Journal of Thrombosis and Haemostasis, **15**: 1–11 DOI: 10.1111/jth.13912

REVIEW ARTICLE

Laboratory testing in patients treated with direct oral anticoagulants: a practical guide for clinicians

J. DOUXFILS,*† W. AGENO,‡ C.-M. SAMAMA,§ S. LESSIRE,¶ H. TEN CATE,** P. VERHAMME,†† J. -M. DOGNÉ* and F. MULLIER‡‡

**Department of Pharmacy, Namur Thrombosis and Hemostasis Centre (NTHC), Namur Research Institute for Life Sciences (NARILIS), University of Namur; †Qualiblood s.a., Namur, Belgium; ‡Department of Clinical and Experimental Medicine, University of Insubria, Varese, Italy; §Cochin University Hospital, Department of Anaesthesiology and Intensive Care, Université Paris Descartes, Paris, France; ¶Department of Anaesthesiology, Namur Thrombosis and Haemostasis Centre (NTHC),Namur Research Institute for Life Sciences (NARILIS), Université catholique de Louvain, CHU UCL Namur, Yvoir, Belgium; **Department of Internal Medicine, Maastricht University Medical Centre and Cardiovascular Research Institute (CARIM), Maastricht, the Netherlands; ††Department of Cardiovascular Sciences, Vascular Medicine and Haemostasis, University of Leuven, Leuven; and ‡‡CHU UCL Namur, Laboratory Hematology, Namur Thrombosis and Haemostasis Centre (NTHC), Namur Research Institute for Life Sciences (NARILIS), Université catholique de Louvain, Yvoir, Belgium*

To cite this article: Douxfils J, Ageno W, Samama C-M, Lessire S, ten Cate H, Verhamme P, Dogné J-M, Mullier F. Laboratory testing in patients treated with direct oral anticoagulants: a practical guide for clinicians. *J Thromb Haemost* 2017; https://doi.org/10.1111/jth.13912.

Click to hear Dr Baglin's perspective on the role of the laboratory in treatment with new oral anticoagulants

Summary. One of the key benefits of the direct oral anticoagulants (DOACs) is that they do not require routine laboratory monitoring. Nevertheless, assessment of DOAC exposure and anticoagulant effects may become useful in various clinical scenarios. The five approved DOACs (apixaban, betrixaban, dabigatran etexilate, edoxaban and rivaroxaban) have different characteristics impacting assay selection and the interpretation of results. This article provides an updated overview on (i) which test to use (and their advantages and limitations), (ii) when to assay DOAC levels, (iii) how to interpret the results relating to bleeding risk, emergency situations and perioperative management, and (iv) what is the impact of DOACs on routine and specialized coagulation assays. Assays for anti-Xa or anti-IIa activity are the preferred methods when quantitative information is useful, although the situations in which to test for DOAC levels are still debated. Different reagent sensitivities and variabilities in laboratory calibrations impact assay results. International calibration standards for all specific tests for each DOAC are needed to reduce the inter-laboratory variability and allow inter-study comparisons. The impact of the DOACs on hemostasis testing may cause false-positive or false-negative results; however, these can be minimized by using specific assays and collecting blood samples at trough concentrations. Finally, prospective clinical trials are needed to validate the safety and efficacy of proposed laboratory thresholds in relation to clinical decisions. We offer recommendations on the tests to use for measuring DOACs and practical guidance on laboratory testing to help patient management and avoid diagnostic errors.

Keywords: apixaban; dabigatran; edoxaban; laboratory testing; practical management; rivaroxaban.

Correspondence: Jonathan Douxfils, University of Namur, Faculty of Medicine, Department of Pharmacy, Namur Thrombosis and Haemostasis Centre (NTHC), Namur Research Institute for Life Sciences (NARILIS), 61, Rue de Bruxelles, B-5000 Namur, Belgium
Tel.: +32 8172 4342
E-mail: jonathan.douxfils@unamur.be

Received: 7 June 2017
Manuscript handled by: M. Carrier
Final decision: F. R. Rosendaal, 18 November 2017

Introduction

The direct oral anticoagulants (DOACs; apixaban, betrixaban [not discussed in this review], dabigatran etexilate, edoxaban and rivaroxaban) have become widely used since their approval in several thromboembolic disorders, including the treatment and secondary prevention of recurrent venous thromboembolism (VTE) and the prevention of stroke in patients with non-valvular atrial fibrillation (NVAF). These agents are administered as either once-daily (od) or twice-daily (bid) fixed-dose regimens, with dosage determined mainly by indication, age and/or creatinine clearance, body weight, and the use of concomitant drugs [1–4].

© 2017 The Authors. *Journal of Thrombosis and Haemostasis* published by Wiley Periodicals, Inc. on behalf of International Society on Thrombosis and Haemostasis. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

The DOACs exhibit more predictable pharmacokinetic and pharmacodynamic profiles than vitamin K antagonists (VKAs); consequently, routine coagulation monitoring is not required [5]. However, assessment of drug exposure and its anticoagulant effect may be helpful in certain clinical situations such as detection of drug accumulation in acute renal/liver failure or overdose, assessing anticoagulant activity in patients with bleeding or thrombosis, planning the timing of urgent surgery or intervention, special patient characteristics such as obesity or gastrointestinal malabsorption, determining the suitability for thrombolytic therapy for acute ischemic stroke or guiding the physician in the administration of reversal agents [6,7]. The different circumstances for laboratory testing, and the clinical relevance of such testing, remain debated, particularly with regard to the interpretation of test results and potential clinical implications.

In addition, because of their modes of action, DOACs affect commonly used global coagulation assays [8–10] and therefore influence coagulation function testing (e.g. for thrombophilia or lupus anticoagulant). As DOAC use continues to increase, it is also important to highlight their impact on coagulation testing.

What test to use to determine the presence of DOACs and their plasma concentrations?

The laboratory medicine specialist and clinician should collaborate to establish an institutional protocol on when and how to test DOACs, to guide the choice of the test based on what information the clinician needs, and to provide a multidisciplinary approach in its interpretation [8].

General considerations

The advantages and drawbacks of coagulation tests that could be used to estimate plasma concentrations of the DOACs or the relative intensity of anticoagulation are summarized in Table 1. It may be noted that one important parameter, the turnaround time (TAT), defined as time from registration of the blood sample in the laboratory to first result communicated (including centrifugation and analyses [reconstitution of the reagents when lyophilized, preparation of calibration curve, validation of control plasma and sample analysis/validation]), is subject to ongoing improvements (e.g. reduction of centrifugation times and implementation of specific tests on a 24/7 basis). To illustrate the feasibility of this, a recent study in patients with acute stroke showed a median TAT of 34 min when specific assays are routinely implemented [11].

Interpretation of the coagulation test results should take account of the likely DOAC plasma concentration range (Fig. 2), the timing of the last dose, the test reagents and underlying pathologies that can influence PT and APTT prolongation [12].

Direct thrombin inhibitor

Dabigatran The peak plasma concentration (C_{MAX}) and maximal anticoagulant effect of dabigatran is achieved within 3 h after oral dosing [2]. The activated partial thromboplastin time (APTT) can provide a qualitative assessment of dabigatran activity, but the sensitivity depends on the reagent and the coagulometer, which complicates the interpretation of the results (Fig. 1) [8]. Most patients treated with dabigatran etexilate will present with a prolonged APTT (ratio > 1.2; [APTT of the patient]/[reference[1] APTT]). A normal APTT excludes above on-therapy levels of dabigatran but does not exclude the presence of dabigatran in the on-therapy range (Figs. 1 and 2) [13]. Conversely, a normal thrombin time (TT) excludes the presence of dabigatran with a high negative predictive value [14–16]. On the other hand, a prolonged thrombin time could suggest either the presence of clinically relevant or trivial levels of dabigatran because the TT is sensitive to dabigatran.

