Journal of Thrombosis and Haemostasis, **14**: 2194–2201 DOI: 10.1111/jth.13486

ORIGINAL ARTICLE

Poor comparability of coagulation screening test with specific measurement in patients receiving direct oral anticoagulants: results from a multicenter/multiplatform study

S. TESTA,* C. LEGNANI,† A. TRIPODI,‡ O. PAOLETTI,* V. PENGO,§ R. ABBATE,¶ L. BASSI,* P. CARRARO,** M. CINI,† R. PANICCIA,¶ D. POLI¶ and G. PALARETI††

**Department of Laboratory Medicine, Hemostasis and Thrombosis Center, AO Istituti Ospitalieri, Cremona, Italy; †Angiology and Blood Coagulation, University Hospital of Bologna, Bologna; ‡Department of Clinical Sciences and Community Health, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Università degli Studi di Milano, IRCCS Cà Granda Maggiore Hospital Foundation, Milan; §Department of Cardiothoracic and Vascular Sciences, University Hospital of Padua, Padua; ¶Thrombosis Center, Department of Heart and Vessels, University Hospital of Florence, Florence; **Department of Laboratory Medicine, ULSS 16 and University-Hospital of Padova, Padova; and ††Cardiovascular Diseases, University of Bologna, Bologna, Italy*

To cite this article: Testa S, Legnani C, Tripodi A, Paoletti O, Pengo V, Abbate R, Bassi L, Carraro P, Cini M, Paniccia R, Poli D, Palareti G. Poor comparability of coagulation screening test with specific measurement in patients receiving direct oral anticoagulants: results from a multicenter/multiplatform study. *J Thromb Haemost* 2016; **14**: 2194–201.

Essentials

- Prothrombin and partial thromboplastin time (PT/PTT) measure direct oral anticoagulants (DOACs).
- PT, PTT and specific tests for DOACs were performed on patients treated for atrial fibrillation.
- Normal PT/PTT don't exclude DOAC activity and their prolongation doesn't confirm DOAC action.
- The use of PT or PTT to evaluate DOAC activity could cause dangerous misinterpretations.

Summary. *Background:* Prothrombin time (PT) and activated partial thromboplastin time (APTT) have been proposed to measure the effect of oral anti-activated factor X (FXa) or anti-activated FII drugs, respectively. *Aims:* To evaluate the relationships and responsiveness of PT and APTT versus direct oral anticoagulant (DOAC) concentrations measured with specific coagulation tests performed with different platforms in four Italian anticoagulation clinics. *Methods:* Six hundred and thirty-five patients with atrial fibrillation participated in the study: 240 were receiving dabigatran, 264 were receiving rivaroxaban, and 131 were receiving apixaban. Blood was taken at trough and peak within the first month (15–25 days) of treatment. PT, APTT, diluted thrombin time (dTT) calibrated for dabigatran and anti-FXa calibrated for rivaroxaban or apixaban were determined. *Results:* For dabigatran, the correlation between APTT and dTT ranged from $r = 0.80$ to $r = 0.62$. For rivaroxaban, the correlation between the anti-FXa assay and PT ranged from $r = 0.91$ to $r = 0.73$. For apixaban, the correlation between the anti-FXa assay and PT was lower than for the two other drugs ($r = 0.81$ to $r = 0.54$). Despite the above significant correlations, the responsiveness of PT or APTT was relatively poor. A discrepancy between global testing and DOAC plasma concentrations was shown in a considerable proportion of patients, depending on the platform and drug, with values ranging from 6% to 62%. *Conclusions:* Overall, poor responsiveness of the screening tests to DOAC concentrations was observed. PT and APTT normal values cannot exclude DOAC anticoagulant activity, and PT or APTT prolongation is not always associated with DOAC anticoagulant effect as determined with specific tests.

Keywords: activated partial thromboplastin time; anticoagulant drugs; atrial fibrillation; blood coagulation test; prothrombin time.

Correspondence: Sophie Testa, Hemostasis and Thrombosis Center, AO Istituti Ospitalieri, Viale Concordia 1, 26100 Cremona, Italy.
Tel.: +39 37 240 5453; fax: +39 37 240 5457.
E-mail: s.testa@ospedale.cremona.it

Received 25 January 2016
Manuscript handled by: S. Kitchen
Final decision: F. R. Rosendaal, 19 August 2016

Introduction

Oral anticoagulant therapy is recommended for the treatment and prevention of venous and arterial thromboembolism [1]. Until a few years ago, vitamin K antagonists (VKAs) were the only available drugs; however, owing to their pharmacologic characteristics, these require frequent laboratory monitoring and expert dose adjustment. More

recently, the direct oral anticoagulants (DOACs) have been introduced into clinical practice for the prevention of stroke and systemic embolism in patients with atrial fibrillation, and for the prevention and treatment of venous thromboembolism. In contrast to VKAs, the use of DOACs has been proposed without the need for laboratory testing and dose adjustment, because phase III clinical trials showed efficacy and safety at fixed doses based only on clinical criteria [2–5].

However, there are situations in which DOAC anticoagulant activity should be measured. These include bleeding or thromboembolic events, before surgery/invasive procedures, and when a decision on thrombolytic therapy in stroke patients needs to be made. Furthermore, testing could be useful in a number of other situations, including: (i) patients with renal/liver disease; (ii) whenever a possible interaction with other drugs is suspected; (iii) in patients with extreme body weight; (iv) to assess adherence to the therapy; and (v) when overcoagulation/under-anticoagulation is suspected [6–9]. In addition, because specific antidotes will soon become available, DOAC measurement might be useful to ensure their appropriate administration in cases of immediate reversal of anticoagulation, to prevent overuse of these expensive medications [10,11].

