXa generation is high, FXa will become incorporated into in the prothrombinase complex where the fondaparinux–antithrombin complex cannot get to and inhibit FXa making fondaparinux a less effective anticoagulant than inhibitors of FIIa inhibitors in the circumstance of high FXa generation [11,22–24].

The current study has a number of limitations. We observed that for some patient samples > 200 ng mL^{-1} of DOACs, a large discrepancy could occur between the calibrated specific assay and the dFiix-PT and dPT. The discrepancies corrected by mixing the samples with normal plasma at ratios of 1:4 and 1:8. Second, our dTP assays were only tested with a single coagulation instrument that uses mechanical detection of clot formation, and other clot detection methods need to be tested. Third, the dTP assays are not specific so that knowledge of what anticoagulant is present is required for reporting a concentration. However, the addition of Fiix deficient plasma improved the assay results for each drug, presumably due to the correction for potential confounding reductions in fibrinogen and coagulation FV and FVII that may occur in the test sample, such as after freezing and thawing. Fourth, the dTP assays, resembling the tissue TP inhibition test [11], are likely to be sensitive to lupus anticoagulants (LAs), and the presence of LAs, therefore, could lead to a false assumption of high anticoagulant concentration. Again,

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however, the predilution of the test sample with buffer and with Fiix-deficient plasma will make the dFiix-PT less susceptile to LAs than the dPT, which is performed on undiluted test plasma.

In conclusion, a single-dilution diluted Fiix-PT may be suitable to screen for the presence of warfarin, dabigatran, rivaroxaban, apixaban, UFH, and enoxaparin in plasma. Using drug-specific calibration curves, estimations of plasma concentrations can be made in samples from patients taking these agents. The test may be suitable for emergency testing of anticoagulant concentrations or effect, although clinical value needs further studies.

Addendum

L. R. Letertre designed and performed experiments and wrote the first manuscript draft. P. T. Onundarson designed the study and experiments and edited the manuscript. B. R. Gudmundsdottir designed and performed experiments. C. W. Francis co-wrote and edited the manuscript. R. C. Gosselin, M. Skeppholm, R. E. Malmstrom, S. Moll, E. Hawes, and S. Francart provided patient samples and edited the manuscript.

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Disclosure of Conflict of Interests

C. W. Francis reports grants from Eisai and support from Janses outside of submitted work. B. R. Gudmundsdottir and P. T. Onundarson have patent P8151US00 issued. The other authors state that they have no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Proportions of test plasma and reagents used in experiments.

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