Specific tests are therefore required and the commercially available diluted thrombin times (dTTs) that use dabigatran calibrators can accurately determine dabigatran level as they display a direct linear relationship with drug concentration and a good accuracy in the 50–500 ng mL^{-1} concentration range [13]. For lower concentrations, an adapted procedure is proposed for some dTTs (i.e. the Hemoclot® Thrombin Inhibitor LOW, Hyphen BioMed, Neuville sur-Oise, France, which uses a lower dilution of the sample and incorporates a standard at 0 ng mL^{-1} in the calibration curve) (Fig. 2) [14]. On the other hand, other dTT assays (i.e. the HemoSIL® Direct thrombin inhibitor (DTI), Instrumentation Laboratory, Bedford, MA, USA) are accurate within a broader range of concentration (from < 50 to 500 ng mL^{-1}) without requiring different methodologies and calibration curves.

Ecarin-based assays provide a direct measure of dabigatran activity. Of these, even if currently recommended in the European Summary of Product Characteristics (EU-SmPC) [2], the ecarin clotting time (ECT) assay is not readily available or useful in the absence of specific kits and standards (i.e. standardization of the concentration of ecarin in the test), is not approved by regulatory bodies (i.e. CE-marked or FDA 510k approved) in this application and is therefore not recommended [17]. However, the STA®-ECA-II (Diagnostica Stago®, Asnière-sur-Seine, France), an ecarin-based chromogenic assay, can accurately assess dabigatran plasma concentrations in the low (< 50 ng mL^{-1}) and the normal range (from 50 to 500 ng mL^{-1}; in this context, the term 'normal range' is used as an analytical terminology to mention that there is a normal calibration curve that is applicable from 50 to

[1]Reference APTT/PT is the APTT/PT of a normal pooled plasma (as in Figure 1) or the mean APTT/PT of at least 20 normal subjects.

© 2017 The Authors. *Journal of Thrombosis and Haemostasis* published by Wiley Periodicals, Inc. on behalf of International Society on Thrombosis and Haemostasis.

Table 1 Characteristics of coagulation tests for estimating plasma concentrations of direct oral anticoagulants or their relative intensity of anticoagulation*

Drugs	Laboratory tests	Utility/interpretation	Availability	Dependence of the reagent
Dabigatran	APTT*	Interpretation: Normal APTT excludes above on-therapy dabigatran levels but does not exclude the presence of dabigatran in the on-therapy range	24/7, all laboratories	Yes
	TT	Interpretation: Normal TT excludes the presence of dabigatran. A prolonged TT could suggest either the presence of clinically relevant or trivial levels of dabigatran.	24/7, all laboratories	Yes
	dTT	Interpretation: Based on plasma concentration estimation in relation to the clinical context. Note: Some methodologies (i.e. the Hemoclot Thrombin Inhibitors (HTI)) require specific calibrators for plasma concentrations < 50 ng mL^{-1}	Can be implemented with all coagulometers	No
	ECA	Interpretation: Based on plasma concentration estimation in relation to the clinical context	Can be implemented with all coagulometers	No
Rivaroxaban (Edoxaban)	PT*	Interpretation: Rivaroxaban: normal PT (with sensitive reagents) excludes above on-therapy rivaroxaban levels but does not exclude the presence of rivaroxaban in the on-therapy range. Edoxaban: normal PT (with sensitive reagents) would exclude above on-therapy edoxaban levels at peak but would not exclude the presence of above on-therapy edoxaban at trough.	24/7, all laboratories	Yes
Rivaroxaban Apixaban Edoxaban	Chromogenic anti-Xa assays*	Interpretation: Based on plasma concentration estimation in relation to the clinical context. Note: Some methodologies (i.e. the Biophen Direct Factor Xa Inhibitors (DiXaI)) require specific calibrators for plasma concentrations < 30–50 ng mL^{-1}. Note: If near to the LOQ, heparin or LMWH-calibrated chromogenic anti-Xa assays can be used to rule out the presence of clinically relevant direct FXa inhibitors.	Can be implemented with all coagulometers	No
Dabigatran Rivaroxaban Apixaban Edoxaban	LC-MS/MS	Interpretation: Based on plasma concentration estimation in relation to the clinical context	Requires trained staff; only in specialized laboratories	Not applicable
	dRVV-T (DRVV-DOAC)*	Interpretation: Normal dRVV result can exclude DOAC concentrations > 50 ng mL^{-1}.	Can be implemented with all coagulometers	Yes, but < than PT or APTT

APTT, activated partial thromboplastin time; dRVVT, diluted Russell's viper venom time; dTT, dilute thrombin time; ECA, ecarin chromogenic assay; ECT, ecarin clotting time; HPLC-MS/MS, high-performance liquid chromatography–tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantitation; PT, prothrombin time; TT, thrombin time. *None of these tests are able to discriminate between therapies. Thrombin-specific tests can easily identify dabigatran because it is the only direct oral thrombin inhibitor, but also other direct thrombin inhibitors such as argatroban or hirudin can influence them. For direct factor (F) Xa inhibitors, only the Biophen® Direct Factor Xa Inhibitor assay can discriminate between heparins and direct FXa inhibitors but cannot differentiate between direct FXa inhibitors. Mass spectrometry is the only technique able to directly discriminate between therapies.

500 ng mL^{-1}, in opposition to the 'low range', which requires adjusted calibration with some assays to assess plasma level below 50 ng mL^{-1}; 50–500 ng mL^{-1} does not correspond to the on-therapy ranges, which are depicted in Fig. 2). Importantly, ecarin assays are not sensitive to heparins, which may be valuable in the case of concomitant heparin administration (i.e. switching therapy from heparins to dabigatran etexilate or vice versa) [14]. A chromogenic anti-IIa assay has also been proposed but requires further validations [18].

Direct factor Xa inhibitors

Apixaban, edoxaban and rivaroxaban The maximal effect of apixaban and rivaroxaban is reached 3 h after the drug intake, whereas 2 h are needed for edoxaban [3,19]. Among the DOACs, rivaroxaban shows the strongest effect on prothrombin time (PT), followed by edoxaban and then apixaban, although different PT reagents show variations in their sensitivities towards these drugs [20,21]. Thus, as stated above, the establishment of an

© 2017 The Authors. *Journal of Thrombosis and Haemostasis* published by Wiley Periodicals, Inc. on behalf of International Society on Thrombosis and Haemostasis.