Originally, prothrombin time (PT) and activated partial thromboplastin time (APTT) were proposed as methods for measuring the levels of oral anti-activated factor X (FXa) drugs and dabigatran, respectively, because of their simplicity and prompt availability [12–14]. The implicit assumption was that PT and APTT are responsive and specific for DOACs. However, it was not considered that they may be potentially affected by interference. PT and APTT are, in fact, functional global tests that measure the time of clot formation, which can be altered in several clinical conditions, including liver disease, acquired/congenital factor deficiencies, or the presence of antiphospholipid antibodies [15]. Furthermore, because of their anti-FXa or anti-activated FII activity, DOACs may interact differently with screening coagulation tests, depending on the composition of the reagents and the type of coagulometer used for testing [16–22]. Finally, there might be considerably different levels of responsiveness of PT and APTT to increasing DOAC concentrations. All of these analytic and biological variables can significantly affect PT and APTT, thus limiting their value for measuring DOAC in practice. Although the limitations of PT and APTT have been highlighted by previous studies [18–22], the numbers of investigated patients were relatively small, or investigations were based on limited numbers of testing platforms. In this study, we sought to evaluate the relationship between DOAC concentrations, measured with specific tests, and PT and APTT, measured with different commercial platforms, for a relatively large number of patients treated with the three DOACs presently available.

Design, patients, and methods

Design

This was an observational multicenter multiplatform study on patients treated with dabigatran, rivaroxaban, or apixaban, and was approved by the ethical committee of the general hospital of Cremona. Four large Italian anticoagulation clinics (Bologna [A], Cremona [B], Padua [C], and Florence [D]), affiliated with the Italian Federation of Anticoagulation Clinics and engaged in the Survey on Anticoagulated Patients (START) Register (www.start-register.org), were asked to join the collaborative study by collecting and testing plasma from patients treated with DOACs.

Patients

Dabigatran, rivaroxaban and apixaban were introduced at different time from June 2013, and medical prescription was allowed with different rules in individual Italian regions. Consequently, during the study period (year 2014), the four anticoagulation clinics enrolled patients receiving dabigatran and rivaroxaban, whereas only three of them enrolled patients receiving apixaban (A, B, and D). After giving informed consent, 635 consecutive patients with atrial fibrillation seen at the anticoagulation clinics from 1 January 2014 to 31 December 2014 were enrolled in the study, provided that they had been treated with DOACs for at least 1 week and were available to attend the clinics for blood sampling at the specified time points (see below). Two hundred and forty patients were receiving dabigatran (122 and 118 taking 150 mg or 110 mg twice daily, respectively), 264 were receiving rivaroxaban (183 and 81 taking 20 mg or 15 mg once daily, respectively), and 131 were receiving apixaban (98 and 33 taking 5 mg or 2.5 mg twice daily, respectively). Patients were evaluated at enrollment, and the type and the dose of drug were prescribed at the discretion of the attending physician, on the basis of both clinical characteristics and renal function. Patients were followed within the first month of treatment (from 15 days to 25 days from the beginning), when trough and peak blood samples were taken. The trough sample was obtained 12 h after the last dose intake for dabigatran and apixaban, and 24 h after the last dose intake for rivaroxaban. The peak sample was obtained 2 h after ingestion of the drug, ensuring concomitant food intake for patients receiving rivaroxaban. Plasma samples were collected in vacuum plastic tubes (Vacutainer; Becton Dickinson, Plymouth, UK), containing 3.2% trisodium citrate (9 : 1 v/v, blood/anticoagulant). Blood was centrifuged at 2000 × *g* for 20 min, and plasma was quickly frozen and stored at − 80 °C until testing. One thousand two hundred and seventy blood samples were collected in the four clinics and used for analysis: 480 for dabigatran, 528 for rivaroxaban, and 262 for apixaban (Table S1).

Table 1 Patients and number of samples, instruments, reagents, prothrombin time (PT) and activated partial thromboplastin time (APTT) upper limits of normal range used in the four anticoagulation clinics

	Clinic A	Clinic B	Clinic C	Clinic D
Dabigatran (patient no./sample no.)	47/94	70/158	89/178	25/50
Rivaroxaban (patient no./sample no.)	72/144	108/216	61/122	23/46
Apixaban (patient no./sample no.)	30/60	91/182	–	10/20
Coagulometer	STA compact (Stago)	STA-R (Stago)	CA 7000 (Sysmex)	ACL TOP 700 (Werfen)
Reagents				
PT	Recombiplastin (Werfen)	Neoplastin (Stago)	Innovin (Siemens)	Recombiplastin (Werfen)
APTT	Actin (Siemens)	PTT (Stago)	Actin-FS (Siemens)	SynthASil (Werfen)
Dabigatran	Thrombin Siemens	Thrombin Stago	Hyphen Hemoclot	Hyphen Hemoclot
Rivaroxaban	Liquid Anti-Xa Stago	Liquid Anti-Xa Stago	Hyphen DiXal	Hyphen DiXal
Apixaban	Liquid Anti-Xa Stago	Liquid Anti-Xa Stago	–	Technochrome anti-Xa Kit
Calibrators				
Dabigatran	Hyphen Biomed	Hyphen Biomed	Hyphen Biomed	Hyphen Biomed
Rivaroxaban	Calibrator Stago	Calibrator Stago	Biophen Rivaroxaban	Hyphen Biomed
Apixaban	Calibrator Stago	Calibrator Stago	–	Technoview Apixaban
PT upper limit of normal range	< 1.20	< 1.20	< 1.13	< 1.20
APTT upper limit of normal range	< 1.25	< 1.22	< 1.30	< 1.27