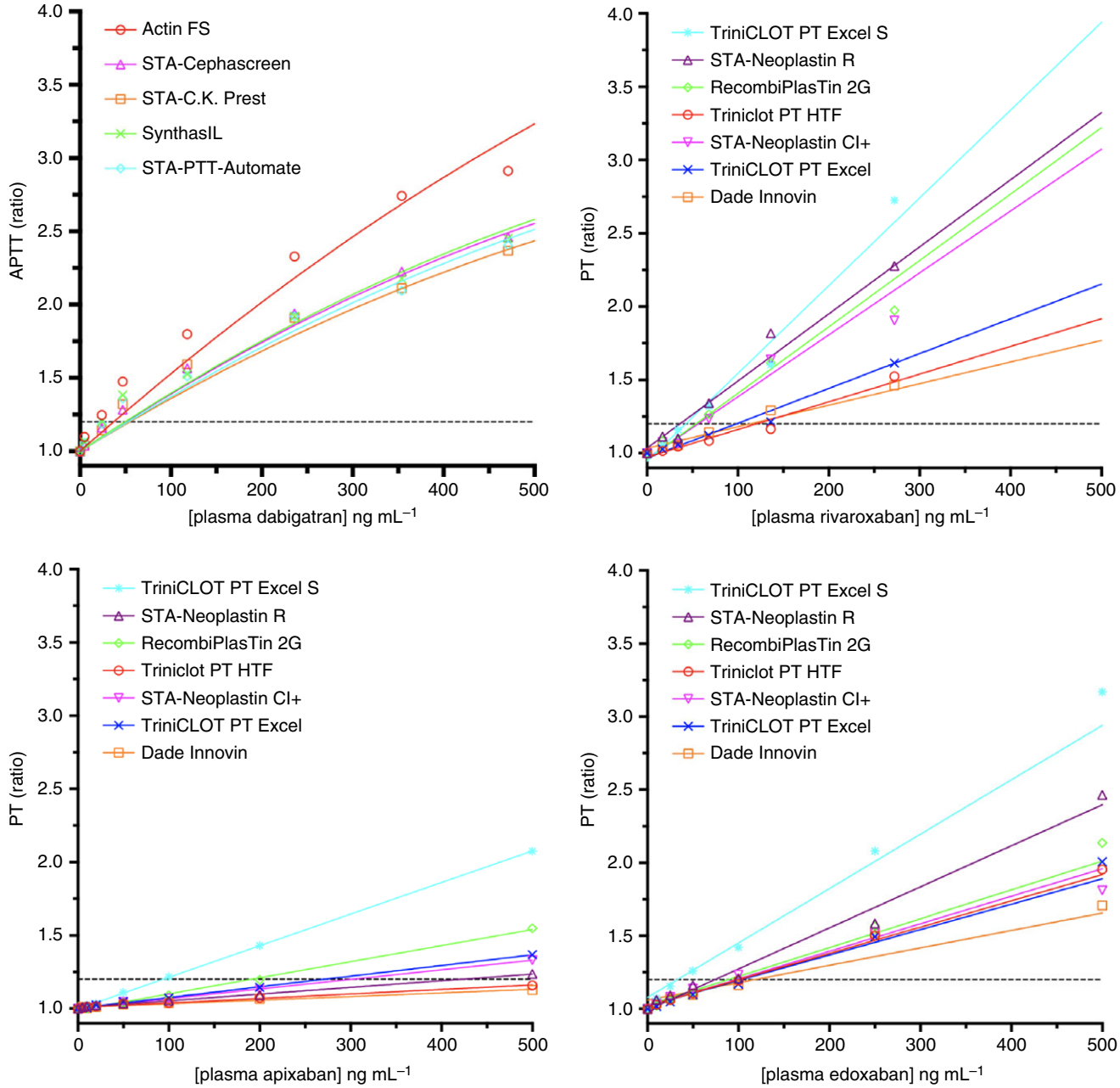

Fig. 1. Impact of dabigatran on APTT, and of rivaroxaban, apixaban and edoxaban on PT: variations in reagent sensitivity. The dotted line represents a ratio of 1.2, which is considered to be a relevant prolongation of the clotting time. APTT, activated partial thromboplastin time; PT, prothrombin time.

institutional protocol by a multidisciplinary team able to address the question of the sensitivity of the PT reagent is mandatory.

Rivaroxaban prolongs PT in a concentration-dependent manner. The sensitivity highly depends on the reagent used. If more sensitive reagents (reagents are considered sensitive when a low drug level (< 50 ng mL^{-1}) is sufficient to prolong the assay above the normal reference range of the assay), such as RecombiPlasTin®2G (Instrumentation Laboratory®) and STA®-Neoplastin®CI +/Neoplastin®R (Diagnostica Stago®), are used, PT can inform on the presence of rivaroxaban at both trough (24 h after last intake) and peak (3 h after last intake) levels and PT will be prolonged (ratio[2] > 1.2; [PT of the patient)/(reference PT]) in most patients. By contrast, when less sensitive reagents such as Dade® Innovin (a reagent routinely used in many laboratories across Europe) are used, PT is not affected (ratio < 1.2) by 'on-therapy' plasma

[2]For PT, the ratio is different from the international normalized ratio (INR), which is the same ratio corrected by an international sensitivity index (ISI) specific to each batch of each reagent.

© 2017 The Authors. *Journal of Thrombosis and Haemostasis* published by Wiley Periodicals, Inc. on behalf of International Society on Thrombosis and Haemostasis.

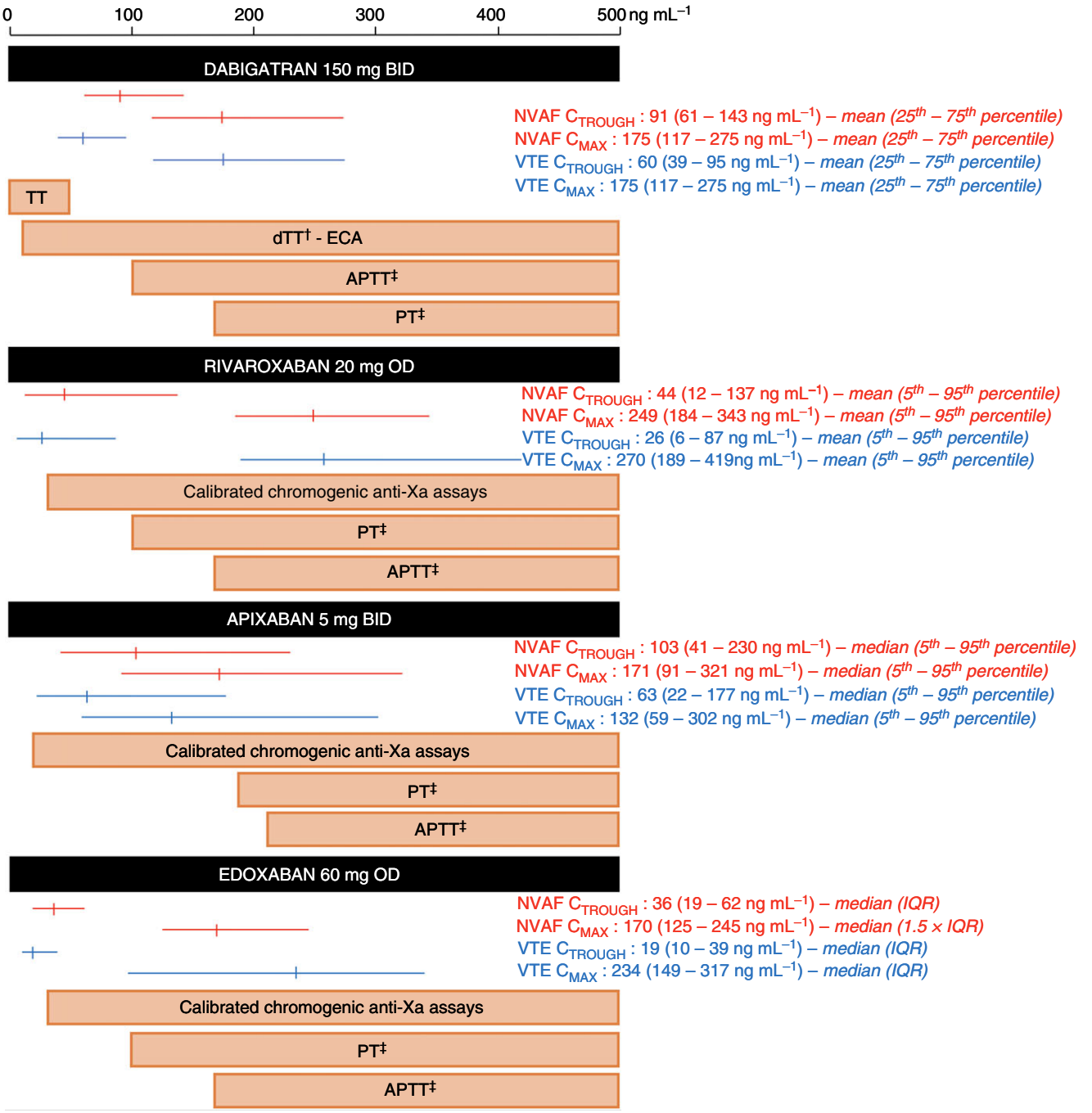

Fig. 2. Laboratory testing of direct oral anticoagulants and expected plasma concentrations after therapeutic doses. Red and blue lines represent plasma concentrations at peak and trough in NVAF and VTE, respectively. Orange boxes represent ranges of applicability of the corresponding test. †Depending on the dTT procedure used, the LOQ may be higher (i.e. 50 ng mL^{-1} instead of 10 ng mL^{-1}). ‡This represents the range of quantitation for sensitive reagents. Depending on the reagent, the sensitivity may be lower. Please refer to Fig. 1 for more details on relevant testing. APTT, activated partial thromboplastin time; CMAX, maximum plasma concentration during the dosing interval; CTROUGH, minimum plasma concentration during the dosing interval; dTT, diluted thrombin time; ECA, ecarin chromogenic assay; IQR, interquartile range; LOQ, limit of quantitation; NVAF, non-valvular atrial fibrillation; PT, prothrombin time; TT, thrombin time; VTE, venous thromboembolism. Notes: (i) Data on plasma concentration were extracted from current SmPC for dabigatran etexilate and apixaban or according to Mueck *et al.* [19] for rivaroxaban and to Ruff *et al.* [54], Weitz *et al.* [53] and Verhamme *et al.* [61] for edoxaban. (ii) For dabigatran edoxaban, plasma concentration ranges are expressed as mean or median (± IQR) representing only 50% of the population studied (except for edoxaban NVAF CMAX extracted from Weitz *et al.*, for which the range represents 75% of the population). Thus, for all dabigatran and edoxaban concentrations, the 5th to 95th percentile ranges are broader than the results expressed here.

© 2017 The Authors. *Journal of Thrombosis and Haemostasis* published by Wiley Periodicals, Inc. on behalf of International Society on Thrombosis and Haemostasis.

concentrations of rivaroxaban at trough and is prolonged (ratio between 1.2 and 1.5) at peak (Fig. 1) [22].

For apixaban, depending on the reagent, PT may be normal in the presence of on-therapy (± 200 ng mL^{-1}) concentrations (Fig. 2). Therefore, PT is not recommended for estimating plasma drug concentrations of apixaban or assessing the relative intensity of anticoagulation at on-therapy doses. One alternative is the dilute Russell's viper venom time (dRVVT, a test which activates FX and triggers the formation of the prothrombinase complex), which may suggest the presence of apixaban if the dRVVT is prolonged and the PT is normal [23]. However, this will not distinguish DOAC therapy from non-specific inhibitors if performed in isolation.

For edoxaban, prolongation of PT and APTT reaches peak within 2 h, but the modest effect and variability make these assays unsuitable for routine clinical assessment of the anticoagulant effect. Conversely, data from a recent study suggest that PT can be informative for ruling out excess edoxaban plasma concentrations at trough (prior to the next dose) but this would require sensitive reagents (i.e. TriniCLOT PT Excel S [TCoag®], STA®-Neoplastin®R [Fig. 1]). For most reagents, PT will be prolonged (ratio > 1.2) only at peak levels. [21].

Dedicated anti-Xa chromogenic assays using specific apixaban, edoxaban or rivaroxaban calibrators are able to measure a wide range of plasma concentrations covering the expected levels after therapeutic doses with results expressed in ng/ml (Fig. 2) [24]. They are more robust than global assays, but are influenced by heparins, so caution is advised in the interpretation of the result in the case of switching/bridging therapy. One chromogenic anti-Xa assay is not generally influenced by heparins: the Biophen® Direct Factor Xa Inhibitor (Hyphen BioMed®) test [25]. However, it should also be noted that the limit of quantitation of most specific tests is around 30–50 ng mL^{-1} and that adapted methodologies (i.e. as for the Hemoclot® Thrombin Inhibitor LOW [Hyphen BioMed®] for dabigatran) are suggested to assess plasma concentrations < 50 ng mL^{-1}. There is no accurate assay available to assess low plasma concentrations in patients switched to or from LMWH [26].

DOAC-calibrated chromogenic anti-Xa assays are not currently available in all hospitals, although with the emergence of liquid stable reagents and more routine use, they could be made available for emergency situations (Table 1). Non-dedicated anti-Xa chromogenic assays used to monitor heparin therapy are able to reliably exclude the presence of direct factor (F) Xa inhibitors but are affected by a high inter-assay variability and thus should not be used to quantify direct anti-Xa inhibitors [3,4,20].

When to measure direct oral anticoagulants and how to interpret the results of the different assays?

Measurement of DOACs may be required in several situations such as bleeding, thrombosis, urgent invasive procedures, to approve thrombolysis, to guide elective procedures (in specific populations or when the elimination of the DOAC could be impaired), in the case of overdose, or to ensure on-therapy levels in patients with multiple factors that interfere with pharmacokinetics of DOACs. Recommendations for test selection depend upon the clinical indication and the required information (i.e. accurate plasma measurement of drug levels or estimation of anticoagulant effect).

Emergency situations: is a direct oral anticoagulant present and how much?

Cases of emergent situations include bleeding, thrombosis, urgent invasive procedures and thrombolysis. In all these cases, physicians aimed to identify levels within or above the on-therapy range (Fig. 2). Studies in the real-world setting revealed that testing is useful if immediately available in urgent clinical situations where assessment of drug presence is judged to be needed [27,28].

Management of emergency situations where an urgent invasive procedure is indicated requires a fine assessment of the urgency of the situation and the hemostatic status of the patient to guide the potential use of specific reversal agents or non-specific pro-hemostatic factors. Guidance has been given by the Subcommittee on Control of Anticoagulation of the International Society on Thrombosis and Hemostasis (ISTH) on the use of specific antidotes for the reversal of DOACs (e.g. idarucizumab and Praxbind®, Boehringer Ingelheim, Ingelheim am Rhein, Germany, for reversing dabigatran's effect or andexanet alpha for FXa inhibitors). According to this document, a drug concentration > 30 ng mL^{-1} in patients requiring an urgent intervention associated with a high risk of bleeding is likely to be sufficiently high to warrant antidote administration, whereas in patients with serious bleeding, antidote administration should be considered if the drug concentration exceeds 50 ng mL^{-1} [29]. However, delaying the intervention or the administration of an antidote until normalization or availability of coagulation test results may be detrimental in the case of life-threatening bleeding (e.g. intracranial bleeding, or in emergency surgery for life-threatening conditions such as a ruptured aortic aneurysm) and neutralization of the anticoagulant effect should not be delayed while awaiting test results [29,30].

In other cases, the use of specific coagulation tests may also help to document the correction of hemostasis after administration of the antidote or to guide the clinician in the use of the antidote [29].