Laboratory tests

PT and APTT, expressed as a ratio (patient clotting times/normal clotting times), were measured with the combination reagent/instrument as detailed in Table 1. DOAC anticoagulant activity, expressed as drug concentration-equivalent (ng mL^{-1}), was also measured according to diluted thrombin time (dTT) calibrated for dabigatran [23], and specific anti-FXa assays calibrated for rivaroxaban or apixaban [24–27]. Testing was performed in each clinic with appropriate coagulation platforms, according to the manufacturers' recommendations.

Statistical analysis

Linear regression (least-squares method) was used to evaluate the relationship between DOAC concentrations as measured with specific tests and the prolongation of PT or APTT for each clinic. Responsiveness, defined as the extent of prolongation of PT or APTT at increasing DOAC concentrations, was assessed for each clinic and drug as follows. The entire range of concentrations observed in the investigated patients (i.e. from < 30 ng mL^{-1} to > 350 ng mL^{-1}) was subdivided into classes of 20 ng mL^{-1} each. These concentrations were then plotted (vertical axis) against the corresponding PT or APTT ratio, and the results were compared with the upper limit of the reference interval. The responsiveness was also evaluated by interpolation of the drug (arbitrary) concentration of 200 ng mL^{-1} from the regression lines to determine the corresponding PT or APTT ratio. By definition, the higher the PT or APTT ratio, the greater the test responsiveness.

Statistical analyses were performed with GRAPHPAD software (GraphPad, San Diego, CA, USA); a two-sided P-value of ≤ 0.05 was considered to be statistically significant.

Results

Six hundred and thirty-five patients were enrolled, and 1270 blood samples were tested. The distributions of numbers of patients and numbers of samples in each clinic are shown in Table 1. Peak values were significantly higher than trough values for each drug and clinic, regardless of the test used (Table S2).

Relationship of DOAC plasma concentration with PT or APTT

We analyzed the relationship of the concentrations of dabigatran and anti-FXa drugs with APTT and PT, respectively. For this analysis, peak and trough values were combined in order to increase the numbers of observations. Regression lines, equations describing the relationship of drug concentration with PT or APTT ratio, correlation coefficients (r-values) and coefficients of determination (r^2-values) are summarized in Figs S1–S3. For dabigatran, the correlation between APTT and dTT ranged from $r = 0.80$ to $r = 0.62$. For rivaroxaban, the correlation between the anti-FXa assay and PT ranged from $r = 0.91$ to $r = 0.73$. For apixaban, the correlation between the anti-FXa assay and PT was lower than for the other two drugs ($r = 0.81$ to $r = 0.54$). Although r-values were acceptable in most cases, the slopes of the regression lines were relatively small, indicating that the PT and APTT tests were not particularly responsive to the DOAC plasma concentrations (see below).

Responsiveness of PT and APTT to increasing DOAC concentrations

The responsiveness of PT or APTT to DOAC concentration was assessed by selecting classes of DOAC concentrations from low to high, and comparing them with PT