In those requiring thrombolysis, plasma concentrations of 10 (apixaban), 50 (dabigatran) and 100 (rivaroxaban) ng/mL have been proposed as cut-offs for considering intravenous (i.v.) thrombolysis with r-tPA in patients with acute ischemic stroke after an individual risk–benefit assessment [31]. According to a study in rivaroxaban

© 2017 The Authors. *Journal of Thrombosis and Haemostasis* published by Wiley Periodicals, Inc. on behalf of International Society on Thrombosis and Haemostasis.

patients, i.v. thrombolysis is recommended if the plasma concentration is < 20 (or 30 ng mL^{-1}). When the plasma level is between 20 and 100 ng mL^{-1} i.v. thrombolysis can be considered, whereas plasma concentrations > 100 ng/ml preclude the possibility of performing i.v. thrombolysis [32]. Patients with intracranial artery occlusion were recommended i.v. thrombolysis plus endovascular treatment or endovascular treatment alone if plasma levels were ≤ 100 ng/mL or > 100 ng/mL, respectively. In this study, determination of rivaroxaban plasma levels enabled i.v. thrombolysis in one-third of patients taking rivaroxaban who would otherwise be ineligible for acute treatment. In addition, no bleedings were reported in this study including 114 rivaroxaban patients. This clearly justifies the setting up of future studies to investigate this approach.

Elective perioperative setting: is a direct oral anticoagulant still present?

A discussion regarding the 2015 American Society of Regional Anesthesia and Pain Medicine guidelines suggests that interruption of DOACs should be based not only on their respective half-lives, but also on the residual drug concentration [33–36]. Although routine monitoring is not required in the perioperative setting, there is still insufficient data to endorse the pharmacokinetic (PK) strategy in all circumstances [37–39].

For elective procedures, routine monitoring is not required in the majority of the cases if the clinician respects the time windows according to the risk of the procedure as stated in the SmPC or guidelines [1–4,40]. However, some patients (e.g. patients with multiple factors that interfere with the pharmacokinetics of DOACs) or cases where the time window is unsure could benefit from a laboratory approach, especially for interventions associated with a high bleeding risk (e.g. neuraxial procedure) that require minimal to no anticoagulant effect at the time of the procedure. Indeed, the empirical treatment cessation of 1–3 days before the intervention, as suggested in the EU-SmPC and in the Food and Drug Administration (FDA) Label Information, revealed plasma concentrations of > 30 ng mL^{-1} at least for dabigatran etexilate and rivaroxaban [41–43]. However, a recent prospective multicenter study confirmed that a last DOAC intake 3 days before a procedure resulted in minimal (< 30 ng mL^{-1}) pre-procedural anticoagulant effect for almost all patients. Moderate renal impairment, especially in dabigatran-treated patients, and the use of antiarrhythmics in anti-Xa-treated patients should result in a longer DOAC interruption [44]. Yet, the proposed cut-off level of < 30 ng mL^{-1} is still a matter of debate [37–39]. Thus, in anticipation of prospective clinical studies designed to address this issue, the ability to measure drugs levels may help to guide the timing of invasive procedures in special circumstances.

To accurately measure low plasma drug concentrations in the perioperative setting, specific tests calibrated for the assessment of low plasma concentrations (i.e. < 50 ng mL^{-1}) are required. For dabigatran, APTT is not recommended, even in conjunction with PT [13,16]. Some dTT tests are suitable for the low range without procedural modifications (e.g. HemosIL®DTI and STA®-ECA II); others require a dedicated test (e.g. Hemoclot® Thrombin Inhibitor LOW). [14] If specific tests are not available, a normal TT excludes the presence of dabigatran [15,16]. For direct FXa inhibitors, PT is not sufficiently sensitive and even in conjunction with the APTT, it cannot rule out the presence of clinically relevant rivaroxaban levels [16]. Thus, DOAC-calibrated anti-Xa chromogenic assays, using appropriate calibrators and controls, have to be used to ensure a reliable assessment of the residual activity of the direct FXa inhibitor [26]. If not available, a heparin-calibrated anti-Xa chromogenic assay can be used to rule out the presence of clinically relevant levels of a direct FXa inhibitor but each laboratory should assess the sensitivity of their respective heparin assay/calibrator systems for the different direct FXa inhibitors using commercially available direct FXa inhibitors [20,45].

Impact of direct oral anticoagulants on coagulation function assessments

Because of their modes of action, DOACs also interfere with diagnostic tests for thrombophilia or bleeding disorders.

Prothrombin time (and the derived INR) and the APTT are both influenced by DOACs. Depending on the reagents, the coagulometer and the DOAC used by the patient, the sensitivity of the PT/INR and the APTT may vary (Fig. 1). Hence, a prolonged PT and/or APTT in a patient with known DOAC exposure should be expected and is likely to be drug related. However, physicians should keep in mind that PT or APTT may be prolonged due to other causes than DOAC presence, such as vitamin K deficiency, antibiotic use (which may impact intestinal flora and the consequent vitamin K synthesis), lipoglycopeptide antibiotics use (which may interfere with phospholipids included in the reagent), lupus anticoagulant, compromised liver function, dys-/a-fibrinogenemia or consumption coagulopathy (such as disseminated intravascular coagulation [DIC]) [46].

For thrombophilia testing, assays such as activated protein C (APC) resistance, antithrombin (AT), protein C, protein S, lupus anticoagulant and clotting factor assays, may be required. In these cases, testing should be performed preferably at C_{TROUGH} (i.e. 12 or 24 h after the last drug intake for bid and od, respectively) even if interferences are still possible, depending on the sensitivity of the test and the DOAC [47]. In the light of the possibility of invalid results, the real need for these tests should be carefully evaluated in patients on DOAC treatment.

In cases of bleeding diathesis or DIC, specific tests such as fibrinogen (Clauss and PT-derived method [dFib]), TT, clotting factor activity and reptilase time may also be

© 2017 The Authors. *Journal of Thrombosis and Haemostasis* published by Wiley Periodicals, Inc. on behalf of International Society on Thrombosis and Haemostasis.

Table 2 Interference of direct oral anticoagulants with various coagulation assays

Test	Dabigatran	Rivaroxaban	Apixaban	Edoxaban	Notes
PT-based measurements of clotting factors/inhibitors	↓	↓↓/↓↓↓	↓/↓↓	↓↓	• All factors affected • Most sensitive to rivaroxaban • Depends on the reagent
APTT-based measurements of clotting factors/inhibitors	↓↓↓	↓↓	↓/↓↓	↓/↓↓	• All factors affected • Most sensitive to dabigatran • Depends on the reagent • Rivaroxaban also interferes with one-stage and chromogenic factor (F) VIII:C assays • Clotting assays based on activation of coagulation at prothrombinase level unaffected by FXa inhibitors
Lupus anticoagulant: dRVVT	↑/↑↑	↑/↑↑	–/↑	↑	• False positives due to high screen/confirmation assay ratios • Taipan snake venom time and ECT time: alternative assays in rivaroxaban-treated patients • DOACs do not affect ELISA-based antiphospholipid assays
APCR	↑	↑	↑	↑	• APTT-based assays mostly affected • Factor V Leiden APTT-based assay generally satisfactory for apixaban-treated patients • No interference of rivaroxaban with Pefakit APCR Factor V Leiden®
Protein C activity	–/↑	–/↑	–/↑	–/↑	• Chromogenic assays: unaffected • Antigen-based assays: unaffected • Clot-based assays: affected
Protein S activity	–/↑	–/↑	–/↑	–/↑	• Antigen-based assay: unaffected • Clot-based: affected
Antithrombin activity	–/↑	–/↑	–/↑	–/↑	• Anti-thrombin-based assays affected by dabigatran • Anti-FXa-based assays affected by the FXa inhibitors

Abbreviations: ↓ to ↓↓↓, reduction; ↑ to ↑↑, increase; –, no effect; APCR, activated protein C resistance; APTT, activated partial thromboplastin time; dRVVT, diluted Russell's viper venom time; PT, prothrombin time.

used. Again, it is of particular importance for clinicians and laboratories to be aware of whether and how these tests may be influenced by DOACs. DOACs do not interfere with immunoassays and reptilase time but DOAC therapy may affect D-dimer levels.