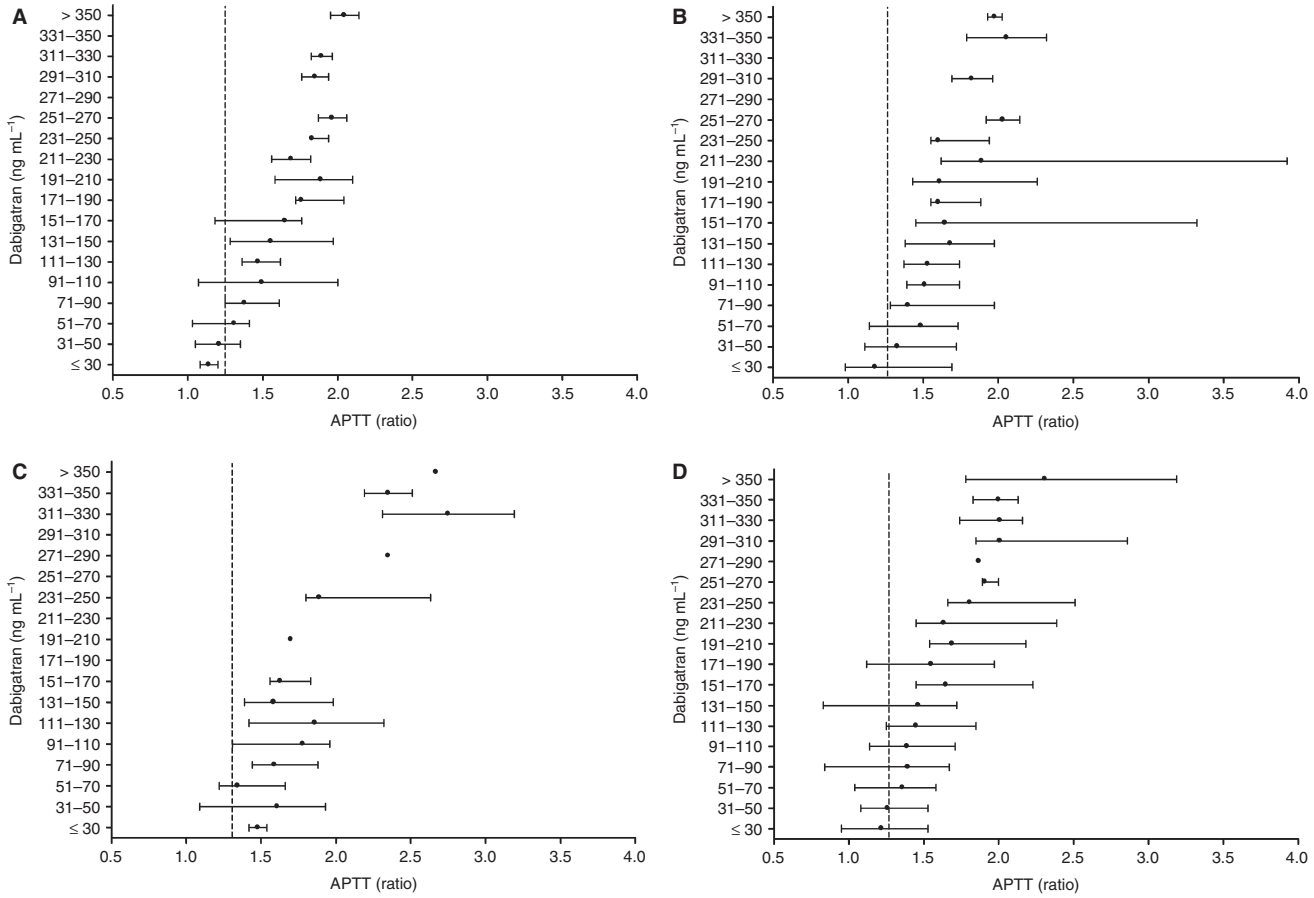

Fig. 1. Activated partial thromboplastin time (APTT) ratio median values and ranges obtained for different arbitrary classes of dabigatran concentrations. (A)–(D) represent results obtained at different clinics. Dotted lines represent the upper limit of the normal range.

or APTT. The results are shown in Figs 1–3. APTT was still within normal limits when dabigatran concentrations were 51–70 ng mL^{-1} (clinics A, B, and C) and 131–150 ng mL^{-1} (clinic D) (Fig. 1). PT was still within normal limits when rivaroxaban concentrations were 91–110 ng mL^{-1} (clinic A), 51–70 ng mL^{-1} (clinic B), and 171–190 ng mL^{-1} (clinic D) (Fig 2). PT was still within normal limits when apixaban concentrations were 151–170 ng mL^{-1} (clinic A) and 231–250 ng mL^{-1} (clinic B) (Fig 3). Detailed analysis of the agreement of PT or APTT with DOAC concentrations are shown in Table 2. There were many instances of PT or APTT results still being normal when the DOAC concentrations were > 50 ng mL^{-1} and vice versa.

The responsiveness of the screening tests to increasing DOAC concentrations was also evaluated by interpolation of the drug (arbitrary) concentration of 200 ng mL^{-1} from the regression lines to determine the corresponding PT or APTT ratio. As shown in Table 2 and Figs S1–S3, the APTT ratio corresponding to 200 ng mL^{-1} dabigatran varied from 1.7 (clinic A) to 2.0 (clinic C). The PT ratio corresponding to 200 ng mL^{-1} rivaroxaban varied from 1.3 (clinic C) to 1.9 (clinic B). The PT ratio corresponding to 200 ng mL^{-1} apixaban was 1.3 (clinics A, B, and D).

Discussion

Previous studies showed poor responsiveness of the coagulation screening tests for DOAC measurements in relation to type of reagent used. Even though some reagents showed acceptable responsiveness to a specific drug (e.g. neoplastin and recombiplastin for rivaroxaban [28], this conclusion cannot be extended to all drugs and all platforms available on the market. In fact, different reagents not only show different levels of responsiveness to DOAC, but also show different levels of responsiveness to individual coagulation factors. This variability creates difficulties in their application for patients receiving treatment, and the harmonization of results across laboratories.

Even though specific tests are not yet widely used in routine clinical practice, dTT and the ecarin tests (clotting or chromogenic) for dabigatran and the chromogenic anti-FXa assays for the anti-FXa drugs are commercially available. They show good linearity and responsiveness to DOACs, and the results can be expressed as drug concentration-equivalents [23–27] by the use of specific calibrators that are commercially available. The results obtained with the above tests correlated with the results obtained with mass spectrometry [29,30].