Thus, physicians should be aware that false-positive or false-negative results are possible in patients receiving DOACs and can lead to diagnostic errors. There are assays unaffected by the DOACs that can be used for coagulation function testing in these patients. If the DOAC-insensitive assays cannot be used, missing one (for od dosing) or two (for bid dosing) doses could be considered to minimize the impact of residual DOACs on testing.

Some diagnostic companies are currently developing strategies to make these tests insensitive to the DOACs or to remove the DOACs from the blood before testing.

Finally, the clinician must inform the laboratory of the drug currently taken by the patient and the expected C_{TROUGH} and C_{MAX}, or the timing of the dose relative to blood sampling. Table 2 summarizes the impact of DOACs on the main coagulation function assays [21,48].

Discussion and conclusions

A wealth of knowledge has emerged over the last 5 years on testing DOAC levels. Although routine assessment of the intensity of anticoagulation is not required with these drugs, several situations may require the use of coagulation testing [49]. Guideline recommendations and consensus documents on laboratory testing of the DOACs are generally consistent and provide clear guidance for clinicians [8,50,51]. Specific tests have emerged as the most suitable solution for the determination of DOAC plasma concentrations and may be used in emergency situations with a turnaround time around 30 min. Studies are ongoing to further reduce this turn around time or to implement point-of-care tests [52]. The cost of these specific tests has also been questioned. They are more expensive than PT/APTT, but if one considers that their use will be restricted to special situations, it is likely that the burden for health systems will be lower than that presently incurred in managing patients on VKAs.

Nevertheless, although determination of DOAC plasma concentrations is now feasible, thresholds are yet to be validated to ensure that clinical decisions based on laboratory thresholds guarantee the optimal balance between avoiding bleeding and preventing thrombosis. Expert societies have proposed algorithms and/or thresholds for clinical situations based on extrapolation from pharmacokinetic studies, which need to be validated in prospective studies specifically designed for that purpose.

© 2017 The Authors. *Journal of Thrombosis and Haemostasis* published by Wiley Periodicals, Inc. on behalf of International Society on Thrombosis and Haemostasis.

Indeed, there is currently no consensus on a therapeutic range for these drugs even if some information can be extracted from phase 2–3 clinical trials on the 'on-therapy' range [2,3,19,53,54]. Publications [5,54,55] and/or data from regulators [56,57] showed association between plasma concentrations and bleeding risk but clear cut-offs (i.e. evidence-based thresholds for antidote administration, risk of (perioperative) bleeding or eligibility for thrombolysis) are not yet established for all DOACs for the different clinical situations physicians may be facing.

Given the widespread use of DOACs, well-designed prospective studies are required to support these preliminary proposals on the management of patients in the periprocedural setting, for both elective and urgent procedures. Appropriate strategies to guide the administration and monitor the effect of reversal agents should also be further investigated.

Studies in the real-world setting evaluating how drug level testing is currently used in clinical practice revealed that, to date, there is little urgency to make the tests widely available for routine use outside of the acute settings discussed above [27,28]. However, specific populations, such as patients with a history of bleeding, patients on polypharmacy with expected drug–drug interactions, patients on immunomodulatory drugs, those with extremes of bodyweight or gastrointestinal malabsorption, patients with liver and/or renal dysfunction or those with multiple interfering factors, should be further studied. To illustrate this, approximately 12–13% of the patients demonstrated plasma concentrations above the 95th percentile observed in phase-3 clinical trials in a study assessing the interpatient variation of apixaban and rivaroxaban in the routine care setting. Drug levels also tend to be more variable (50 to 60-fold interpatient variation) than predicted [58]. The high inter-individual variability, the numerous factors interfering with the pharmacokinetics and the dose–response relationship observed in phase-3 studies [5,54,55], suggest that the benefit–risk balance could be improved by a proper dose titration, which could be guided by determination of the response at the individual level in selected patients. Such an approach has been suggested by some manufacturers [59] and evidence demonstrated that trough plasma levels (for edoxaban and dabigatran), PT prolongation (for rivaroxaban) and AUC (for apixaban) are all linked with bleeding risk [5]. Reports of unexpected low plasma levels linked with thromboembolic events also suggest that more frequent measurements may add value in the routine care setting [60]. However, there are to date no clearly established therapeutic ranges and for a given DOAC it is difficult to titrate the dose using registered doses. Furthermore, the effectiveness of such an approach has to be confirmed by clinical data [5].

Importantly, in the elective setting, recording of the time between the last dose of DOAC and the blood sampling is required for all coagulation assays in DOAC-treated patients. Laboratories should also know the sensitivity of their own reagent/coagulometer combinations. Patients' samples are preferred to *in vitro* experiments to determine this sensitivity. Thus, there is an urgent need to develop international standards for each DOAC comparable to those for thromboplastin and heparin. This will improve the inter-laboratory reproducibility for all specific tests and allow direct comparisons between studies in order to develop and implement international guidelines for the optimal management of patients treated with DOACs.

Beside these strategies that aim to improve the safe use of DOACs, the influence of DOACs on coagulation function testing or hemostasis diagnostic tests has also to be clearly understood.

Addendum

J. Douxfils was responsible for the first draft and the revision of the manuscript. W. Ageno, C.-M. Samama, H. ten Cate, P. Verhamme, J.-M. Dogné, F. Mullier and S. Lessire provided comments and expertise during the revision of the manuscript. All authors were responsible for and approved the final version of the manuscript.

Disclosure of Conflict of Interests

W. Ageno reports personal fees and non-financial support from Stago and Daiichi Sankyo; grants, personal fees and non-financial support from Bayer Pharma and Boehringer Ingelheim; and personal fees from BMS/Pfizer and Aspen, outside the submitted work. H. ten Cate reports personal fees for being a consultant to STAGO, during the conduct of the study. J. Douxfils is CEO and founder of QUALIblood sa and reports personal fees from Stago and Daiichi-Sankyo, outside the submitted work. P. Verhamme reports grants and personal fees from Bayer Pharma, Boehringer Ingelheim and LeoPharma; personal fees from Daiichi Sankyo, BMS/Pfizer and Portola; and grants from Sanofi, outside the submitted work. C.-M. Samama reports speaker's fees from Bayer, BMS, Pfizer, Siemens and Stago; he has sat on advisory committees for Bayer, Daiichi-Sankyo, Portola and Roche, and has been a primary investigator for Bayer, BMS and Boehringer-Ingelheim.

References

1 European Medicines Agency. Xarelto - Summary of Product Characteristics. 2017. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000944/WC500057108.pdf (accessed 26 September 2017).
2 European Medicines Agency. Pradaxa - Summary of Product Characteristics. 2017. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/

© 2017 The Authors. *Journal of Thrombosis and Haemostasis* published by Wiley Periodicals, Inc. on behalf of International Society on Thrombosis and Haemostasis.