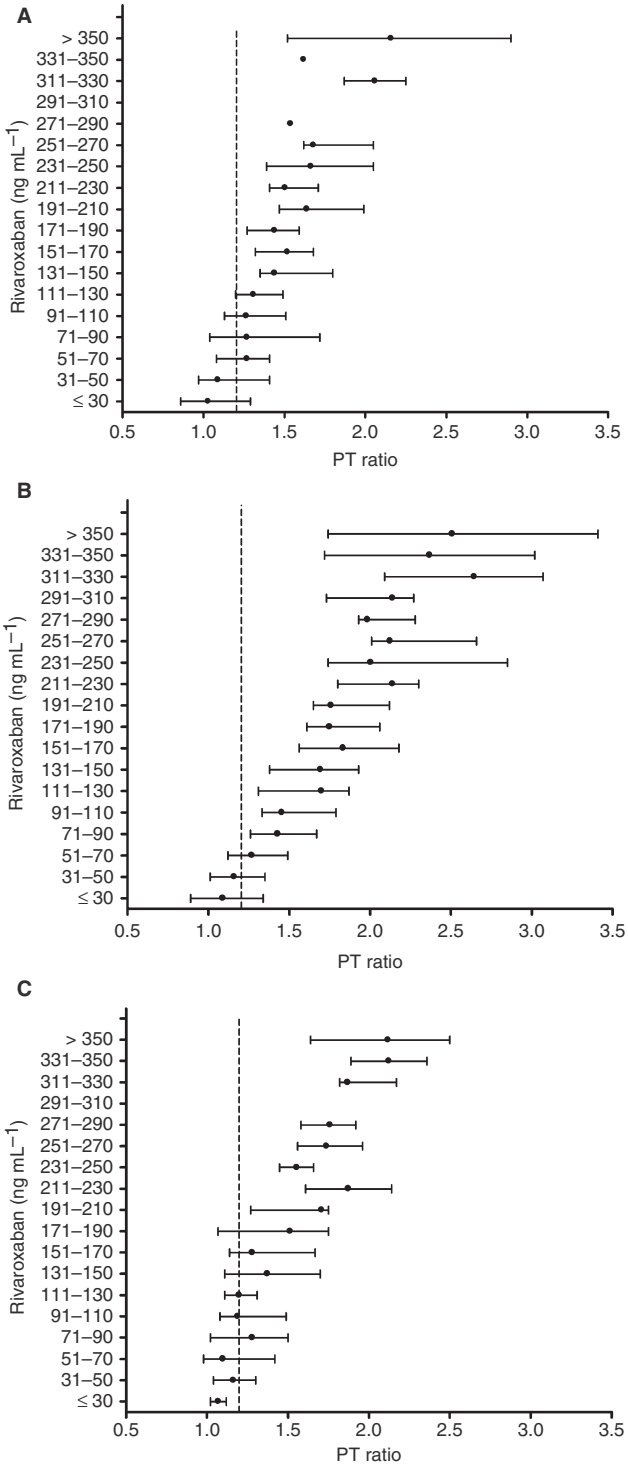

Fig. 2. Prothrombin time (PT) ratio median values and ranges obtained for different arbitrary classes of rivaroxaban concentrations. (A), (B) and (D) represent results obtained at different clinics. Dotted lines represent the upper limit of the normal range.

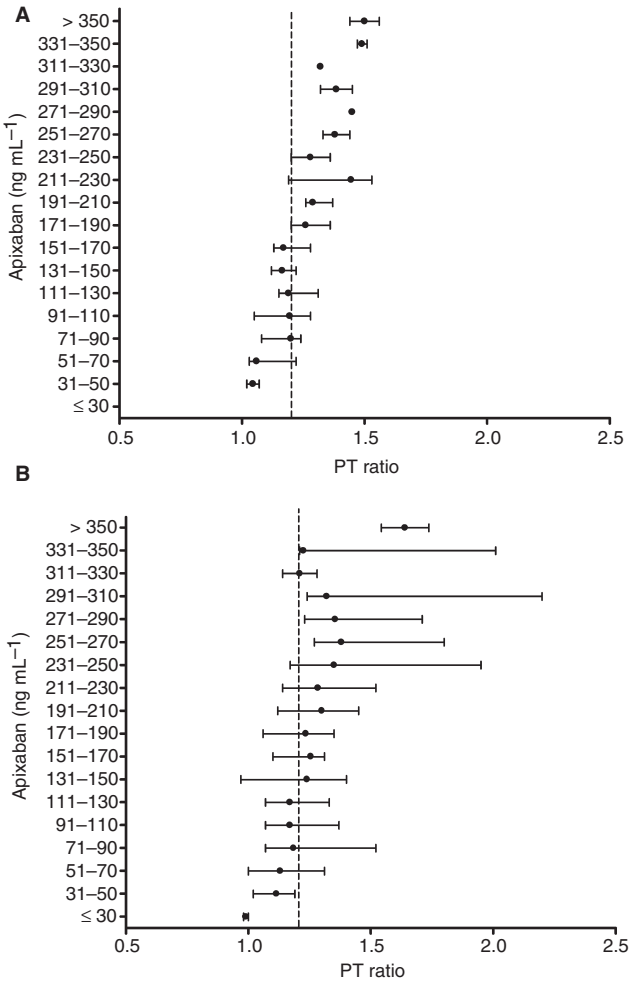

Fig. 3. Prothrombin time (PT) ratio median values and ranges obtained for different arbitrary classes of apixaban concentrations. (A) and (B) represent results obtained at different clinics. Dotted lines represent the upper limit of the normal range.

There is an ongoing debate in the literature regarding DOAC laboratory testing. Some authors argue that specific tests should be recommended for DOAC measurements, highlighting the inappropriateness of PT or APTT [31,32], whereas others suggest that PT or APTT should be used, if not to quantify DOACs, at least to assess for the presence/absence of drug and the anticoagulant activity [12–14].

The present study, carried out on a large number of patients, confirms the poor concordance between DOAC plasma concentrations and PT or APTT, highlighting that a normal test result is not always associated with the absence of or minimal residual concentrations of drugs. These observations raise concerns about the value of PT or APTT for assessing the individual anticoagulant activity of DOACs or for assessing the presence or absence of circulating drugs. This aspect represents a clinical problem, because of the risk of misinterpretation, which may endanger patients. As an example, patients who present with normal PT or APTT could be erroneously considered to be safe for surgery; on, in contrast, surgery, invasive procedures or thrombolysis could be postponed or contraindicated in patients showing prolonged PT or APTT. In addition, the availability of specific antidotes to be used for immediate neutralization of

Table 2 Responsiveness of prothrombin time (PT) or activated partial thromboplastin time (APTT) to direct oral anticoagulants (DOACs)

	Clinic A	Clinic B	Clinic C	Clinic D
Dabigatran (APTT), ratio (95% CI)	1.67 (1.61–1.75)	1.74 (1.66–1.80)	1.97 (1.82–2.14)	1.68 (1.63–1.75)
Rivaroxaban (PT), ratio (95% CI)	1.57 (1.54–1.62)	1.88 (1.85–1.94)	1.31 (1.26–1.38)	1.53 (1.49–1.59)
Apixaban (PT), ratio (95% CI)	1.28 (1.25–1.34)	1.29 (1.26–1.36)	NA	1.32 (1.21–1.47)
Normal APTT and dabigatran > 50 ng mL^{-1}, *n* (%)	6/87 (6.9)	6/107 (5.6)	3/42 (7.1)	19/158 (12.0)
Prolonged APTT and dabigatran ≤ 50 ng mL^{-1}, *n* (%)	1/7 (14.3)	22/51 (43.1)	5/8 (62.5)	9/20 (45.0)
Normal PT and rivaroxaban > 50 ng mL^{-1}, *n* (%)	7/75 (9.3)	3/119 (2.5)	2/26 (7.7)	34/109 (31.2)
Prolonged PT and rivaroxaban ≤ 50 ng mL^{-1}, *n* (%)	7/69 (10.1)	11/97 (11.3)	5/20 (25.0)	3/13 (23.1)
Normal PT and apixaban > 50 ng mL^{-1}, *n* (%)	25/58 (43.1)	73/172 (42.4)	NA	6/18 (33.3)
Normal PT and apixaban ≤ 50 ng mL^{-1}, *n* (%)	0/2 (0)	0/10 (0)	NA	1/2 (50.0)

CI, confidence interval; NA, not available because clinic C did not enroll patients receving apixaban. Ratio (95% CI): the APTT or PT ratio (95% CI) of patient clotting time/normal clotting time corresponding to a DOAC concentration of 200 ng mL−1 (see also Figs 1–3). *n* (%): number and percentage of discrepancies between global test results and DOAC anticoagulant activity measured with specific tests.

anticoagulation in patients with life-threatening hemorrhage might call for DOAC measurement to ensure appropriate use of these medications [11]. They would therefore require specific and reliable DOAC testing, which cannot be guaranteed with PT or APTT.

On the other hand, PT and APTT show many of the characteristics that make them 'ideal' (e.g. inexpensive, widely available, and rapid), but, as shown in this and other reports, they are not adequately responsive to the DOAC concentrations. These limitations could be overcome by the use of specific tests. They are now commercially available from many manufacturers, can be easily set up in any of the ordinary coagulometers, and can be performed even in emergency situations without special expertise. Even though they are more expensive than PT or APTT, their use in special and selected situations will ultimately counterbalance their costs.

The strengths of our evaluation are the real-life nature of the study, dealing with a large number of samples from anticoagulated patients, and the use of different commercial platforms in four different clinics. Some limitations of the study should be recognized. First, we did not measure DOAC concentrations with gold standard methods that would have made comparison with PT and APTT more valuable. However, previous studies have shown that specific tests for DOAC are highly correlated with mass spectrometry results for the measurement of DOAC concentrations [29,30]. Second, we could not directly compare the different platforms for PT and APTT, as samples collected in different clinics were not centralized for measurement within the same laboratory. However, it should be recognized that, although a direct comparison is not possible, the indirect comparison is still valuable for giving some indication of which brand are more or less responsive to DOACs. Third, owing to the limited experience with DOACs, clinical endpoints relating adverse events (hemorrhage or thrombosis) with DOAC plasma concentrations are not available, so a fair comparison between PT/APTT and specific tests is not possible. A clinically meaningful comparison would require retrieval of data from clinical trials, as was done for dabigatran by Reilly *et al*. [32], but this was beyond the scope of the present study. Fourth, we did not assess the reasons why PT/APTT and the specific tests do not agree. For this evaluation, a larger sample of well-characterized patients would have been required.

In conclusion, the present study shows that: (i) PT and APTT react differently to DOACs in relation to the type of drug and to the type of reagent, suggesting that each laboratory should be aware of the performance of the reagent used; (ii) patients having the same DOAC plasma concentrations may present with different PT or APTT results; and (iii) normal APTT or PT test results obtained in patients treated with dabigatran or anti-FXa drugs, respectively, cannot exclude significantly high plasma concentrations of the relevant DOAC, especially with poorly responsive APTT or PT reagents. Consequently, the use of PT or APTT in clinical practice to evaluate DOAC anticoagulant activity could cause dangerous misinterpretations.

Addendum

S. Testa, C. Legnani, A. Tripodi, and O. Paoletti conceived the study, reviewed the data, and wrote the manuscript. S. Testa, C. Legnani, O. Paoletti, V. Pengo, R. Abbate, L. Bassi, P. Carraro, M. Cini, R. Paniccia, O. Paoletti, and D. Poli enrolled patients and supervised laboratory measurements. S. Testa, C. Legnani, and M. Cini analyzed the data. All authors revised and accepted the final version of the manuscript.