human/000829/WC500041059.pdf (accessed 26 September 2017).
3 European Medicines Agency. Eliquis - Summary of Product Characteristics. 2017. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002148/WC500107728.pdf (accessed 26 September 2017).
4 European Medicines Agency. Lixiana - Summary of Product Characteristics. 2017. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002629/WC500189045.pdf (accessed 26 September 2017).
5 Eikelboom JW, Quinlan DJ, Hirsh J, Connolly SJ, Weitz JI. Laboratory monitoring of non-vitamin K antagonist oral anticoagulant use in patients with atrial fibrillation: a review. *JAMA Cardiol* 2017; **2**: 566–74.
6 Weitz JI, Eikelboom JW. Urgent need to measure effects of direct oral anticoagulants. *Circulation* 2016; **134**: 186–8.
7 Douxfils J, Mullier F, Dogne JM. Dose tailoring of dabigatran etexilate: obvious or excessive? *Expert Opin Drug Saf* 2015; **14**: 1283–9.
8 Gosselin RC, Adcock DM. The laboratory's 2015 perspective on direct oral anticoagulant testing. *J Thromb Haemost* 2016; **14**: 886–93.
9 Bonar R, Favaloro EJ, Mohammed S, Ahuja M, Pasalic L, Sioufi J, Marsden K. The effect of the direct factor Xa inhibitors apixaban and rivaroxaban on haemostasis tests: a comprehensive assessment using *in vitro* and *ex vivo* samples. *Pathology* 2016; **48**: 60–71.
10 Bonar R, Favaloro EJ, Mohammed S, Pasalic L, Sioufi J, Marsden K. The effect of dabigatran on haemostasis tests: a comprehensive assessment using *in vitro* and *ex vivo* samples. *Pathology* 2015; **47**: 355–64.
11 Seiffge DJ, Traenka C, Polymeris A, Hert L, Fisch U, Peters N, De Marchis GM, Guzman R, Nickel CH, Lyrer PA, Bonati LH, Tsakiris D, Engelter S. Feasibility of rapid measurement of Rivaroxaban plasma levels in patients with acute stroke. *J Thromb Thrombolysis* 2017; **43**: 112–6.
12 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.
13 Douxfils J, Dogne JM, Mullier F, Chatelain B, Ronquist-Nii Y, Malmstrom 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.
14 Douxfils J, Lessire S, Dincq AS, Hjemdahl P, Ronquist-Nii Y, Pohanka A, Gourdin M, Chatelain B, Dogne JM, Mullier F. Estimation of dabigatran plasma concentrations in the perioperative setting. An *ex vivo* study using dedicated coagulation assays. *Thromb Haemost* 2015; **113**: 862–9.
15 Lessire S, Douxfils J, Baudar J, Bailly N, Dincq AS, Gourdin M, Dogne JM, Chatelain B, Mullier F. Is Thrombin Time useful for the assessment of dabigatran concentrations? An *in vitro* and *ex vivo* study. *Thromb Res* 2015; **136**: 693–6.
16 Jabet A, Stepanian A, Golmard JL, Flaujac C, Joly B, Gaussem P, Veyradier A, Gouin-Thibault I, Siguret V. CA27: Can PT, aPTT and TT reliably be used to detect DOAC at clinically relevant concentrations in emergency situations? results from a 660-patient cohort study. *J Thromb Haemost* 2016; **14**: 20.
17 Douxfils J, Pochet L, Lessire S, Vancraeynest C, Dogné J-M, Mullier F. Mass spectrometry in the therapeutic drug monitoring of direct oral anticoagulants. Useful or useless? *TrAC, Trends Anal Chem.* 2016; **84**, Part B: 41–50.
18 Brunetti L, Sanchez-Catanese B, Kagan L, Wen X, Liu M, Buckley B, Luyendyk JP, Aleksunes LM. Evaluation of the chromogenic anti-factor IIa assay to assess dabigatran exposure in geriatric patients with atrial fibrillation in an outpatient setting. *Thromb J* 2016; **14**: 10.
19 Mueck W, Stampfuss J, Kubitza D, Becka M. Clinical pharmacokinetic and pharmacodynamic profile of rivaroxaban. *Clin Pharmacokinet* 2014; **53**: 1–16.
20 Gosselin R, Grant RP, Adcock DM. Comparison of the effect of the anti-Xa direct oral anticoagulants apixaban, edoxaban, and rivaroxaban on coagulation assays. *Int J Lab Hematol* 2016; **38**: 505–13.
21 Douxfils J, Chatelain B, Chatelain C, Dogne JM, Mullier F. Edoxaban: impact on routine and specific coagulation assays. A practical laboratory guide. *Thromb Haemost* 2016; **115**: 368–81.
22 Douxfils J, Mullier F, Loosen C, Chatelain C, Chatelain B, Dogne 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.
23 Lippi G, Favaloro EJ. Recent guidelines and recommendations for laboratory assessment of the direct oral anticoagulants (DOACs): is there consensus? *Clin Chem Lab Med* 2015; **53**: 185–97.
24 Douxfils J, Gosselin RC. Laboratory assessment of direct oral anticoagulants. *Semin Thromb Hemost* 2017; **43**: 277–90.
25 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; **104**: 1078–9.
26 Lessire S, Douxfils J, Pochet L, Dincq AS, Larock AS, Gourdin M, Dogne JM, Chatelain B, Mullier F. Estimation of Rivaroxaban Plasma Concentrations in the Perioperative Setting in Patients With or Without Heparin Bridging. *Clin Appl Thromb Hemost* 2018; **24**: 129–38.
27 Martin K, Moll S. Direct oral anticoagulant drug level testing in clinical practice: a single institution experience. *Thromb Res* 2016; **143**: 40–4.
28 Wright C, Brown R, Cuker A. Laboratory measurement of the direct oral anticoagulants: indications and impact on management in clinical practice. *Int J Lab Hematol* 2017; **39**(Suppl. 1): 31–6.
29 Levy JH, Ageno W, Chan NC, Crowther M, Verhamme P, Weitz JI, The subcommittee on control of anticoagulation. When and how to use antidotes for the reversal of direct oral anticoagulants: guidance from the SSC of the ISTH. *Journal of Thrombosis and Haemostasis: JTH* 2016; **14**: 623–7.
30 Pernod G, Albaladejo P, Godier A, Samama CM, Susen S, Gruel Y, Blais N, Fontana P, Cohen A, Llau JV, Rosencher N, Schved JF, deMaistre E, Samama MM, Mismetti P, Sie 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) - March 2013. *Arch Cardiovasc Dis* 2013; **106**: 382–93.
31 Steiner T, Bohm M, Dichgans M, Diener HC, Ell C, Endres M, Epple C, Grond M, Laufs U, Nickenig G, Riess H, Rother J, Schellinger PD, Spannagl M, Veltkamp R. Recommendations for the emergency management of complications associated with the new direct oral anticoagulants (DOACs), apixaban, dabigatran and rivaroxaban. *Clin Res Cardiol* 2013; **102**: 399–412.
32 Seiffge DJ, Traenka C, Polymeris AA, Thilemann S, Wagner B, Hert L, Muller MD, Gensicke H, Peters N, Nickel CH, Stippich C, Sutter R, Marsch S, Fisch U, Guzman R, De Marchis GM, Lyrer PA, Bonati LH, Tsakiris DA, Engelter ST. Intravenous thrombolysis in patients with stroke taking rivaroxaban using

© 2017 The Authors. *Journal of Thrombosis and Haemostasis* published by Wiley Periodicals, Inc. on behalf of International Society on Thrombosis and Haemostasis.