Acknowledgements

This study was supported by the START Laboratory Register.

Disclosure of Conflict of Interests

G. Palareti reports receiving personal fees from Alfa-Wassermann, Daiichi-Sankyo, Instrumentation Laboratory, Siemens, and Stago, outside the submitted work. 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:

Fig. S1. Dabigatran and APTT ratio.
Fig. S2. Rivaroxaban and PT ratio.
Fig. S3. Apixaban and PT ratio.
Table S1. Clinical characteristics, center distribution of patients, and number of samples.
Table S2. DOAC measurement with specific and global tests at peak and trough levels.

References

1 Ageno W, Gallus AS, Wittkowsky A, Crowther M, Hylek EM, Palareti G; American College of Chest Physicians. Oral anticoagulant therapy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest* 2012; **41**(Suppl. 2): e44S–88S.

2 Connolly SJ, Ezekowitz MD, Yusuf S, Eikelboom J, Oldgren J, Parekh A, Pogue J, Reilly PA, Themeles E, Varrone J, Wang S, Alings M, Xavier D, Zhu J, Diaz R, Lewis BS, Darius H, Diener HC, Joyner CD, Wallentin L; RE-LY Steering Committee and Investigators. Dabigatran versus warfarin in patients with atrial fibrillation. *N Engl J Med* 2009; **361**: 1139–51.

3 Patel MR, Mahaffey KW, Garg J, Pan G, Singer DE, Hacke W, Breithardt G, Halperin JL, Hankey GJ, Piccini JP, Becker RC, Nessel CC, Paolini JF, Berkowitz SD, Fox KA, Califf RM; ROCKET AF Investigators. Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. *N Engl J Med* 2011; **365**: 883–91.

4 Granger CB, Alexander JH, McMurray JJ, Lopes RD, Hylek EM, Hanna M, Al-Khalidi HR, Ansell J, Atar D, Avezum A, Bahit MC, Diaz R, Easton JD, Ezekowitz JA, Flaker G, Garcia D, Geraldes M, Gersh BJ, Golitsyn S, Goto S, *et al.*; ARISTOTLE Committees and Investigators. Apixaban versus warfarin in patients with atrial fibrillation. *N Engl J Med* 2011; **365**: 981–92.

5 Giugliano RP, Ruff CT, Braunwald E, Murphy SA, Wiviott SD, Halperin JL, Waldo AL, Ezekowitz MD, Weitz JI, Špinar J, Ruzyllo W, Ruda M, Koretsune Y, Betcher J, Shi M, Grip LT, Patel SP, Patel I, Hanyok JJ, Mercuri M, *et al.*; ENGAGE AF-TIMI 48 Investigators. Edoxaban versus warfarin in patients with atrial fibrillation. *N Engl J Med* 2013; **369**: 2093–104.

6 Pengo V, Crippa L, Falanga A, Finazzi G, Marongiu F, Palareti G, Poli D, Testa S, TiraferriE, Tosetto A, Tripodi A, Siragusa S, Manotti C. Questions and answers on the use of dabigatran and perspectives on the use of other new oral anticoagulants in patients with atrial fibrillation. A consensus document of the Italian Federation of Thrombosis Center (FCSA). *Thromb Haemost* 2011;**106**: 868–76.

7 Baglin T. The role of the laboratory in treatment with new oral anticoagulants. *Br J Haematol* 2013; **163**: 160–7.

8 Tripodi A. The laboratory and the direct oral anticoagulants. *Blood* 2013; **121**: 4032–5.

9 Dale BJ, Chan NC, Eikelboom JW. Laboratory measurement of the direct oral anticoagulants. *Br J Haematol* 2016; **172**: 315–36.

10 Pollack CV Jr, Reilly PA, Eikelboom J, Glund S, Verhamme P, Bernstein RA, Dubiel R, Huisman MV, Hylek EM, Kamphuisen PW, Kreuzer J, Levy JH, Sellke FW, Stangier J, Steiner T, Wang B, Kam CW, Weitz JI. Idarucizumab for dabigatran reversal. *N Engl J Med* 2015; **6**: 511–20.

11 Bauer KA. Targeted anti-anticoagulants. *N Engl J Med* 2015; **373**: 569–71.

12 Krishnamoorthy A, Sherwood MW, Lopes RD, Becker RC. The periprocedural management of novel oral anticoagulants in patients with nonvalvular atrial fibrillation: rationale and a summary of the available evidence from phase 3 clinical trials. *Am Heart J* 2015; **169**: 315–22.

13 Daniels PR. Peri-procedural management of patients taking oral anticoagulants. *BMJ* 2015; **351**: h2391.

14 Pernod G, Albaladejo P, Godier A, Samama CM, Susen S, Gruel Y, Blais N, Fontana P, Cohen A, Llau JV, Rosencher N, Schved JF, de Maistre E, Samama MM, Mismetti P, Sié P; Working Group on Perioperative Haemostasis. Management of major bleeding complications and emergency surgery in patients on long-term treatment with direct oral anticoagulants, thrombin or factor Xa inhibitors: proposals of the Working Group on Perioperative Haemostasis (GIHP). *Arch Cardiovasc Dis* 2013; **106**: 382–93.