drug specific plasma levels: experience with a standard operation procedure in clinical practice. *J Stroke* 2017; **19**: 347–55.
33 Narouze S, Benzon HT, Provenzano DA, Buvanendran A, De Andres J, Deer TR, Rauck R, Huntoon MA. Interventional spine and pain procedures in patients on antiplatelet and anticoagulant medications: guidelines from the American Society of Regional Anesthesia and Pain Medicine, the European Society of Regional Anaesthesia and Pain Therapy, the American Academy of Pain Medicine, the International Neuromodulation Society, the North American Neuromodulation Society, and the World Institute of Pain. *Reg Anesth Pain Med* 2015; **40**: 182–212.
34 Benzon HT, Lindholm PF, Huntoon MA. Direct oral anticoagulants: correlation of laboratory monitoring with safe interventional pain procedures. *Reg Anesth Pain Med* 2016; **41**: 123–4.
35 Douketis JD, Syed S, Schulman S. Periprocedural management of direct oral anticoagulants: comment on the 2015 American Society of Regional Anesthesia and Pain Medicine Guidelines. *Reg Anesth Pain Med* 2016; **41**: 127–9.
36 Lessire S, Douxfils J, Dincq AS, Mullier F. Periprocedural Management of Direct Oral Anticoagulants Should Be Guided by Accurate Laboratory Tests. *Reg Anesth Pain Med* 2016; **41**: 787–8.
37 Tripodi A. To measure or not to measure direct oral anticoagulants before surgery or invasive procedures: reply. *J Thromb Haemost* 2016; **14**: 2559–61.
38 Tripodi A. To measure or not to measure direct oral anticoagulants before surgery or invasive procedures. *J Thromb Haemost* 2016; **14**: 1325–7.
39 Spyropoulos AC, Al-Badri A, Sherwood MW, Douketis JD. To measure or not to measure direct oral anticoagulants before surgery or invasive procedures: comment. *J Thromb Haemost* 2016; **14**: 2556–9.
40 Heidbuchel H, Verhamme P, Alings M, Antz M, Diener HC, Hacke W, Oldgren J, Sinnaeve P, Camm AJ, Kirchhof P. Group ESCSD. Updated European Heart Rhythm Association practical guide on the use of non-vitamin-K antagonist anticoagulants in patients with non-valvular atrial fibrillation: Executive summary. *Eur Heart J* 2017; **38**: 2137–49.
41 Godier A, Martin AC, Leblanc I, Mazoyer E, Horellou MH, Ibrahim F, Flaujac C, Golmard JL, Rosencher N, Gouin-Thibault I. Peri-procedural management of dabigatran and rivaroxaban: duration of anticoagulant discontinuation and drug concentrations. *Thromb Res* 2015; **136**: 763–8.
42 Douketis JD, Wang G, Chan N, Eikelboom JW, Syed S, Barty R, Moffat KA, Spencer FA, Blostein M, Schulman S. Effect of standardized perioperative dabigatran interruption on the residual anticoagulation effect at the time of surgery or procedure. *J Thromb Haemost* 2016; **14**: 89–97.
43 Byron M, Zochert S, Hellwig T, Gavozdea-Barna M, Gulseth MP. Successful use of laboratory monitoring to facilitate an invasive procedure for a patient treated with dabigatran. *Am J Health Syst Pharm* 2017; **74**: 461–5.
44 Godier A, Dincq AS, Martin AC, Radu A, Leblanc I, Antona M, Vasse M, Golmard JL, Mullier F, Gouin-Thibault I. Predictors of pre-procedural concentrations of direct oral anticoagulants: a prospective multicentre study. *Eur Heart J* 2017; **38**: 2431–9.
45 Sabor L, Raphael M, Dogne JM, Mullier F, Douxfils J. Heparin-calibrated chromogenic anti-Xa assays are not suitable to assess the presence of significant direct factor Xa inhibitors levels. *Thromb Res* 2017; **156**: 36–8.
46 Gosselin RC, Gosselin R, Douxfils J, Adcock D. Clinical pearls: laboratory assessments of direct oral anticoagulants (DOACS). *Hamostaseologie* 2017; **37**: 295–301.
47 Ratzinger F, Lang M, Belik S, Jilma-Stohlawetz P, Schmetterer KG, Haslacher H, Perkmann T, Quehenberger P. Lupus-anticoagulant testing at NOAC trough levels. *Thromb Haemost* 2016; **116**: 235–40.
48 Favaloro EJ, Lippi G. Laboratory testing in the era of direct or non-vitamin K antagonist oral anticoagulants: a practical guide to measuring their activity and avoiding diagnostic errors. *Semin Thromb Hemost* 2015; **41**: 208–27.
49 Cuker A, Siegal DM, Crowther MA, Garcia DA. Laboratory measurement of the anticoagulant activity of the non-vitamin K oral anticoagulants. *J Am Coll Cardiol* 2014; **64**: 1128–39.
50 Ageno W, Gallus AS, Wittkowsky A, Crowther M, Hylek EM, Palareti G. Oral anticoagulant therapy: antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest* 2012; **141**: e44S–88S.
51 Heidbuchel H, Verhamme P, Alings M, Antz M, Diener HC, Hacke W, Oldgren J, Sinnaeve P, Camm AJ, Kirchhof P. Updated European Heart Rhythm Association Practical Guide on the use of non-vitamin K antagonist anticoagulants in patients with non-valvular atrial fibrillation. *Europace* 2015; **17**: 1467–507.
52 Fontana P, Alberio L, Angelillo-Scherrer A, Asmis LM, Korte W, Mendez A, Schmid P, Stricker H, Studt JD, Tsakiris DA, Wuillemin WA, Nagler M. Impact of rivaroxaban on point-of-care assays. *Thromb Res* 2017; **153**: 65–70.
53 Weitz JI, Connolly SJ, Patel I, Salazar D, Rohatagi S, Mendell J, Kastrissios H, Jin J, Kunitada S. Randomised, parallel-group, multicentre, multinational phase 2 study comparing edoxaban, an oral factor Xa inhibitor, with warfarin for stroke prevention in patients with atrial fibrillation. *Thromb Haemost* 2010; **104**: 633–41.
54 Ruff CT, Giugliano RP, Braunwald E, Morrow DA, Murphy SA, Kuder JF, Deenadayalu N, Jarolim P, Betcher J, Shi M, Brown K, Patel I, Mercuri M, Antman EM. Association between edoxaban dose, concentration, anti-Factor Xa activity, and outcomes: an analysis of data from the randomised, double-blind ENGAGE AF-TIMI 48 trial. *The Lancet* 2015; **385**: 2288–95.
55 Reilly PA, Lehr T, Haertter S, Connolly SJ, Yusuf S, Eikelboom JW, Ezekowitz MD, Nehmiz G, Wang S, Wallentin L, Investigators R-L. 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.
56 Food and Drug Administration. Eliquis - Clinical Pharmacology and Biopharmaceutics Review(s). 2012. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/202155Orig1s000ClinPharmR.pdf (accessed 26 September 2017).
57 Food and Drug Administration. Xarelto - Clinical Pharmacology and Biopharmaceutics Review(s). 2011. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/022406Orig1s000ClinPharmR.pdf (accessed 26 September 2017).
58 Gulilat M, Tang A, Gryn SE, Leong-Sit P, Skanes AC, Alfonsi JE, Dresser GK, Henderson SL, Rose RV, Lizotte DJ, Teft WA, Schwarz UI, Tirona RG, Kim RB. Interpatient Variation in Rivaroxaban and Apixaban Plasma concentrations in routine care. *Can J Cardiol* 2017; **33**: 1036–43.
59 Boehringer Ingelheim. An Idea for a mid to long term strategy for pradaxa, showing EMA range comparisons. *BMJ*, 2014. Available at: http://www.bmj.com/highwire/powerpoint/761319 (accessed 26 September 2017).
60 Savage RL, Castellano M, Tatley MV. International reports of unexpected low plasma concentrations of dabigatran suggest that more frequent measurements will add value. *Semin Thromb Hemost* 2017; **43**: 635–8.
61 Verhamme P, Wells PS, Segers A, Ageno W, Brekelmans MP, Cohen AT, Meyer G, Grosso MA, Raskob G, Weitz JI, Zhang G, Buller H. Dose reduction of edoxaban preserves efficacy and safety for the treatment of venous thromboembolism. An analysis of the randomised, double-blind HOKUSAI VTE trial. *Thromb Haemost* 2016; **116**: 747–53.

© 2017 The Authors. *Journal of Thrombosis and Haemostasis* published by Wiley Periodicals, Inc. on behalf of International Society on Thrombosis and Haemostasis.