15 Kamal AH, Tefferi A, Pruthi RK. How to interpret and pursue an abnormal prothrombin time, activated partial thromboplastin time, and bleeding time in adults. *Mayo Clin Proc* 2007; **82**: 864–73.

16 Tripodi A. Interference of new oral anticoagulants with frequently used coagulation tests. *Clin Chem Lab Med* 2012; **50**: 1501–3.

17 van Blerk M, Bailleul E, Chatelain B, Demulder A, Devreese K, Douxfils J, Jochmans K, Mullier F, Wijns W, Soumali MR, Coucke W, Vernelen K, van de Walle P. Influence of dabigatran and rivaroxaban on routine coagulation assays. A nationwide Belgian survey. *Thromb Haemost* 2015; **113**: 154–64.

18 Douxfils J, Mullier F, Robert S, Chatelain C, Chatelain B, Dogné JM. Impact of dabigatran on a large panel of routine or specific coagulation assays. Laboratory recommendations for monitoring of dabigatran etexilate. *Thromb Haemost* 2012; **107**: 985–97.

19 Douxfils J, Mullier F, Loosen C, Chatelain C, Chatelain B, Dogné JM. Assessment of the impact of rivaroxaban on coagulation assays: laboratory recommendations for the monitoring of rivaroxaban and review of the literature. *Thromb Res* 2012; **130**: 956–66.

20 Douxfils J, Chatelain C, Chatelain B, Dogné JM, Mullier F. Impact of apixaban on routine and specific coagulation assays: a practical laboratory guide. *Thromb Haemost* 2013; **110**: 283–94.

21 Hawes EM, Deal AM, Funk-Adcock D, Gosselin R, Jeanneret C, Cook AM, Taylor JM, Whinna HC, Winkler AM, Moll S. Performance of coagulation tests in patients on therapeutic doses of dabigatran: a cross-sectional pharmacodynamic study based on peak and trough plasma levels. *J Thromb Haemost* 2013; **11**: 1493–502.

22 Douxfils J, Mani H, Minet V, Devalet B, Chatelain B, Dogné JM, Mullier F. Non-VKA oral anticoagulants: accurate measurement of plasma drug concentrations. *Biomed Res Int*. 2015; **2015**: 345138.

23 Stangier J, Feuring M. Using the HEMOCLOT direct thrombin inhibitor assay to determine plasma concentrations of dabigatran. *Blood Coagul Fibrinolysis* 2012; **23**: 138–43.

24 Samama MM, Amiral J, Guinet C, Perzborn E, Depasse F. An optimised, rapid chromogenic assay, specific for measuring direct factor Xa inhibitors (rivaroxaban) in plasma. *Thromb Haemost* 2010; **103**: 815–25.

25 Samama MM, Contant G, Spiro TE, Perzborn E, Guinet C, Gourmelin Y, Le Flem L, Rohde G, Martinoli JL; Rivaroxaban Anti-Factor Xa Chromogenic Assay Field Trial Laboratories. Evaluation of the anti-factor Xa chromogenic assay for the measurement of rivaroxaban plasma concentrations using calibrators and controls. *Thromb Haemost* 2012; **107**: 379–87.

26 Becker RC, Yang H, Barrett Y, Mohan P, Wang J, Wallentin L, Alexander JH. Chromogenic laboratory assays to measure the factor Xa-inhibiting properties of apixaban – an oral, direct and

selective factor Xa inhibitor. *J Thromb Thrombolysis* 2011; **32**: 183–7.

27 Tripodi A, Chantarangkul V, Guinet C, Samama MM. The International Normalized Ratio calibrated for rivaroxaban has the potential to normalize prothrombin time results for rivaroxaban-treated patients: results of an in vitro study. *J Thromb Haemost* 2011; **9**: 226–8.

28 Samama MM, Martinoli JL, LeFlem L, Guinet C, Plu-Bureau G, Depasse F, Perzborn E. Assessment of laboratory assays to measure rivaroxaban – an oral, direct factor Xa inhibitor. *Thromb Haemost* 2010; **103**: 815–25.

29 Douxfils J, Dogné JM, Mullier F, Chatelain B, Rönquist-Nii Y, Malmström RE, Hjemdahl P. Comparison of calibrated dilute thrombin time and aPTT tests with LC-MS/MS for the therapeutic monitoring of patients treated with dabigatran etexilate. *Thromb Haemost* 2013; **110**: 543–9.

30 Douxfils J, Tamigniau A, Chatelain B, Chatelain C, Wallemacq P, Dogné JM, Mullier F. Comparison of calibrated chromogenic anti-Xa assay and PT tests with LC-MS/MS for the therapeutic monitoring of patients treated with rivaroxaban. *Thromb Haemost* 2013; **110**: 723–31.

31 Kitchen S, Gray E, Mackie I, Baglin T, Makris M on behalf of the BCSH committee. Measurement of non-coumarin anticoagulants and their effects on test of haemostasis: guidance from the British Committee for Standards in Haematology. *Br J Haematol* 2014; **166**: 830–41.

32 Reilly PA, Lehr T, Haertter S, Connolly SJ, Yusuf S, Eikelboom JW, Ezekowitz MD, Nehmiz G, Wang S, Wallentin L; RE-LY Investigators. The effect of dabigatran plasma concentrations and patient characteristics on the frequency of ischemic stroke and major bleeding in atrial fibrillation patients: the RE-LY Trial (Randomized Evaluation of Long-Term Anticoagulation Therapy). *J Am Coll Cardiol* 2014; **63**: 321–8.